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The ARDS, Pneumonia, and Sepsis (APS) Consortium: Rationale, Design, and Feasibility of a National Platform for Phenotyping Critical Illness Syndromes

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Title:

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Appendix A: APS Consortium study protocol A for participants with research informed consent
Appendix B: APS Consortium study protocol B for participants using alteration of informed consent
Appendix C: APS Consortium biospecimen collection standard operating procedures

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TITLE

The ARDS, Pneumonia, and Sepsis (APS) Consortium: Rationale, Design, and Feasibility of a National Platform for Phenotyping Critical Illness Syndromes

ABSTRACT

Background: To enhance biological understanding of acute respiratory distress syndrome (ARDS), pneumonia, and sepsis and accelerate therapeutic development in these areas, the National Institutes of Health developed the ARDS, Pneumonia, and Sepsis (APS) Consortium.

Research Question: Is the APS Consortium study rapidly generating data and biospecimens from a large cohort of critically ill adults with ARDS, pneumonia, and sepsis that will facilitate phenotyping of these syndromes?

Study Design and Methods: The APS Consortium Phenotyping Study is a multicenter longitudinal prospective observational cohort study aimed at enrolling 4,000 critically ill adults with ARDS, pneumonia, and/or sepsis over 4 years. Data and biospecimens are collected to characterize many aspects of each participant's chronic health, acute illness, and long-term recovery to facilitate phenotyping—that is, subclassifying ARDS, pneumonia, and sepsis into precise biologically-based subsets with shared pathophysiology. Feasibility of the study was assessed by evaluating the first 1,000 participants in terms of recruitment pace, participant characteristics, biospecimen collection, and proportion with confirmed ARDS, pneumonia, and sepsis based on expert adjudication.

Results: The first 1,000 participants were recruited ahead of schedule in less than 13 months. Median age was 64 years, 75% received vasopressors, 50% received invasive mechanical ventilation, and 25% died in the hospital within 4 weeks of enrollment. Biospecimen collection rates were high, with 99% of participants with blood, 98% with upper respiratory swabs, 37% with lower respiratory samples, 80% with urine, and 65% with gastrointestinal samples. Expert adjudication resulted in 40% classified with ARDS, 52% with pneumonia, and 89% with sepsis.

Interpretation: The APS Consortium Phenotyping Study is producing a cohort of critically ill adults with ARDS, pneumonia, and sepsis with high severity of disease and a rich set of data and biospecimens. The study will continue to full enrollment of 4,000 participants.

Registration: clinicaltrials.gov NCT06521502

Key words: acute respiratory distress syndrome; pneumonia; sepsis; phenotype; precision medicine; critical care; recovery

Abbreviations:

Abbreviation	Full text
AI	artificial intelligence
AIDS	acquired immunodeficiency syndrome
APS	ARDS, Pneumonia, and Sepsis
ARDS	acute respiratory distress syndrome
BAL	bronchoalveolar lavage
CD4	cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CPT	cell preparation tubes
CT	computed tomography
DNA	deoxyribonucleic acid
ECMO	extracorporeal membrane oxygenation
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GDF	growth differentiation factor
HIV	human immunodeficiency virus
HME	heat moisture exchange
ICAM-1	intercellular adhesion molecule-1

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Abbreviation	Full text
ICU	intensive care unit
IL	interleukin
IMV	invasive mechanical ventilation
IQR	interquartile range
IRB	institutional review board
MCP1	monocyte chemoattractant protein 1
MCP1	monocyte chemoattractant protein 1
NBBAL	non-bronchoscopic bronchoalveolar lavage
NFL	neurofilament light chain
NHLBI	National Heart, Lung, and Blood Institute
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
PAI-1	plasminogen activator inhibitor-1
PBMC	peripheral blood mononuclear cells
PCP3	procollagen peptide-3
PETAL	Prevention and Early Treatment of Acute Lung Injury
PEth	Phosphatidylethanol
RAGE	receptor for advanced glycation end-products
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RUCA	rural-urban commuting area
SOFA	sequential organ failure assessment
TNF	tumor necrosis factor
US	United States

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The overlapping syndromes of acute respiratory distress syndrome (ARDS), pneumonia, and sepsis are major contributors to morbidity and mortality in the United States (US) and globally.[1–3] While mortality from ARDS, pneumonia, and sepsis in high-income countries has declined over the past 30 years,[4,5] most of this decline is attributable to improvements in early recognition of disease, prompt antibiotics, and advances in supportive care.[3,6,7] Therapies that prevent or reverse the dysregulated host immune response that underlies ARDS, pneumonia, and sepsis remain elusive.[8,9] Hundreds of pharmacological agents have been tested as potential therapies for interrupting the maladaptive host response to infection, yet none are approved by the US Food and Drug Administration (FDA).[10–12] Many groups have noted that substantive further improvements in outcomes for patients with ARDS, pneumonia, and sepsis are likely to require therapies that fundamentally alter dysregulated human biological responses to infection.[10,11,13–18]

The experiences of clinical trial networks funded by the US National Heart, Lung, and Blood Institute (NHLBI) to study therapies for ARDS, pneumonia and sepsis during the past 30 years are illustrative. The ARDS Network (1996-2013) and Prevention and Early Treatment of Acute Lung Injury (PETAL) Network (2014-2023) conducted 18 clinical trials.[19,20] These studies contributed to major improvements in supportive care practices, including low tidal volume ventilation,[21] fluid administration practices,[22,23] and avoidance of routine use of pulmonary artery catheters[24] and neuromuscular blockade.[25] However, trials for targeted pharmacological therapies repeatedly showed null results when conducted in heterogeneous, clinically-defined populations.[26–32] The investigators pointed to the heterogenous nature of ARDS and sepsis as a potential reason why pharmacological interventions targeting specific host pathways failed to show benefit.[33]

During the past decade, secondary analyses using a phenotyping approach of several previously completed trials in ARDS and sepsis have shown promise.[34–39] In this approach, patients were classified based on unsupervised machine learning algorithms using baseline patient characteristics and protein biomarkers to create separate sub-populations (phenotypes). Efficacy was then re-evaluated within these phenotypes. Results have demonstrated important heterogeneity of treatment effect, with some therapies that had null results in the overall trial population appearing to have benefit in some specific phenotypes

and harm in others. For example, activated protein C failed to show benefit in a heterogenous population of patients with sepsis in the PROWESS-SHOCK trial.[40] However, reanalysis of the trial suggested patients with a hyperinflammatory phenotype did benefit from activated protein C.[37] These types of observations have motivated an emerging approach to testing interventions in ARDS, pneumonia, and sepsis in which patient phenotypes are prospectively built into trial design, with an ultimate goal of including patients in trials who have biological features that match the intervention's mechanism of action (predictive enrichment).[41] This type of approach to therapeutic development and evaluation requires an advanced understanding of the biological mechanisms underlying ARDS, pneumonia, and sepsis.

Thus, in 2023, NHLBI and the National Institute of General Medical Sciences (NIGMS) launched a new national consortium in the US to fundamentally advance biological understanding of ARDS, pneumonia, and sepsis, and to identify specific subsets of patients with biological profiles likely to benefit from targeted therapies. As a result, the ARDS, Pneumonia, and Sepsis (APS) Consortium was born. This manuscript describes the rationale and design of the APS Consortium and the feasibility assessment of its phenotyping study after enrollment of the initial 1,000 participants.

STUDY DESIGN AND METHODS

APS Consortium Structure

The APS Consortium is funded jointly by NHLBI and NIGMS of the National Institutes of Health (NIH) via grants to a coordinating center and 6 clinical centers, which each serve as hubs for multiple clinical sites **(Figure 1)**. Sites include academic, community, and safety-net hospitals in the US. The consortium is governed by a steering committee composed of representatives from each clinical center and coordinating center. During 2023, we developed a longitudinal observational cohort study to enroll 4,000 critically ill adults with ARDS, pneumonia, and/or sepsis. This study, called the APS Consortium Phenotyping Study (clinicaltrials.gov: NCT06521502), provides the basis for participant enrollment, data collection, and biospecimen collection. The study was approved by an NHLBI-appointed protocol review committee and is overseen by a single institutional review board at Vanderbilt University Medical Center

and an Observational Study Monitoring Board. Enrollment was launched in 2024 and is projected to continue through 2028.

The study protocol is designed to facilitate 3 levels of investigations: (1) *consortium-wide studies* funded by NIH APS Consortium grants, governed by the APS Consortium Phenotyping Study protocol, and conducted collaboratively by the entire consortium; (2) *clinical center studies* funded by NIH APS Consortium grants and led by one of the clinical centers within the consortium; and (3) *ancillary studies* that are funded outside APS Consortium grants, use banked data and/or biospecimens from the consortium, and conducted by researchers either within or outside the consortium.

Access to APS Consortium Data and Biospecimens for Ancillary Studies

An important aspect of the APS Consortium is the collection of biospecimens beyond what we plan to use in consortium-wide and clinical center studies. These biospecimens will be banked along with participant data and made broadly available to researchers to pursue additional investigations in ancillary studies. Data will be iteratively posted at BioDataCatalyst [https://biodatacatalyst.nhlbi.nih.gov/]. Biospecimens will be banked initially at the APS Consortium coordinating center and ultimately at BioLINCC [https://biolincc.nhlbi.nih.gov]. Researchers wishing to gain access to APS Consortium data or biospecimens for ancillary studies may request access at the APS Consortium website [apsconsortium.org]. The Ancillary Studies Sub-committee reviews these requests and grants access for meritorious applications that do not overlap with work already scheduled. After conclusion of APS Consortium funding, access will be governed through BioDataCatalyst and BioLINCC.

APS Consortium Phenotyping Study Design

The APS Consortium Phenotyping Study is a longitudinal prospective observational cohort study designed to collect clinical data, biospecimens, laboratory measurements, physiological measurements, cognitive assessments, patient-reported outcomes, and radiographic images to characterize a patient's biological and clinical course during acute illness and recovery from ARDS, pneumonia, or sepsis. These

data will be used to develop phenotypes, which are clusters of patients who share key biological similarities.

The eligible population includes adults critically ill from an acute inflammatory condition with cardiovascular dysfunction, defined by use of vasopressors to treat hypotension, and/or pulmonary dysfunction, defined as mechanical ventilation or use of ≥6 liters per minute of supplemental oxygen to treat hypoxemia. The full list of eligibility criteria is available in the study protocols in the supplemental materials **(Appendix A and B)**. These eligibility criteria are designed to enroll patients with or at risk for developing ARDS, pneumonia, and/or sepsis.

The presence of ARDS, pneumonia, and sepsis was not included in eligibility criteria to avoid the need for study personnel to make decisions at the bedside regarding whether these syndromes are present, which is often not conclusively possible in real-time. This enables rapid enrollment of a large population of critically ill patients with acute organ dysfunction thought to be due to an acute inflammatory event, such as infection, aspiration, or pancreatitis. Adjudication for the presence of ARDS, pneumonia, and sepsis is retrospectively performed by investigators who review all available information from the patient's hospitalization from two days before enrollment (study day -2) through 7 days after enrollment (study day 7). This approach enables classification of ARDS, pneumonia, and sepsis based on as much information as possible, which maximizes accuracy of the classifications. Investigators classify ARDS based on the New Global Definition of ARDS,[42] pneumonia based on Centers for Disease Control and Prevention (CDC) criteria,[43] and sepsis based on Sepsis-3 criteria.[44]

To capture biospecimens early in the course of critical illness, participants are enrolled as soon as possible and no later than 48 hours after meeting organ dysfunction criteria. At the time of eligibility confirmation, if a participant has capacity for consent or a surrogate decision maker is available, informed consent for research participation is obtained before study procedures commence. If the participant lacks capacity and a surrogate is not available, study personnel may begin study procedures using an alteration of informed consent paradigm. If a participant is enrolled under alteration of informed consent, study personnel iteratively attempt to obtain consent over time. While a participant is on study under alteration

of informed consent, only minimal risk study procedures are performed. The study protocols for participants with informed consent **(Appendix A)** and alteration of informed consent **(Appendix B)** are available in the supplemental materials.

Biospecimen Collection

We collect and bank biospecimens from enrolled participants at multiple timepoints, with the exact time of collection recorded **(Table 1)**. Each specimen is collected and processed locally according to study-specific standard operating procedures (**Appendix C**). We followed several principles when developing the schedule of biospecimen collection. First, we emphasize collection from multiple anatomical compartments, including the blood, respiratory tract, gastrointestinal tract, and urine to ensure samples are available to evaluate the human host as a system. Second, recognizing the central importance of the respiratory tract, we collect respiratory samples using multiple approaches, including protocolized collection of tracheal aspirates, fluid from heat and moisture exchange (HME) filters inserted into the mechanical ventilator circuit,[45,46] and non-bronchoscopic bronchoalveolar lavage (NBBAL). Third, understanding that ARDS, pneumonia, and sepsis are dynamic conditions, we collect serial samples throughout the first two weeks of acute illness so biological changes can be assessed. Fourth, post-hospital recovery is an essential phase of disease to understand, leading us to collect biospecimens in a subset of participants through 12 months after enrollment. Fifth, blood collection is scheduled so participants on study under alteration of informed consent can have the full volume of blood collected during the first 3 days without exceeding the threshold for a minimal risk study procedure (50 ml).[47] This provides time for research personnel to obtain consent before participants on the alteration of informed consent protocol miss blood draws compared to those on the consented protocol.

Data Collection

We collect clinical data via participant and surrogate interviews and recording of information from the medical record. These data are used to describe the characteristics of participants prior to acute illness, at

the time of enrollment, and throughout their hospital stay. Key in-hospital outcome data include: mortality; hospital and ICU length of stay; sequential organ failure assessment score version 2 (SOFA-2)[48] every day the patient is in the hospital between study day -2 and day 7; and organ support free day outcomes, which are ordinal outcomes that include a composite of mortality and duration of supportive therapy, such as vasopressors or invasive mechanical ventilation.[49]

After hospital discharge, we seek to obtain follow-up information from all survivors at 3, 6, and 12 months via surveys administered either online or via telephone. The survey battery is designed to assess quality of life, mental health, dyspnea, frailty, functional limitations, disability, cognition, return to work, financial toxicity, and healthcare utilization.[50] Additionally, we aim to perform in-person follow-up assessments on a convenience sample of 600 survivors at 3-6 months and 12 months. These in-person visits include biospecimen collection, performance-based assessment of physical and cognitive status, and at 12-months only, pulmonary function tests and chest computed tomography (CT).

Imaging and Laboratory Measurements

Serial chest radiographic imaging is banked as part of study procedures, including chest x-rays and CT scans during the acute phase of illness and CT scans during post-hospital follow-up. We will use these images to evaluate whether interpretation of radiographic images, including with artificial intelligence (AI) algorithms, can meaningfully contribute to phenotyping. The images will also be available for future use in ancillary studies.

As part of the initial studies conducted by the APS Consortium investigators, we will perform an array of laboratory measurements selected by the consortium steering committee **(Table 2)**. These measurements will be used to understand mechanisms of acute illness and recovery and to provide data for phenotyping.

Study Feasibility Assessment

After enrollment of 1,000 participants (one-quarter of the target sample size), we analyzed data to assess the feasibility of the study using several metrics: (i) pace of recruitment, with a target to recruit the first 1,000 in ≤15 months; (ii) biospecimen collection with a target for ≥90% having blood and upper respiratory samples collected; (iii) sample size for each clinical syndrome, with a target for ≥25% with each syndrome (ARDS, pneumonia, and sepsis); and (iv) severity of disease, with a target for ≥50% of the participants receiving vasopressors and ≥50% receiving invasive mechanical ventilation

RESULTS

The initial 1,000 participants of the planned 4,000-participant cohort were enrolled between July 2024 and July 2025, ahead of target recruitment pace. Among these 1,000 enrolled participants, median age was 64 years, 75% were living in the community without assistance for daily living prior to their acute illness, and chronic medical conditions were very common, including 24% with active cancer, and 25% with an immunocompromising condition **(Table 3)**. The percentage of participants with biospecimen collection on study day 0 or 2 was: 99% for plasma; 99% for RNA Paxgene; 92% for cell preparation tubes; 98% for upper respiratory (oral or nasal) swabs, 37% for lower respiratory samples (HME filter, tracheal aspirate, or NNBAL); 80% for urine; and 65% for gastrointestinal samples (rectal swab or stool). Adjudication of critical illness syndromes resulted in 40% of participants classified with ARDS, 52% with pneumonia, and 89% with sepsis, including 32% with all three syndromes concurrently. Within 28 days of enrollment, 75% of participants received vasopressors, 50% received invasive mechanical ventilation, and 25% died in the hospital **(Figure 2A)**. Daily SOFA-2 scoring demonstrated a high proportion of participants with organ dysfunction each day for the first week after enrollment **(Figure 2B)**. Free-day outcomes demonstrated broad distributions, illustrating heterogeneity in the timing of organ recovery **(Figure 3)**. The study met each of its feasibility metrics through 1,000 participants and will continue to full enrollment.

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DISCUSSION

The APS Consortium was designed to improve our biological understanding of ARDS, pneumonia, and sepsis. Using rich data, biospecimens, and imaging collected serially during the acute phase of illness and first year of recovery from 4,000 patients, we will develop the most comprehensive repository on ARDS, pneumonia, and sepsis ever assembled. The goal is for this repository to be used by both investigators within the consortium and other researchers to fundamentally improve how the medical community views and approaches these syndromes. An assessment of the first 1,000 participants enrolled demonstrated feasibility of the study, with recruitment ahead of schedule, high rates of biospecimen collection, a distribution of ARDS, pneumonia, and sepsis that will enable robust investigation of each syndrome, and a critically ill study population with a high rate of organ support and mortality.

Moving forward, our aspirations include two ground-breaking advances: (1) redefining the clinical syndromes currently known as ARDS, pneumonia, and sepsis into biologically-derived disease states ("phenotypes") categorized by mechanistic understanding rather than solely on clinical observations; and (2) reshaping how clinical trials evaluating pharmacological interventions for ARDS, pneumonia, and sepsis are designed based on a precision approach in which patients with specific mechanistic derangements are matched to therapies that act to correct those derangements.

Initial analyses will focus on phenotyping, with unsupervised and supervised statistical techniques to classify patients with ARDS, pneumonia, and sepsis into categories based on similar biological profiles. We are seeking to identify phenotypes that are: (1) reproducible across populations; (2) defined by biological characteristics in key mechanistic pathways that drive disease; and (3) therapeutically and/or prognostically important These analyses will include data reflecting the patient's state at initial diagnosis and changes over time. Dimension reduction strategies such as redundancy analysis and principal components analysis will be used for high-dimensional data. Internal validation strategies including k-fold cross-validation and bootstrapping will be pursued in all major analyses to evaluate validity and the potential for model overfitting.

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As our studies progress, we will also pursue development of precision clinical trials based on the findings of these biological investigations, thus translating phenotypes into actionable populations for clinical trials and ultimately clinical care. This will begin with target trial emulation using observational data within the consortium to simulate clinical trials for hypothesized interventions in newly developed phenotypes. In the future, we envision leveraging knowledge gained from studies in the APS Consortium to design phenotype-informed clinical trials that evaluate novel and repurposed drugs where each phenotype is biologically matched to a drug's mechanism of action.

We designed the APS Consortium with several key innovations and strengths in study design. Coordinated, prospective enrollment of 4,000 critically ill patients from 23 hospitals with detailed data collection will provide one of the richest datasets ever assembled for studying ARDS, pneumonia, and sepsis. Most prior research on phenotyping critical illness syndromes has focused on host plasma measurements in the acute phase of illness. In our study, we collect biospecimens from multiple sources, including the respiratory tract, gastrointestinal tract, urinary tract, immune cells, and plasma to characterize infecting pathogens, the microbiome, and the host response in multiple compartments. Additionally, we serially collect data and biospecimens over the course of a year to characterize both the acute and recovery phases of critical illness, enabling longitudinal analyses to understand trajectories. Laboratory analyses are conducted in central laboratories with deep expertise in the assays they are performing, ensuring rigorous methods consistently applied to a large sample size of patients enrolled from across the US. Importantly, the consortium is designed to broadly share data and biospecimens with researchers both inside and outside the consortium to maximize scientific output and knowledge gained.

Limitations of our approach should also be noted. First, all our studies are limited to adults treated in technologically advanced hospitals in the US. It is unknown if our observations will be generalizable to children or resource-limited settings. Second, while our overall sample size of 4,000 participants is large, some analyses that aim to evaluate specific patient subsets are likely to be limited by sample size. Third, the study was designed for mechanistic interrogation and not population epidemiology; thus, concepts such as prevalence of disease states cannot be evaluated. Fourth, sampling

the respiratory tract is challenging. Our approach involves upper respiratory swabs from all participants, and the addition of HME filter fluid, tracheal aspirates, and NBBAL for those on invasive mechanical ventilation. Sampling of the respiratory tract is limited for those not treated with invasive mechanical ventilation. Fifth, many patients with ARDS, pneumonia and sepsis die early in the course of acute illness. Early death prevents assessment of longitudinal biological changes and post-hospital clinical outcomes. We will use statistical techniques to address the competing risk of mortality; however, such techniques cannot completely account for missing data at later timepoints. Sixth, achieving post-hospital follow-up is challenging. In addition to mortality, functional impairment, subsequent illnesses, and other challenges, such as housing insecurity, can interfere with follow-up. Our approach is to complete in-person follow-up with biospecimen collection and functional assessments in a convenience sample of 600 participants, recognizing that this population may not be completely generalizable to the 4,000-patient cohort initially enrolled.

INTERPRETATION

The APS Consortium is pursuing a deeper biological understanding of ARDS, pneumonia, and sepsis by enrolling 4,000 participants with these syndromes into a longitudinal observational cohort study, assessing in-hospital and post-hospital outcomes, performing targeted laboratory and physiological measurements, conducting phenotyping analyses, and making its data, biospecimens, and images broadly available to researchers for further investigations. Assessment of the study through 1,000 participants demonstrates it is on track to achieve its goals and will continue to full sample size.

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TABLES

Table 1. Biological specimens and radiographic images collected and banked from consented, non-pregnant participants in the APS Consortium Phenotyping Study.

Biospecimen/ Image	Time Point							
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)	
	Day 0[a]	NBBAL[b]	Day 2	Day 4[c]	Day 6	Day 14	3-6 months	12 months
Acceptable study days for collection	Day 0-1	+96 hours from intubation	Day 2-3	Day 3-5	Day 6-8	Day 14-17	Day 75-240	Day 330-450
EDTA plasma	✓		✓	✓	✓	✓	✓	✓
RNA Paxgene	✓		✓		✓	✓	✓	✓
CPT (blood)[d]	✓	✓			✓		✓	
Sodium citrate plasma	✓		✓		✓			
Buffy coat from EDTA plasma	✓							
Red blood cells	✓						✓	✓
Urine	✓							
Oral swab	✓		✓		✓		✓	✓
Nasal swab	✓		✓		✓		✓	✓
Rectal swab	✓		✓		✓			
Stool	✓		✓		✓		✓	✓
HME filter fluid[e]	✓		✓	✓	✓	✓		
Tracheal aspirate fluid[e]	✓		✓	✓	✓	✓		
NBBAL fluid[e]		✓						
Chest x-ray (clinically obtained)[f]	✓		✓		✓			
Chest CT scan (clinically obtained) [f]	✓						✓	
Chest CT scan (study dedicated)[g]								✓
Pulmonary function tests (study dedicated)[g]								✓

Table 1 abbreviations:
NBBAL: non-bronchoscopic bronchoalveolar lavage; EDTA: ethylenediaminetetraacetic acid; RNA: ribonucleic acid; CPT: cell preparation tubes; HME: heat and moisture exchange; CT: computed tomography

Table 1 footnotes:

a. Study Day 0 and study enrollment are defined as the time when the first study-specified biospecimen is collected.
b. The NBBAL time point is anchored on the time of completion of a research-dedicated NBBAL and may occur on any study day between Study Day 0 and Study Day 8. The NBBAL time point

specimens are collected once as soon as possible after intubation and no later than 96 hours after intubation.

c. Day 4 specimens are only collected from participants on invasive mechanical ventilation.
d. CPT blood was collected on approximately 1,000 participants. After approximately 1,000 participants, CPT blood is only collected at the same time a NBBAL is performed.
e. HME filter fluid, tracheal aspirate fluid, and NBBAL fluid specimens are only collected from participants on invasive mechanical ventilation.
f. Clinically obtained chest x-rays and CT scans are collected and stored when they are available from clinical care. Participants who did not have clinically obtained images in the time windows for these study visits do not have images available.
g. Study dedicated CT scans and pulmonary function tests are obtained via study protocol and are not dependent on clinical testing.

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Table 2. Laboratory measurements planned within the APS Consortium study.

Class of Analysis	Specific measurements
Plasma protein biomarkers	IL-6, IL-8, soluble tumor necrosis factor receptor-1, protein C, receptor for advanced glycation end products (RAGE), angiopoietin-2, intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein 1 (MCP1), syndecan-1, S100A12, growth differentiation factor (GDF)-15, IL-1 receptor-like 1 (IL1RL1 or sST2), cell free DNA, neurofilament light chain (NFL), total tau protein
Respiratory tract protein biomarkers using HME filter fluid	IL-6, IL-8, angiopoietin-2, RAGE, TNF receptor 1, monocyte chemoattractant protein 1 (MCP1), ICAM, protein C, plasminogen activator inhibitor-1 (PAI-1), procollagen peptide-3 (PCP3), total protein
Host response to critical illness	Host RNA sequencing to characterize the transcriptome (whole blood)
Chronic alcohol consumption	Phosphatidylethanol (PEth) from red blood cells
Respiratory tract immune response to critical illness	Bronchoalveolar lavage (BAL) mononuclear cell analyses
Systemic immune response to critical illness	RNA sequencing of peripheral blood mononuclear cells (PBMCs) to compare immune response in systemic circulation and the lung compartment
Respiratory and gastrointestinal tract microbiome	16S rRNA gene sequencing
Pathogen and microbiota detection	Metagenomic sequencing of plasma, nasal swabs, tracheal aspirates, and NBBAL samples

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Table 3. Characteristics of the first 1,000 participants enrolled in the APS Consortium Phenotyping Study.

Characteristic	**First 1,000 participants enrolled**
Age, median (IQR) [years]	64 (53, 74)
Sex, n (%)	
Female	456 (45.6%)
Male	544 (54.4%)
Race, n (%)	
White	614 (61.4%)
Black	194 (19.4%)
Asian	59 (5.9%)
Hispanic (no other race reported)	58 (5.8%)
American Indian or Alaska Native	5 (0.5%)
Middle Eastern or North African	4 (0.4%)
Native Hawaiian or other Pacific Islander	4 (0.4%)
Multiple races	9 (0.9%)
Other	19 (1.9%)
Unknown	34 (3.4%)
Hispanic ethnicity, n (%)	87 (8.7%)
Location of residence, n (%)[a]	
Urban	876 (87.6%)
Rural	117 (11.7%)
Unknown	7 (0.7%)
Type of residence, n (%)	
Community home without assistance with daily living	751 (75.1%)
Community home with assistance with daily living	96 (9.6%)
Nursing home or skilled nursing facility	71 (7.1%)
Homeless or shelter	28 (2.8%)
Rehabilitation facility	26 (2.6%)
Other	11 (1.1%)
Unknown	16 (1.6%)
Comorbidities (chronic medical conditions)	
Body mass index > 30 kg/m^2, median (IQR)	26 (22, 33)
Heart failure, n (%)	205 (20.5%)
Asthma, n (%)	102 (10.2%)
Chronic obstructive pulmonary disease, n (%)	196 (19.6%)
Home oxygen use, n (%)	83 (8.3%)
Chronic supplemental oxygen flow rate among those with home oxygen use, median (IQR) [liters per minute] [n=83]	3 (2, 4)
Diabetes mellitus, n (%)	341 (34.1%)
Chronic kidney disease on kidney replacement therapy, n (%)	52 (5.2%)
Dementia, n (%)	44 (4.4%)
Prior stroke, n (%)	88 (8.8%)
Solid organ transplant, n (%)	57 (5.7%)
Active cancer[b], n (%)	243 (24.3%)
HIV infection, n (%)	29 (2.9%)
Moderate or severe immunosuppression[c], n (%)	247 (24.7%)

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Currently pregnant, n (%)	3 (0.3%)
SOFA-2 score for participants alive in hospital, by study day, median (IQR)	
Day -2 (n=382)	3 (1, 6)
Day -1 (n=917)	5 (3, 7)
Day 0 (n=1000)	6 (4, 9)
Day 1 (986)	5 (3, 8)
Day 2 (945)	4 (2, 7)
Day 3 (n=898)	4 (2, 6)
Day 4 (n=841)	4 (1, 6)
Day 5 (n=779)	3 (1, 6)
Day 6 (=725)	3 (1, 6)
Day 7 (n=662)	3 (1, 6)
Disease severity at enrollment (study day 0)	
Serum lactate, median (IQR) [mmol/L]	2.0 (1.3, 3.3)
Vasopressor use, n (%)	597 (59.7%)
Invasive mechanical ventilation, n (%)	391 (39.1%)
Clinical syndromes (not mutually exclusive), n (%)	
ARDS	399 (40.0%)
Pneumonia	523 (52.3%)
Sepsis	884 (88.4%)
No ARDS, pneumonia, or sepsis	87 (8.7%)
Bacteremia or fungemia, n (%)[d]	260 (26.0%)
In-hospital 28-day outcomes (not mutually exclusive), n (%)	
Death	250 (25.0%)
Vasopressor use	746 (74.6%)
High flow nasal oxygen	446 (44.6%)
Non-invasive ventilation	298 (29.8%)
Invasive mechanical ventilation	506 (50.6%)
ECMO	6 (0.6%)
New kidney replacement therapy	115 (11.5%)

Table 2 abbreviations: IQR: interquartile range; HIV: human immunodeficiency virus; SOFA 2: sequential organ failure assessment version 2; ARDS: acute respiratory distress syndrome; ECMO: extracorporeal membrane oxygenation

Table 2 footnotes:

a. Classification of residence location as urban versus rural was based on home zip code and the rural-urban commuting area (RUCA) code classification system.
b. Active cancer was defined as a solid organ or hematologic cancer newly diagnosed or treated with cancer therapy in the prior 6 months.
c. Moderate to severe immunosuppression was defined as the participant having any of the following conditions: receipt of systemic corticosteroids at a dose of ≥20 mg prednisone per day for ≥2 weeks; receipt of immunosuppressive medication in the prior 90 days due to an organ transplant; receipt of cancer treatment that suppresses immune function ("chemotherapy") in the prior 90 days; receipt of an immunosuppressive medication other than corticosteroids for a reason other than organ transplant or cancer in the prior 90 days; AIDS (HIV infection with at least one of the following: CD4 count < 200 cells/mcl; AIDS defining illness without immune

reconstitution; clinical manifestations of symptomatic HIV); hematopoietic transplant in the prior 2 years; moderate or severe primary immunodeficiency (such as, severe combined immunodeficiency, DiGeorge syndrome, Wiskott-Aldrich syndrome, common variable immunodeficiency disease).

d. Bacteremia or fungemia was defined as a positive blood culture with a likely bacterial or fungal pathogen from a sample collected between hospital admission and study day 14. The following detected organisms were considered contaminants and not a sign of bacteremia: coagulase negative staphylococci, alpha hemolytic streptococci, corynebacterium species, micrococcus species, Propionibacterium acnes.

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FIGURE LEGENDS

Figure 1. Participating sites in the APS Consortium.

Figure 2. In-hospital mortality, hospital discharge, and sequential organ failure assessment 2 (SOFA-2)[48] scores among the first 1,000 participants in the APS Consortium Phenotyping Study. (A) Time to in-hospital death and index hospital discharge through 28 days (study day 27). Discharged alive from index hospitalization represents leaving the hospital in which study enrollment occurred, by discharge to home, or discharge or transfer to another facility. (B) Stacked bar plot on study days -2 (two day prior to enrollment) through study day 7 showing participant status using a categorical scale. The scale includes these levels: not in the hospital (either due to not being admitted yet for study day -2 and -1 or hospital discharge after study day 0); SOFA-2 score for participants in the hospital; and dead.

Figure 2 Footnote:

SOFA-2 score was calculated using the methods described by Ranzani et al,[48] including the methods of imputation for missing values. Fewer than 6.5% of participant days required imputation for each of the SOFA-2 organ systems except the liver system (total bilirubin concentration), which required imputation for 41% of values. Following the methods recommended by Ranzani et al, we imputed 0 points for the liver system if bilirubin was missing on the first day of data collection in our study and carried forward the last recorded bilirubin value if missing on later days.

Figure 3. Histograms of ordinal in-hospital outcomes through 28 days among the first 1,000 participants in the APS Consortium Phenotyping Study.[a] (A) Hospital-free days; (B) ICU-free days; (C) Invasive ventilator (IMV)-free days; (D) Respiratory support-free days (composite of high flow nasal cannula, non-invasive ventilation, and invasive mechanical ventilation); (E) Vasopressor-free days; (F) Organ

support-free days (composite of respiratory support, vasopressor therapy, and new kidney replacement therapy).

Figure 3 Footnote:

a. Free-day outcomes were calculated using the following principles, using vasopressor-free days as an example. Vasopressor-free days is a composite of in-hospital 28-day mortality and duration of vasopressor use for 28 days after enrollment (day 0). The outcome "vasopressor-free days" is an ordinal outcome with 30 levels, with -1 representing death, 0 representing vasopressor use for the full 28-day follow-up period, and the values between 1 and 28 representing the number of days alive and free of vasopressor use through 28 days of follow-up. Participants who die in the hospital at any time before the end of study day 27 are coded as having -1 vasopressor-free days. Continuous infusion of a vasopressor of any duration on a calendar day results in classification of that day as a day with vasopressor use. Days of vasopressor use is calculated as the number of days between the first and last use of vasopressors between study day 0 and study day 27, inclusive of the day vasopressors were started and ended (this is a first on/last off method of calculating free-day outcomes). Participants discharged from the hospital prior to the end of study day 27 without vasopressors infusing are considered to complete the remainder of the follow-up period off vasopressors, while those discharged on vasopressors are considered to have vasopressors continued through the end of 28-day follow-up. For participants who did not die, vasopressor-free days is calculated as 28 minus the number of days of vasopressor use.

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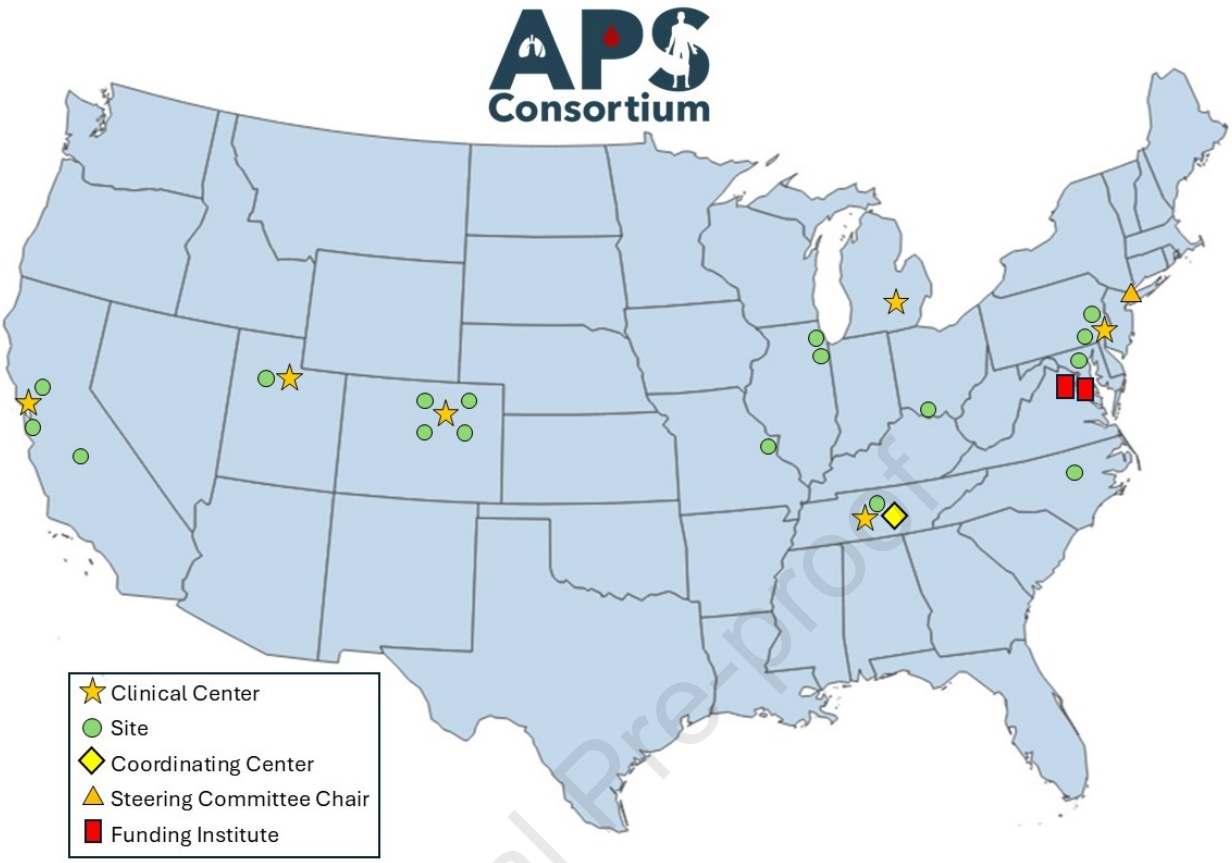

Clinical center (hub) sites [stars on map] - University of California San Francisco (UCSF) - Intermountain Medical Center - University of Colorado - Vanderbilt University Medical Center - University of Michigan - University of Pennsylvania	**Participating sites [circles on map]** - UCSF Fresno - Zuckerberg San Francisco General Hospital - Stanford University - Johns Hopkins University - Duke University - University of Utah - Denver Health - Medical Center of the Rockies - Poudre Valley Medical Center - Saint Joseph Hospital/National Jewish - Meharry Medical College - Washington University - University of Cincinnati - University of Chicago - Northwestern University - University of Pennsylvania Presbyterian - University of Pennsylvania Cedar
Coordinating Center [diamond on map] - Vanderbilt University Medical Center	
Steering Committee Chair [triangle on map] - Montefiore Medical Center	
Funding Institutes [rectangles on map] - National Heart, Lung, and Blood Institute - National Institute of General Medical Sciences	

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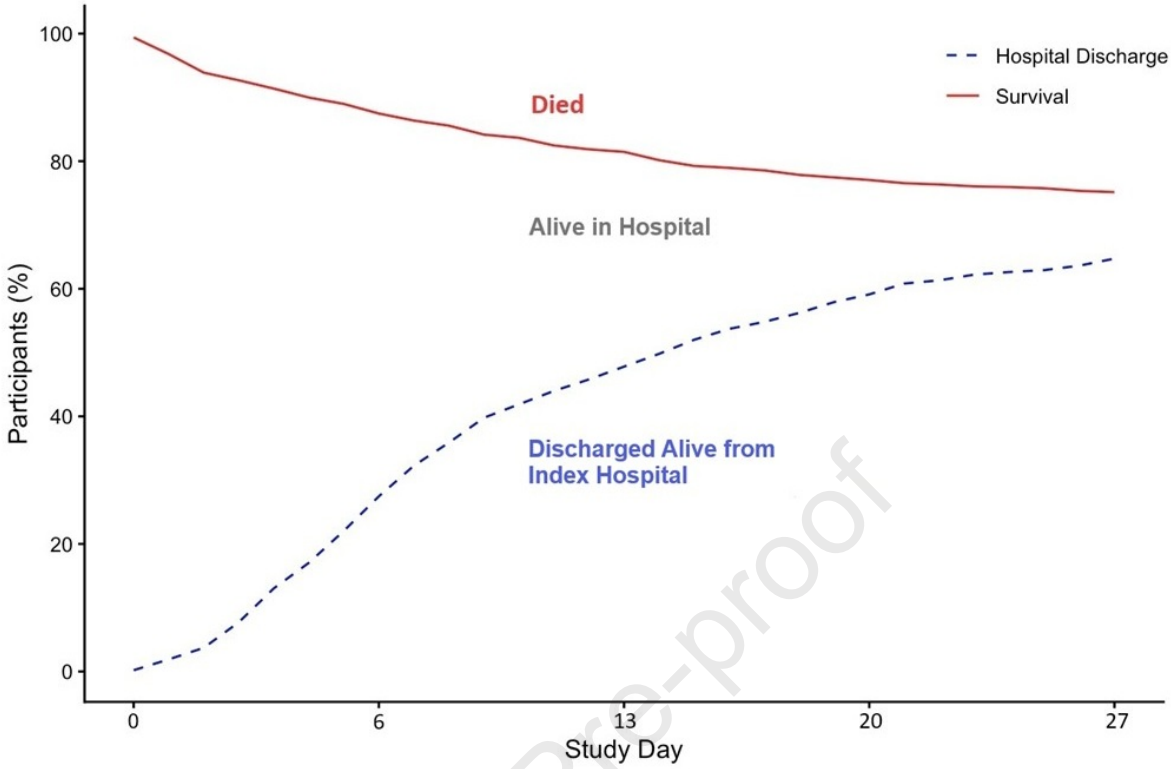

	Day 0	Day 6	Day 13	Day 20	Day 27
Died	6	110	181	225	246
Alive in hospital	992	668	360	195	117
Discharged alive from index hospital	2	220	457	578	635
Withdrawn from study	0	2	2	2	2

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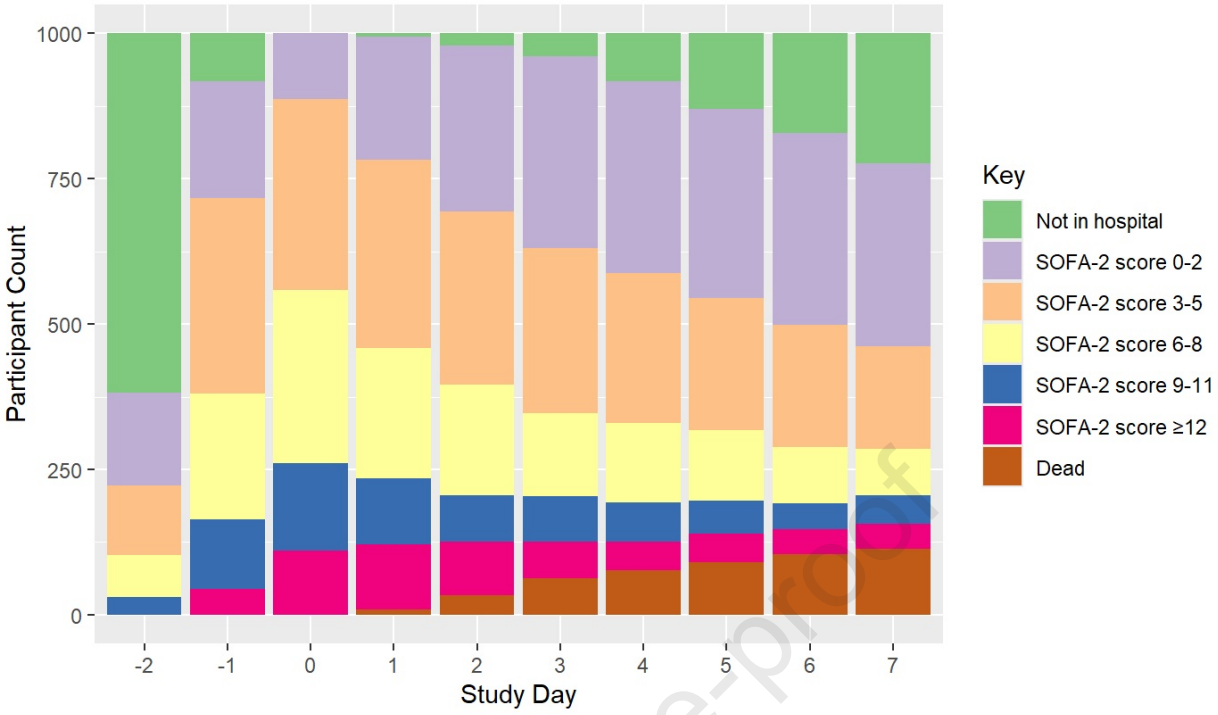

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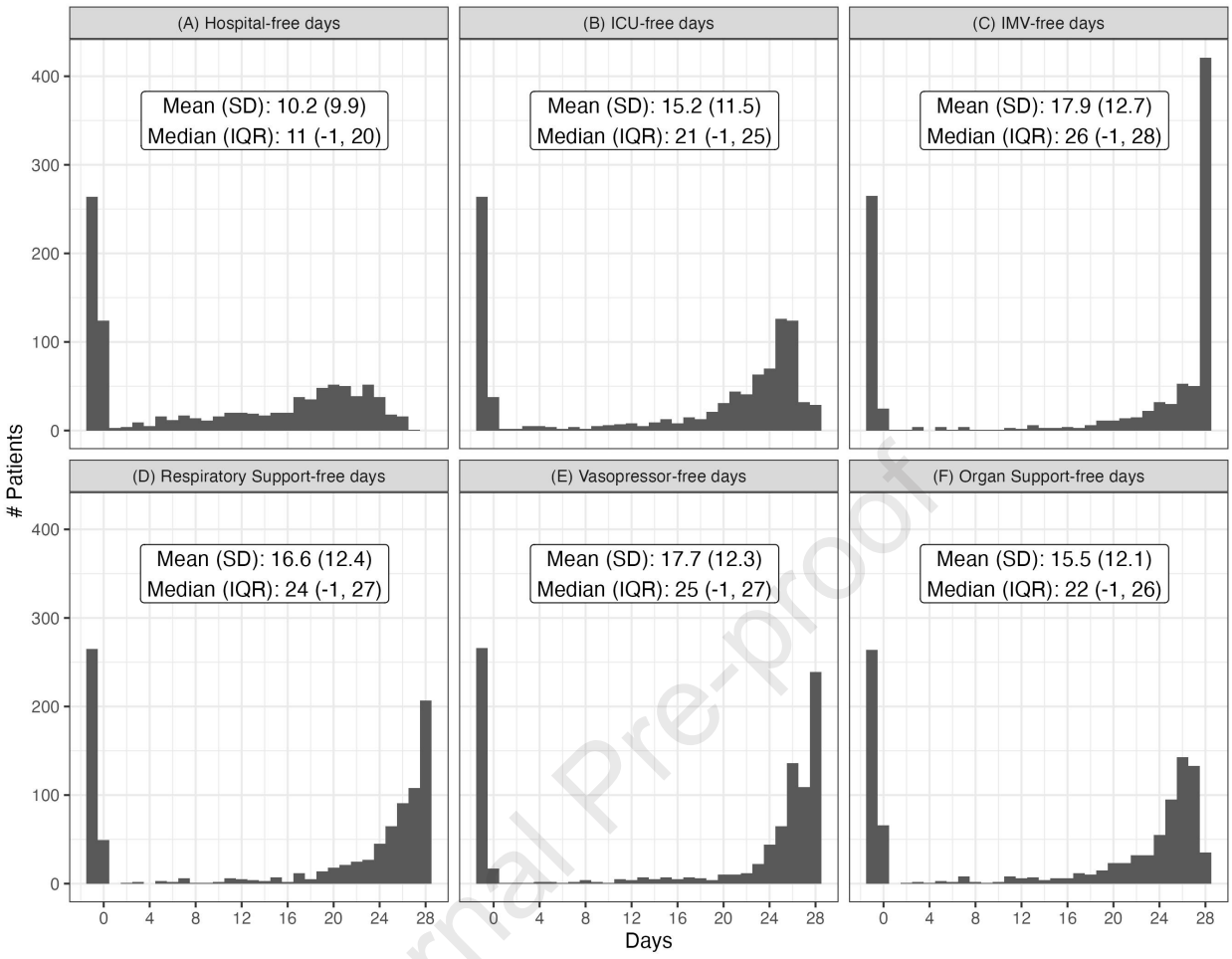

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The ARDS, Pneumonia, and Sepsis (APS) Consortium: Rationale, Design, and Feasibility of a National Platform for Phenotyping Critical Illness Syndromes

TAKE HOME POINTS

Study Question: Is the APS Consortium study rapidly generating data and biospecimens from a large cohort of critically ill adults with ARDS, pneumonia, and sepsis that will facilitate phenotyping of these syndromes?

Results: The APS Consortium study is a multicenter longitudinal prospective cohort study that enrolled 1,000 participants (one-quarter of planned total sample size) in less than 13 months with high disease severity (75% vasopressor use, 50% invasive mechanical ventilation use, 25% in-hospital mortality), a high success rate for collecting biospecimens, and successful adjudication of ARDS, pneumonia, and sepsis.

Interpretation: The APS Consortium study is successfully collecting the data and biospecimens needed to conduct robust phenotyping analyses and will continue to the full planned sample size of 4,000 participants.

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The APS Phenotyping Study Protocol A (full protocol)

Protocol Full Title:	The ARDS, Pneumonia, and Sepsis (APS) Consortium: A Prospective Observational Study to Evaluate Phenotypes (Protocol A – full protocol)
Protocol Short Title:	The APS Phenotyping Study Protocol A (full protocol)
Protocol Version:	6.0
Protocol Date	January 8, 2026
Network:	The ARDS, Pneumonia, and Sepsis (APS) Consortium
Funders:	(1) National Heart, Lung, and Blood Institute (NHLBI) (2) National Institute for General Medical Sciences (NIGMS)
Clinical Center Hub Institutions:	(1) University of California, San Francisco (2) Intermountain Medical Center (3) University of Colorado (4) Vanderbilt University Medical Center (5) University of Michigan (6) University of Pennsylvania
Coordinating Center & Single IRB:	Vanderbilt University Medical Center
Investigators:	See section 1
Corresponding Investigator:	Wesley H. Self, MD, MPH wesley.self@vumc.org
Purpose:	This protocol outlines study procedures for the collection of data and biospecimens to establish and build the APS phenotyping cohort. This protocol (Protocol A) is the full study protocol that describes all study procedures and governs study conduct for participants who have completed informed consent for study participation. A companion protocol (Protocol B) describes alteration of informed consent procedures and outlines study procedures that may be completed with alteration of informed consent. Appendices in this protocol (Protocol A) outline specific uses of the data and biospecimens by APS investigators for Consortium-wide studies.

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1. Investigators

Table 1. APS Consortium investigators

Clinical Center (CC)	Site	Investigator
Colorado	University of Colorado	Marc Moss, MD (CC PI)
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Colorado	University of Colorado	Kristine Erlandson, MD
Colorado	University of Colorado	Adit Ginde, MD
Colorado	University of Colorado	Sarah Jolley, MD
Colorado	University of Colorado	Diana Quan, MD
Colorado	Denver Health	Jason Mansoori, MD
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Colorado	Medical Center of the Rockies	Eric Stevens, MD
Colorado	Medical Center of the Rockies	Landon Hamilton, PhD
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Colorado	National Jewish Health	Nir Goldstein, MD, FCCP
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Intermountain/InterACT	Intermountain Medical Center	Ithan Peltan, MD
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Intermountain/InterACT	Duke University	Elias Pratt, MD
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Intermountain/InterACT	Johns Hopkins University	Jack Iwashyna, MD, PhD
Intermountain/InterACT	Johns Hopkins University	Ann Parker, MD, PhD
Intermountain/InterACT	Johns Hopkins University	Sarina Sahetya, MD, MHS
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Intermountain/InterACT	University of Utah	Elizabeth Middleton, MD
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Michigan/Great Lakes	University of Michigan	Robert Dickson, MD (CC PI)
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Michigan/Great Lakes	University of Michigan	Scott Denstaedt, MD
Michigan/Great Lakes	University of Cincinnati	Duncan Hite, MD
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Michigan/Great Lakes	University of Cincinnati	Mihir Atreya, MD, MPH
Michigan/Great Lakes	University of Chicago	JP Kress, MD
Michigan/Great Lakes	University of Chicago	Bhakti Patel, MD
Michigan/Great Lakes	University of Chicago	Krysta Wolfe, MD
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Michigan/Great Lakes	Northwestern University	Chiagozie Pickens, MD

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Clinical Center (CC)	Site	Investigator
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Vanderbilt	Vanderbilt University Medical Center	Julie Bastarache, MD
Vanderbilt	Vanderbilt University Medical Center	James Jackson, PsyD
Vanderbilt	Vanderbilt University Medical Center	Tatsuki Koyama, PhD
Vanderbilt	Meharry Medical Center	Richard Fremont, MD
Vanderbilt	Meharry Medical Center	Rajbir Singh, MD
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Vanderbilt	Washington University, St. Louis	Bryan Kraft, MD
Vanderbilt	Washington University, St. Louis	Phillip Mudd, MD, PhD
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UCSF	UCSF San Francisco	Michael Matthay, MD
UCSF	UCSF San Francisco	Kathleen Liu, MD, PhD
UCSF	UCSF San Francisco	Chaz Langelier, MD, PhD
UCSF	Stanford University	Angela Rogers, MD
UCSF	Stanford University	Jenny Wilson, MD, MS
UCSF	Stanford University	Joseph Levitt, MD, MS
UCSF	UCSF Fresno	Eyad Almasri, MD
UCSF	San Francisco General Hospital	Carolyn Hendrickson, MD
UCSF	San Francisco General Hospital	Lucy Kornblith, MD
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Consortium Coordinating Center	Vanderbilt University Medical Center	Gordon Bernard, MD (CCC PI)
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Consortium Coordinating Center	Vanderbilt University Medical Center	Matthew Shotwell, PhD
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Consortium Coordinating Center	Duke University	Chris Lindsell, PhD (CCC PI)
Steering Committee Chair	Montefiore Medical Center	Michelle N. Gong, MD

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Clinical Center (CC)	Site	Investigator
NIGMS / NIH		Xiaoli Zhao, PhD
NHLBI / NIH		Ingrid Espinoza. PhD

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2. Protocol Notes

2.1 Protocol Revision History

Protocol Version Number	Protocol Version Date	Summary of Revisions
1.0	January 7, 2024	Original protocol submitted to sIRB
1.1	March 18, 2024	Revision submitted to sIRB, with splitting of protocol documents into two protocols: Protocol A (full protocol), and Protocol B (alteration protocol)
1.2	March 20, 2024	Revision submitted to sIRB, with the addition of this sentence to section 13.2.1: Remote consent must use a procedure compliant with Title 21 CFR Part 11, such as signature through Adobe Acrobat Sign or DocuSign.
1.3	March 26, 2024	Revised section 14.2.3 to change the time frame of reporting adverse events to the sIRB from within 14 days to within 7 days of site awareness of event.
2.0	April 13, 2024	Protocol version used to initiate study enrollment (approved by single IRB at Vanderbilt University Medical Center). (1) Revised sections 9.2 and 10 to include data collection on whether a participant is pregnant at 3-month, 6-month, and 12-month surveys. (2) Revised section 13.2.4 to note that, "If a participant attends a long-term outcome in-person visit without having previously provided informed consent for study participation, informed consent for study participation will be obtained at that time using ICD#1."
2.1	June 6, 2024	- Section 8.1.1: This item removed from in-hospital interview: Cognitive function (PROMIS Cognitive Function Short Form 8a) - Section 9: clarified that study day number started at enrollment (study day 0) and not hospital admission. - Table 5: corrected typographical error - Table 7: corrected typographical error - Throughout the document, especially section 13, language was revised to more precisely outline the use of identifiable information. Enrolling sites will enter identifiable information, such as date of hospital admission and date of birth, into the study's database. These data will be visible by APS coordinating center personnel. De-identified datasets will be created for long-term storage of data outside the APS Consortium.

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Protocol Version Number	Protocol Version Date	Summary of Revisions
2.2	July 13, 2024	- Section 1: Added Xiaoli Zhao as a consortium investigator. - Sections 9.2.2 and 13.2.4: Added language outlining study procedures for the long-term outcome surveys when the patient had previously been participating in the study via surrogate consent. In this situation, at the 3-month phone call, the study team will seek consent for study participation from the patient with waiver of documentation of signature prior to administering survey questions via phone call. If the patient is unable to provide consent for study participation, a limited number of survey questions may be administered to the surrogate.
3.0	August 5, 2024	- Sections 7.1.3, 9.3, and 10 and Table 8: The protocol was altered so that pregnant participants only undergo study procedures that are no greater than minimal risk. - Section 8.2: Language added to clarify that aliquots of biospecimens collected on this research protocol may be used for clinical laboratory testing if the local team judges such tests to be potentially beneficial for the patient.
4.0	October 23, 2024	- Section 7.1.2: Exclusion criterion #4 changed to: "The clinical team has initiated transfer of the patient to a lower level of care that does not meet inclusion criterion #2 (such as placement of a transfer order out of the ICU). - Section 8.1.1: Language added to note that up to 3 surrogates will be identified while the participant is in the hospital who may answer questions about the participant's health in this study if the patient lacks capacity. - Section 8.2.1: These two sentences were added: Additionally, if residual volumes of biospecimens collected clinically meet the volume and collection time needed to fulfill biospecimens outlined in protocol schedule of events of this protocol, those residual samples may be used for research purposes in this protocol. For example, if a patient undergoes bronchoscopic bronchoalveolar lavage within 96 hours of intubation for clinical purposes, and residual bronchial samples are available from that procedure to use in this research protocol, those samples collected by the clinically orchestrated bronchoscopic bronchoalveolar lavage may be used in lieu of the patient undergoing a non-bronchoscopic bronchoalveolar lavage for research purposes. - Section 9.2.2: Language added to increased clarity around the use of surrogates to answer long-term outcome survey questions: "In cases where the participant was enrolled via surrogate consent, the surrogate who initially consented for the participant to enroll in the study will be prioritized as the first surrogate option for answering the limited battery of questions. If the surrogate who

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		consented for the participant is not available, the other two surrogates may be contacted, in any order compliant with state law and individual institutional policy regarding legally authorized representatives to assess their availability and ability to complete the surveys. Similarly, if the participant consented for herself/himself initially for the study but lacks capacity at a follow-up time point, the surrogates identified during the in-hospital stay may be contacted to complete surveys, with the order of surrogate outreach compliant with state law and individual institutional policy." - Section 9.2.3: additional safety criteria added to NBBAL exclusion checklist: "Tracheal or bronchial stent" - Section 13.2.1: The following sentence was removed: Remote consent must use a procedure compliant with Title 21 CFR Part 11, such as signature through Adobe Acrobat Sign or DocuSign. - Section 13.2.4: The following sentence was added: If participant does not possess capacity at the 3-month follow-up time point, surrogate consent may be obtained, including with waiver of documentation of signature.
5.0	August 10, 2025	- Section 1 (Investigators): updated the investigators list. - Section 7.2: enrollment targets based on demographic characteristics removed. - Section 9.2.2: language changed to allow electronic surveys for long-term outcome collection to be delivered to participants who had original consent obtained via a surrogate. - Section 9.2.4: This language was added: "The local clinical center/site may contact participants to remind them about surveys and in-person study visits. These reminders may be delivered via a variety of methods, such as telephone, email, text message, mail, and communication via the electronic health record." - Section 13.2.2: This language was added: "The study's informed consent document will be available in multiple languages. When an LAR/surrogate does not speak any of these languages, the study team may use a consent short form. When a short form is used, an IRB-approved translated Surrogate Rider will be attached as an addendum to the end of the translated Short Form and used to document the surrogate's written consent." - Throughout the protocol: renumbering of tables to account for removal of Table 3 in protocol version 4.0. - Throughout the protocol: minor revisions for clarity

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Protocol Version Number	Protocol Version Date	Summary of Revisions
6.0	January 8, 2026	- Section 9.2.2: the language describing the methods for survey data collection were simplified for clarity. - Section 9.2.4: eligibility criteria for the post-hospital in-person long-term outcome visits were modified to include two new concepts. Participants will be excluded from in-person long-term outcome visits if they meet either of these two new exclusion criteria: new exclusion criterion #3: discharged from the index hospitalization on hospice, palliative, or comfort care; new exclusion criterion #4: Known severe physical, neurological, cognitive, psychological, or other condition that existed prior to the participant's acute illness that would prevent completion of planned assessments at the in-person visits (note: impairments caused by the participant's acute illness should not result in the participant being excluded; only severe chronic conditions present before the participant's ARDS/pneumonia/sepsis event should be considered when assessing this exclusion criterion). - Section 9 and 10: the post-hospital in-person long-term outcome visits at 3 months and 6 months were collapsed into one visit. Hence, moving forward the in-person long-term outcome visits consist of: timepoint 1 (3 to 6 months; window: day 75 -240) and timepoint 2 (12 months; window: day 330-450). - Section 16: Scientific aims were adjusted to account for collapsing the in-person long-term outcome visits at 3-months and 6-months into a single combined visit (timepoint 1 at 3 to 6 months).

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2.2 Abbreviations

Table 2. Abbreviations

Abbreviation	Full term
AE	Adverse Event
AESI	Adverse Event of Special Interest
APS	ARDS, Pneumonia, and Sepsis
ARDS	Acute Respiratory Distress Syndrome
AUDIT-C	Alcohol Use Disorder Identification Test – Concise
CC	Clinical Center
CCC	Consortium Coordinating Center
CNS	Central Nervous System
COI	Covariate of Interest
COPD	Chronic Obstructive Pulmonary Disease
COS	Core Outcomes Set
COX-IV	Cytochrome c oxidase subunit IV
CPT	Cell Preparation Tube
CT	Computed Tomography
CXR	Chest X-Ray
DAMP	Damage-Associated Molecular Pattern
ddPRC	Droplet digital Polymerase Chain Reaction
DICOM	Digital Imaging and Communications in Medicine
DLCO	Diffusing Capacity of the Lungs for Carbon Monoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EHR	Electronic Health Record
ELISA	Enzyme-Linked Immunosorbent Assay
EQ-5D-5L	EuroQol 5 Dimensions 5 Level
FVC	Forced Vital Capacity
GDF-15	Growth differentiation factor 15
HADS	Hospital Anxiety and Depression Scale
HME	Heat Moisture Exchanger
ICAM-1	Intracellular adhesion molecule 1
ICAP-Revised	Inventory for Client and Agency Planning-Revised

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Abbreviation	Full term
ICC	Intraclass correlation coefficient
ICU	Intensive Care Unit
IES-6	Impact of Event Scale-6
IQ-CODE	Informant Questionnaire on Cognitive Decline in the Elderly
IRB	Institutional Review Board
Katz ADL	Katz Index of Independence in Activities of Daily Living
LAR	Legally Authorized Representative
Lawton IADL	Lawton Instrumental Activities of Daily Living scale
LCA	Latent class analysis
lpm	liters per minute
LRM	Logistic-Regression Model
LTO	Long Term Outcome
MCP1	Monocyte chemoattractant protein 1
mMRC dyspnea scale	Modified Medical Research Council dyspnea scale
mNGS	metagenomic Next Generation Sequencing
MODS	Multiple Organ Dysfunction Syndrome
mtDNA	Mitochondrial DNA
NBBAL	Non-Bronchoscopic Bronchoalveolar Lavage
ND-1	NADH dehydrogenase 1
NET	Neutrophil-Endothelial Traps
NHATS	National Health and Aging Trends Study
NHLBI	National Heart, Lung, and Blood Institute
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
OSMB	Observational Study Monitoring Board
PAI-1	Plasminogen activator inhibitor 1
PEEP	Positive End-Expiratory Pressure
PERMANOVA	Permutational multivariate analysis of variance
P:F ratio	Partial pressure of oxygen: Fraction of inspired oxygen ratio
PFT	Pulmonary function testing
PICS	Post-Intensive Care Syndrome
PRC	Protocol Review Committee
PTSD	Post-Traumatic Stress Disorder

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Abbreviation	Full term
qPCR	Quantitative Polymerase Chain Reaction
RAGE	Receptor for advanced glycation end-products
RALE score	Radiographic Assessment of Lung Edema score
RBM	Rules-Based Model
RNA	Ribonucleic acid
rRNA	ribosomal RNA
SAE	Serious Adverse Event
SC	Steering Committee
sIRB	Single Institutional Review Board
SIRS criteria	Systemic Inflammatory Response criteria
SMS	Short Message Service
SOFA score	Sequential Organ Failure Assessment score
SP-D	Surfactant protein D
SPPB	Short Physical Performance Battery
STAR	Spliced Transcripts Alignment to a Reference
sTM	Soluble thrombomodulin
TA	Tracheal aspirate
TNFR1	Tumor necrosis factor receptor 1
UMAP	Uniform Manifold Approximation and Projection
UP	Unanticipated Problem
WHODAS-12	World Health Organization Disability Assessment Schedule 12-item version

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3. Purpose of this Protocol / Program Overview

3.1 Scope of Study

This protocol describes the study activities that the ARDS, Pneumonia and Sepsis (APS) Consortium will complete together as a group, termed "Consortium-wide study procedures" and "Consortium-wide science." The main body of this protocol is a *Master Protocol* and includes the overall rationale for the APS program, and the procedures for enrolling participants, collecting data, and collecting biospecimens.

Data and biospecimens collected using this master protocol will be used for three types of scientific studies:

i. **APS Consortium-Wide Science:** Studies the APS Consortium investigators will complete and disseminate together using data and biospecimens collected under this master protocol. This science is described in the appendices of this protocol. Each of the 7 appendices describes one aim (study) of the APS Consortium-wide science. Consortium-wide science is funded by the NIH as part of the APS program.

ii. **APS Clinical Center-Specific Studies:** Studies that individual Clinical Centers within the APS program will complete with data and biospecimens collected under this master protocol. The science of these studies will be completed without involvement of the full APS consortium and is not governed by this protocol. Use of data and biospecimens in Clinical Center-specific studies will be governed by other protocols, as necessary. Clinical Center-specific studies are funded by NIH as part of the APS program through grants to individual Clinical Centers.

iii. **Ancillary Studies:** Studies that use banked data and biospecimens collected under this master protocol for conducting science not part of the APS Consortium-wide science nor the APS Clinical Center-specific studies. Use of data and biospecimens in ancillary studies will be governed by other protocols, as necessary. Ancillary studies are not funded as part of the APS program. Ancillary studies may be completed by APS Consortium investigators or investigators from outside the APS Consortium.

In summary, this protocol governs the collection of data and biospecimens for Consortium-wide study procedures and, in the appendices, describes the use of some of these data and biospecimens for consortium-wide scientific studies. Other studies, including Clinical Center-specific studies and Ancillary Studies, that will use data and biospecimens banked from this master protocol will be governed by other protocols managed by the investigators leading those studies.

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3.2 Protocol Documents

The APS Consortium study is governed by two study protocols:

- Protocol A (full protocol): This document is Protocol A. It describes all study procedures that a participant may complete during the course of the APS study and governs study procedures for participants who have completed informed consent for research participation. Protocol A contains two parts:
    - Master protocol: Study procedures that govern enrollment, data collection, and biospecimen collection. The master protocol is described in the main text of this document.
    - Consortium-wide science: specific analyses that will use data and biospecimens collected in this study and will be undertaken by the APS Consortium investigators collaboratively across the entire consortium. Consortium-wide science is described in appendices to this document.

- Protocol B (alteration protocol): This is a separate document not contained in the document presented herein. It describes a procedure for participation in the APS Consortium study with alteration of informed consent. Protocol B will be used for participants for whom informed consent for research cannot be obtained via the participant or a legally authorized representative prior to initiation of study procedures. Minimal risk procedures within the APS study may be completed using alteration of informed consent. Protocol B outlines the minimal risk procedures that may be completed with alteration of informed consent.

Participants who have provided informed consent will complete study procedures described in Protocol A (this document), and participants who have not provided informed consent will complete study procedures described in Protocol B (a separate document). Protocol B includes a subset of study procedures in Protocol A and does not include any study procedures not in Protocol A. Hence, patients participating under alteration of informed consent will complete a subset of study procedures (Protocol B) completed by participants with informed consent for study participation (Protocol A). Participants who enter the study on the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A. For participants who started study participation under alteration of informed consent, greater than minimal risk procedures may be completed after informed consent is obtained. Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

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4. Master Protocol Synopsis

This master protocol presented in the main text of this document describes the enrollment of participants into the APS Consortium Cohort and the collection of data and biospecimens from these participants. Eligible hospitalized adult patients ≥18 years old will be identified and consented for participation in a multi-site cohort study that seeks to understand risk factors, molecular phenotypes, and illness trajectory during acute and recovery phases for ARDS, pneumonia, and sepsis as common and potentially overlapping critical illness syndromes. Baseline and longitudinal clinical data, questionnaires, and biospecimens from approximately 4000 participants will be collected during the index hospitalization and post-hospitalization periods. Attempts will be made to contact all surviving participants for post-hospitalization assessments, including up to 600 participants who will return for additional in-person study visits and biospecimen collection.

Site investigators will adjudicate for the presence of ARDS, pneumonia, and sepsis syndromes among enrolled participants by reviewing the electronic health records (EHR) and other available data during a defined period of the index hospitalization.

Clinical data and biospecimens will be shared with the Consortium Coordinating Center (CCC) for cataloging, analysis to complete the Consortium-wide scientific aims, and distribution to other investigators for use. Data and biospecimens will ultimately be deposited in the NHLBI BioDataCatalyst and BioLINCC respectively, to support future use among APS Consortium and non-Consortium researchers.

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5. Introduction

5.1 Background

ARDS, pneumonia, and sepsis are common critical illness syndromes inflicting significant morbidity and mortality, both during the acute phase and in the years following the initial illness.[1] Hallmarks of APS include high mortality; complexity of illness; a high burden of pre-existing chronic illness; the possibility of rapid shifts in clinical status; a plethora of adverse events resulting from multiple, simultaneous interventions that can exacerbate and confound the acute disease state; and long-term physical, psychological, and cognitive morbidity.

There is substantial heterogeneity in each of these syndromes – ARDS, pneumonia, and sepsis – both in terms of the insults that incite them and in patients' physiological and biological responses.[1] Currently, there are no host-targeted pharmacotherapies with established efficacy against ARDS, pneumonia, or sepsis and their sequelae.[2] One of the leading theories as to why pharmacotherapy development has been unsuccessful in this area is due to the heterogeneity of these syndromes.[2] Study treatments applied homogenously in heterogeneous populations, as has historically been done in most APS clinical trials, may have concealed important benefits in specific sub-phenotypes of APS patients.[2] Indeed, increasing evidence has suggested the presence of distinct clinical and biological phenotypes within critical illness syndromes in which differential treatment responses are expected.[2] The presence of significant heterogeneity within each syndrome, combined with the overlapping phenotypes among syndromes, suggests that study of common critical illness syndromes in an interrelated manner may improve the understanding of mechanisms that determine short- and long-term outcomes and enhance the identification of sub-phenotypes across syndromes.[2]

The overarching goal of the APS Consortium is to support the development of deeper mechanistic understandings of critical illness syndromes to facilitate precision-based therapies that will curtail the devastating morbidity and mortality caused by ARDS, pneumonia and sepsis.[1] The goals of the APS Consortium were established by the NIH/NHLBI in the request for applications (RFA) soliciting applications for the clinical centers and are described in detail at (https://grants.nih.gov/grants/guide/rfa-files/RFA-HL-23-001.html). In brief, the Consortium seeks to understand the heterogeneity and underlying mechanisms of critical illness syndromes and recovery in adults with ARDS, pneumonia, and/or sepsis, as well as the relationship and biological overlap among these syndromes, through a prospective, longitudinal observational study with common data and biospecimen collection. The scientific focus of the consortium is on identifying novel phenotypes of critical illness, describing the clinical and biological features that define these phenotypes, establishing their prognostic and clinical value, and identifying their fundamental mechanisms; in addition, as set out in the RFA, the Consortium will generate a richly characterized clinical dataset and biobank for future investigations.

The ultimate goal of this research endeavor is to advance our ability to deliver precision critical care by more precisely matching interventions to specific phenotypes that are more likely to respond to those interventions, following the precision medicine paradigm of "right drug, right patient, right time." At the end of the Consortium, we aspire to have identified tangible novel phenotypes with strong evidence for their specific mechanistic underpinnings and practical methods for identification in clinical practice so as

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to lay the foundation for precision-targeted clinical trials. We anticipate that by combining careful clinical characterization according to currently accepted syndromic definitions with the novel deep molecular characterization will allow us to describe the relationship of novel phenotypes to existing syndromes, and potentially may refine clinical syndromes to those that are more biologically homogeneous.

By focusing on the recovery post-ARDS, pneumonia, and sepsis in a large and rigorously characterized population, we expect to inform clinicians about which baseline characteristics and dynamic features predict favorable or delayed recovery in specific domains, helping clinicians better prognosticate and helping future trials identify the highest risk patients to enroll for new tested interventions. Finally, we believe that some aspects of our clinical and/or molecular characterization for the APS consortium may display enough utility to spur development as clinically certified tests that are adopted into clinical care in the future.

To achieve these goals, the Consortium will conduct a longitudinal cohort study of approximately 4,000 adults hospitalized in the United States with ARDS, pneumonia and/or sepsis, and collect multidimensional data and biospecimens for up to one year from the time of index hospitalization. These data and biospecimens will be used both within the Consortium and by others to enhance our understanding of the mechanistic underpinnings of ARDS, pneumonia and sepsis.

5.2 Study objectives

5.2.1 Master protocol objectives

The central objective of Consortium-wide procedures described in this master protocol is to generate a deeply phenotyped cohort of participants with ARDS, pneumonia, and/or sepsis patients, and create a comprehensive, longitudinal biobank of specimens collected from these patients during acute illness and recovery that will be available to investigators both inside and outside the original APS Consortium investigator group.

5.2.2 Consortium-wide science objectives

The APS Consortium Investigators will use some of the data and biospecimens collected under the master protocol to complete scientific aims (Consortium-wide science). At initiation of this protocol, the APS Consortium Investigators have outlined the following 7 scientific aims they will complete. Details of each of these aims are contained in appendices to this protocol. Additional consortium-wide scientific aims may be added in the future via additional protocol appendices.

The initial 7 consortium-wide scientific aims are:

1. To determine and interrogate the role of microbiota (pathogen and microbiome) in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis, and their long-term outcomes.
2. To determine the host response phenotypes that most strongly associate with organ failure and 3, 6, and 12-month health status, and test the incremental utility of markers of vascular dysfunction, plasma DAMPs, and leukocyte expression for APS short- and long-term outcomes.

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3. To test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, sepsis, and pneumonia will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term outcomes, including pulmonary dysfunction.
4. To determine trajectories of established phenotypes over time and utilize longitudinal data to identify novel phenotypes of APS during the acute phase and recovery.
5. To determine and interrogate the role of patient comorbidities, exposures, and biophysical constitution in the clinical and biological heterogeneity of ARDS, sepsis, and pneumonia.
6. To develop approaches for translating phenotypes to the bedside to enable follow-on precision clinical trials.
7. To determine whether APS phenotypes identify differences in multiple organ dysfunction syndrome (MODS).

6. Study Design

This is a multicenter observational cohort study enrolling participants with acute cardiovascular or pulmonary organ dysfunction in the context of ARDS, pneumonia, sepsis, or a condition at high risk to progress to one of these syndromes. Acute cardiovascular and pulmonary dysfunction are highly relevant clinical manifestations of ARDS, pneumonia, and sepsis, and hence the target of enrollment strategies. Targeted sample size for enrollment is approximately 4,000 participants. Baseline and longitudinal molecular samples including blood, respiratory samples, circulating blood cells, urine, stool, and oral, nasal, and rectal swabs will be collected. Rich clinical data will be collected from the EHR, and participants will be asked to complete surveys and tests that will be used to phenotype multiple domains of health. Following discharge, participants will be asked to complete surveys and a subset will be asked to return for in-person assessments that will include biospecimen sampling (blood, stool, microbiome swabs) and assessments of physical, cognitive, and emotional health. Following the index hospitalization, participants will have in-person study visits up to approximately 12 months after enrollment.

7. Participant Enrollment

7.1 Eligibility Criteria

7.1.1 Inclusion Criteria

To be eligible for enrollment, a patient must meet all the following inclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Age ≥ 18 years old.

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2. Admitted (or planned to be admitted) to an ICU or other in-patient hospital location where IV vasopressors or advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula) are routinely provided (referred to as an "eligible unit.")

3. Acute cardiovascular or pulmonary organ dysfunction defined by meeting at least one of the two criteria below:
    i) New receipt of invasive mechanical ventilation, non-invasive ventilation, high flow nasal cannula, or supplemental oxygen at a flow rate of ≥ 6 lpm for acute hypoxemia.
        → Patients who use chronic oxygen therapy are eligible to participate if they are receiving at least 6 lpm higher than their baseline oxygen requirement (e.g., a patient on 3 lpm O2 at baseline is eligible if they require ≥9 lpm for hypoxemia) or are started on advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula).
    ii) Receipt of intravenous infusion of a vasopressor medication for at least one hour.

4. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) is attributed to an acute inflammatory condition, including but not limited to any of the following:
    i) Any infection including pneumonia.
    ii) Aspiration pneumonitis.
    iii) Pancreatitis.
    iv) Auto-inflammatory condition such as:
        a. Hemophagocytic lymphohistiocytosis.
        b. Suspected acute rheumatologic or auto-immune disease with pulmonary or cardiovascular manifestations.
        c. Suspected cryptogenic organizing pneumonia presenting acutely.
        d. Suspected diffuse alveolar hemorrhage.
        e. Suspected acute anaphylaxis.
        f. Suspected acute pulmonary drug toxicity.

7.1.2 Exclusion Criteria

To be eligible for enrollment, a patient must not meet any of the following exclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Patient/LAR declines participation.

2. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) has been present for > 48 hours.

3. Patient has been in an eligible unit (inclusion criterion #2) for more than 120 hours (five days).

4. The clinical team has initiated transfer of the patient to a lower level of care that does not meet inclusion criterion #2 (such as placement of a transfer order out of the ICU).

5. Patient desires comfort measures only.

6. Patient is a prisoner.

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7. Patient had out-of-hospital cardiac arrest leading to this hospitalization.

8. Residence immediately before this hospitalization in a long-term acute care facility.

9. Presence of tracheostomy for respiratory failure.

10. Home invasive mechanical ventilation or non-invasive ventilation (except patients with non-invasive ventilation prescribed as a treatment for a sleep disorder may participate).

11. Suspected cause of the patient's acute cardiovascular and/or pulmonary dysfunction (inclusion criterion #3) is an alternative condition (not ARDS, pneumonia, or sepsis), including but not limited to the list below:

    i) Drug overdose (without aspiration, lung injury, pneumonia, or infection).
    ii) Trauma (without aspiration, pneumonia, or infection).
    iii) Chronic lung disease without suspected infection, aspiration, or inflammation.
    iv) Asthma, COPD, sarcoidosis, interstitial lung disease, neuromuscular respiratory failure.
    v) Status epilepticus.
    vi) Acute pulmonary embolism.
    vii) Acute decompensated heart failure.
    viii) Diabetic ketoacidosis.
    ix) Acute stroke or intracranial hemorrhage.
    x) Acute bleeding (GI bleeding, post-procedural bleeding, hemolysis).
    xi) Cytokine release syndrome due to chemotherapy.

12. Inability or unwillingness to complete study-specified blood draws, for example, due to local policies about hemoglobin thresholds for research blood draws.

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7.1.3 Rationale for eligibility criteria

The goal is to enroll patients with acute cardiopulmonary dysfunction that is due to either (a) ARDS, pneumonia, or sepsis, or (b) an acute inflammatory condition that places patients at high risk for short-term development of ARDS, pneumonia, or sepsis. Patients with common critical care conditions that may result in acute cardiopulmonary dysfunction, but which have established pathophysiologic mechanisms distinct from ARDS, pneumonia, and sepsis will be excluded. This approach will optimize the ability to understand the pathophysiology of ARDS, pneumonia, and sepsis, whereas an approach that limited enrollment to patients who meet the historical syndrome definitions would limit our ability to identify new, meaningful phenotypes. In addition, this approach recognizes the difficulty in making definitive clinical diagnosis of historical syndromes (ARDS, pneumonia, and sepsis) at the time of enrollment among critically ill patients. By taking this approach to inclusion criteria, the APS Consortium is expected to further improve our ability to make diagnoses in real time.

Potential participants of all sexes, races, ethnicities, language proficiencies, and nationalities are invited to participate. Enrollment in the cohort will not be limited by the language(s) spoken by the participant.

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Individual components of APS studies, such as long-term outcome surveys, may be limited to participants who speak certain languages to ensure adequacy of data collection for those components. All adult patients across the age span are invited to participate; pediatric participants are not the focus of this project and will not be enrolled.

Pregnant individuals are eligible to participate in the APS cohort because pregnant patients experience APS syndromes at rates higher than the general population of similar age, and there is no strong rationale to exclude such participants. The risks of the procedures in this study are not greater for pregnant participants than for other participants. Due to potential risks to a fetus posed by study procedures that are greater than minimal risk, study procedures for pregnant participants will be limited to procedures that are not greater than minimal risk. The altered schedule of procedures for pregnant participants is detailed in the schedule of events section of this protocol.

Prisoners are excluded because there is concern that their participation may not be fully voluntary.

Patients who are unable to consent for themselves due to acute illness, cognitive impairment, or psychological impairment are eligible to participate. Details of inclusion of these patients are included in the Human Subjects section of this protocol.

Pediatric participants <18 years old are excluded because they are frequently admitted to different, specialized pediatric hospitals, the tools for assessing organ dysfunction and molecular phenotyping are different for children, and the focus of this study is on adults.

7.2 Anticipated Study Population

7.2.1 Enrollment targets

The APS Consortium investigators recognize that phenotyping efforts require adequate numbers of patients within specific groups for which phenotyping is desired. The APS Consortium will closely follow the characteristics of the enrolled cohort throughout the recruitment period to ensure large numbers of participants with each of the historical syndromes of ARDS, pneumonia, and sepsis, and patients in whom deep respiratory samples are available (patients ventilated through an endotracheal tube).

The APS Consortium Steering Committee will regularly monitor participant characteristics of the overall cohort and the subset with in-person long-term outcome visits. The Steering Committee will work to achieve the following enrollment goals:

i. Representation from the historical ARDS, pneumonia, and sepsis categories, with at least 1000 patients with ARDS, 2000 patients with sepsis, and 2000 patients with pneumonia in the overall cohort, recognizing that a given patient may count toward multiple categories;
ii. Representation of patients who received invasive mechanical ventilation in the overall cohort (target: ≥1000 patients) and long-term outcome subset (target: ≥150 patients).

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7.2.2 Anticipated enrollment by APS categories

Most patients enrolled according to the eligibility criteria of this study will have ARDS, pneumonia, and/or sepsis according to current diagnostic criteria for these syndromes at some point during the index hospitalization.[3-8] Eligibility criteria for this study require participants to have evidence of acute cardiopulmonary organ dysfunction, operationalized as receiving vasopressors, new substantial oxygen therapy (at least 6 liters per minute of supplemental oxygen over baseline), or new respiratory support (high-flow nasal cannula, non-invasive ventilation, or invasive ventilation).

Figure 1 displays anticipated enrollment numbers by syndrome. Importantly, there is substantial overlap among ARDS, pneumonia, and sepsis, such that many enrolled patients will have more than one of these syndromes. The number of participants in each category cannot be anticipated perfectly. Throughout the enrollment period of the study, the APS Consortium Steering Committee will issue guidance to enrolling sites to help ensure the final cohort includes at least 1000 patients with ARDS, 2000 patients with pneumonia, and 2000 patients with sepsis.

Eligibility criteria for this study limit enrollment of patients with pneumonia to those on vasopressors or newly treated with ≥6 liters per minute of supplemental oxygen or respiratory support. Therefore, nearly all patients enrolled with pneumonia are expected to have a Sequential Organ Function Assessment (SOFA) score ≥2 via cardiovascular dysfunction (vasopressor use) or respiratory dysfunction (P:F ratio <300),[9] and therefore also meet the Sepsis-3 criteria for sepsis.[8] The enrolled pneumonia cohort will include patients with pneumonia causing acute cardiovascular or respiratory organ dysfunction.

The most common cause of sepsis in this cohort is anticipated to be pneumonia. Patients with sepsis without pneumonia (non-pulmonary sepsis) meeting the eligibility criteria will also be enrolled. The enrolled sepsis cohort will include patients with sepsis causing acute cardiovascular or respiratory organ dysfunction, regardless of the etiology of sepsis. Patients who have suspected infection and a SOFA score ≥2 (a common operationalization of the Sepsis-3 definition) without acute cardiovascular or respiratory dysfunction will not be enrolled. Sepsis with a SOFA score ≥2 can occur without cardiovascular or respiratory dysfunction if organ dysfunction is present in the central nervous system (GCS ≤12), coagulation system (platelets <100,000 /mcl), liver (serum total bilirubin ≥2.0 mg/dl), or kidneys (creatinine ≥2.0 mg/dl).

Patients with ARDS meeting study eligibility criteria will be enrolled regardless of whether the etiology of ARDS is pneumonia (anticipated to be most common), non-pulmonary sepsis (anticipated to be second most common), or an insult other than infection (anticipated to be least common).

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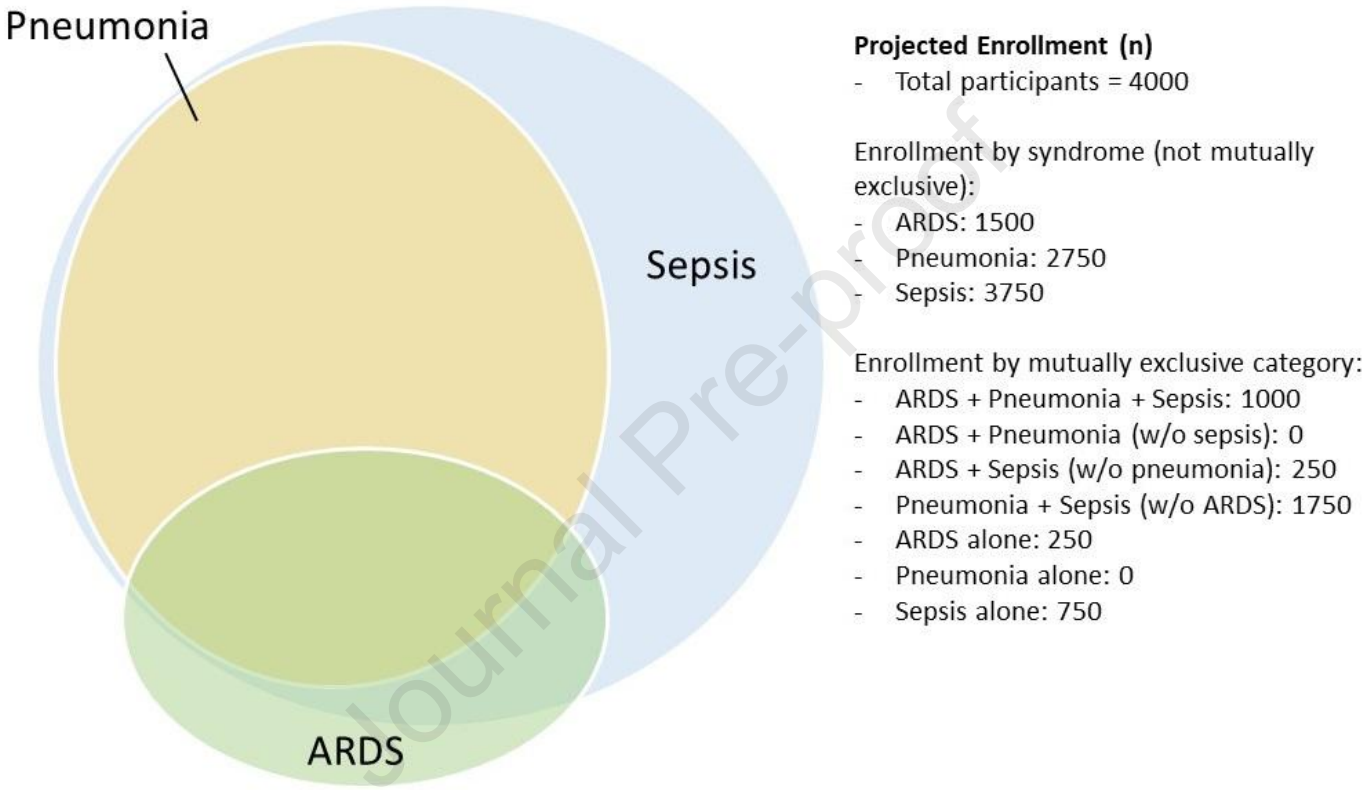
Figure 1. Anticipated overlap of ARDS, pneumonia, and sepsis among participants enrolled in the APS Consortium cohort study. Displayed numbers are projections based on published cohorts and investigator experience; actual enrollment numbers may differ and will be followed closely throughout the recruitment period to ensure adequate numbers of patients with ARDS, pneumonia, and sepsis.



7.3 Definition of Enrollment Time Zero

The calendar day of the first study-specified biospecimen collection is defined as "Day 0." The study schedule of events follows "study day" nomenclature, with enrollment day (the day of the first biospecimen collection) identified as Day 0. Study Day 1 is the calendar day following enrollment. Study Day -1 is the calendar day before enrollment. In some circumstances, consent for participation may be obtained after Day 0 (such as on Day 1) or prior to Day 0 (such as Day -1).

Time zero for enrollment is defined as the time of the first biospecimen collection for the study, regardless of the timing of hospital admission, consent, and development of ARDS, pneumonia, or sepsis. A patient is considered enrolled when the first study-specified biospecimen collection occurs. Time stamps will be collected for key events, such as hospital admission, meeting study eligibility criteria, and collection of biospecimens, so that analyses can evaluate the time between these events.

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8. Study Procedures During Index Hospitalization

8.1 Data collection during index hospitalization

Data collection approaches within the APS Consortium are designed to optimize: (a) participant experience; (b) the scientific integrity of the Consortium-wide scientific aims; and (c) a robust and useable biobank for future research that is consistent with applicable regulations, guidance, and ethical norms.

8.1.1 Participant/surrogate interview

Attempts will be made to interview enrolled participants (or a surrogate) during the index hospitalization. The purpose of the interview (which may be performed with the participant's surrogate if the participant is unable to participate in the interview) is to obtain contact information (which has been demonstrated to improve cohort retention), to assess baseline health and health behaviors, to assess race/ethnicity and skin tone, to understand social drivers of disease at baseline, and to assess for preexisting comorbidities and frailty. Domains of data collection via interview include:

- Identification of up to 3 surrogates who may answer questions for this study related to the participant's health if the participant is unable to answer
- Contact information of patient and potential surrogates
- Skin color (Monk skin tone scale)
- Self-assessment of overall health (5-point Likert scale from Health & Retirement Study)
- Smoking status (current/former/never; pack-years)
- Alcohol use (AUDIT-C)
- Health-related quality of life (EQ-5D-5L)
- Frailty (Clinical Frailty Scale)
- Functional Limitations (Katz ADL/Lawton IADL)
- Disability (WHODAS-12)
- Employment status (ICAP-Revised)
- Cognitive function (IQ-CODE)
- Social isolation (NHATS-6)
- Engagement with healthcare (National Health Interview and All of Us Surveys)
- Education (Health and Retirement Study)
- Housing instability (Council of State Governments)
- Current/former opioid misuse (ARDS Network Long Term Outcomes Study)

8.1.2 Medical record data abstraction

Medical record data abstraction, elements of which may be confirmed via patient/surrogate interview, may occur at varying times during the hospitalization based on operationalization at a given site. These

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data, whether extracted at the time of a given study visit or near or after hospital discharge, will be tied to the schedule of events. Features of data abstraction will be extracted to correspond with the baseline visit as well as subsequent visits. Domains of data collection via medical record review include:

- Contact information of patient and family members, including home address and type of residence
- 9-digit zip code. This allows for linkage to other datasets to capture measures of social vulnerability (e.g., Social Vulnerability Index, Social Deprivation Index, Area Deprivation Index), as well as healthcare availability and rurality. Note: to avoid risk of re-identification of study subjects, zip code will be stripped from shared datasets; data on social vulnerability, healthcare availability, rurality, and other factors obtained by linkage to 9-digit zip code will be retained as categorial variables in shared datasets.
- Demographics
- Home medications
- Biometrics (height, weight)
- Language fluency
- Place of residence
- Chronic health conditions (including tobacco use, alcohol misuse, and opioid misuse)
- Health insurance status
- Prior hospitalizations
- Clinical laboratory results, medications, and vital signs, including SOFA score elements
- Therapies administered (including respiratory support therapies)
- Discharge disposition

8.1.3 Acute Respiratory Distress, Pneumonia, and Sepsis Syndrome Classification

Enrolled participants will be evaluated for ARDS, pneumonia, and sepsis utilizing detailed, investigator driven review and application of published and accepted clinical criteria for each syndrome. To evaluate for ARDS, pneumonia, and sepsis, investigators will evaluate data and the clinical record for each participant for Study Day -2 through Day 7 while the patient is hospitalized (the phenotyping observation window). Individual criteria for each syndrome will be collected and recorded separately allowing for patients to be classified as ARDS, Sepsis, and/or Pneumonia using multiple published criteria (e.g., for sepsis using both “sepsis-2” and “sepsis-3” criteria). These data will then be used to code variables in our dataset that classify patients according to syndrome definitions. Standard operating procedures will be provided to each site to ensure accuracy and consistency of syndrome adjudication.

ARDS will be identified based on the Berlin Definition with the additional modifications proposed in the New Global Definition of ARDS.[3,4] Trained physician investigators will determine the presence of potential precipitating factors for ARDS (e.g., sepsis, aspiration, pancreatitis). Chest radiographs and chest computed tomography scans conducted for clinical purposes during the observation window will be reviewed by trained physician investigators for bilateral infiltrates consistent with ARDS. The type of chest imaging, time and date of imaging acquisition, and bilateral infiltrates present, absent, or equivocal will be recorded in case report forms. The presence or absence of pleural effusions will also be recorded. Arterial blood gas (ABG) values drawn for clinical purposes and corresponding fraction inspired oxygen

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percentage (FiO2) will also be recorded in case report forms. If an ABG is unavailable on a given day, SpO2 and corresponding FiO2 will be recorded to calculate the S/F ratio. Invasive and non-invasive oxygenation and ventilation parameters will also be extracted from the medical record. We will apply strict physiologic criteria in ARDS definitions to determine the presence or absence of ARDS daily, timing when ARDS criteria are met, and the severity of ARDS based the level of hypoxemia (P:F and S:F ratios).

Participants will be assessed for the presence of pneumonia based on radiographic, clinical, and laboratory criteria, including those published by CDC and the Infectious Disease Society of America/American Thoracic Society.[5,6] Chest imaging conducted for clinical purposes during the phenotyping observation window will be reviewed by trained physician investigators for new/persistent/progressive infiltrates, consolidation, or cavitation consistent with a pneumonia event. Clinical signs and symptoms potentially indicating a respiratory infection will be identified, including vital signs, laboratory results, and clinical status. Assessment of clinical status will include: 1) new onset purulent sputum or change in character of sputum, increasing respiratory sections, or increasing suctioning requirements, 2) new onset or worsening cough, dyspnea, or tachypnea, 3) rales or bronchial breath sounds, and 4) worsening gas exchange. These data will form the core dataset for classifying the presence and absence of pneumonia.

Sepsis will be identified based on the Sepsis-2 and Sepsis-3 definitions of sepsis, severe sepsis and septic shock.[7,8] Trained physician investigators will review the medical record to determine if an infection is confirmed, suspected, not suspected, or unknown daily for the observation window. The presence of positive culture will not be required to identify the confirmed or suspected presence of an infection; however, positive cultures can be used to help determine the presence of sepsis. Suspected sources of infection will be adjudicated by the physician investigators. The physiology of sepsis and septic shock will be classified based on the change in Sequential Organ Function Assessment (SOFA) scores, the presence or absence of the systemic inflammatory response (SIRS) criteria, the presence or absence of vasopressors, and values for lactate. These data will be used to facilitate sepsis classification by the investigator adjudicators.

8.2. Biospecimen collection during index hospitalization

8.2.1 Interplay between clinical and research procedures for biospecimen collection

Aliquots of biospecimens collected on this research protocol may be sent for clinical laboratory testing if such testing is judged to be of potential benefit to the patient. Clinical laboratory testing is optional and may be completed at the discretion of the local site team. If clinical laboratory testing is completed, results may be delivered to clinicians caring for the patient according to local practice patterns.

Additionally, if residual volumes of biospecimens collected clinically meet the volume and collection time needed to fulfill biospecimens outlined in protocol schedule of events of this protocol, those residual samples may be used for research purposes in this protocol. For example, if a patient undergoes

bronchoscopic bronchoalveolar lavage within 96 hours of intubation for clinical purposes, and residual bronchial samples are available from that procedure to use in this research protocol, those samples collected by the clinically orchestrated bronchoscopic bronchoalveolar lavage may be used in lieu of the patient undergoing a non-bronchoscopic bronchoalveolar lavage for research purposes.

8.2.2 Blood specimen collection during index hospitalization

During the index hospitalization, blood will be collected from patients at multiple timepoints, collected in EDTA or sodium citrate tubes (for protein or metabolite measurements), PAXgene tubes (for nucleic acids), CPT tubes (for peripheral blood mononuclear cells), and sodium heparin tubes (for whole blood CyTOF and flow cytometry). Blood will be collected at the timepoints indicated in the schedule of events for biospecimen collection (Section 10). Participants known to be pregnant will only have blood collected during the index hospitalization on Day 0 and Day 2, ensuring <50 ml of blood volume collected in any 8-week period and ≤2 blood draws in any 1-week period for research purposes, which is considered to be no greater than minimal risk in guidance from the Office Human Research Protections (OHRP) [https://www.hhs.gov/ohrp/regulations-and-policy/guidance/categories-of-research-expedited-review-procedure-1998/index.html].

8.2.3 Respiratory specimen collection during index hospitalization

During the index hospitalization, the following respiratory specimens may be collected: 1) nasal swabs to facilitate both microbiome profiling and pathogen detection, 2) oral swabs, 3) tracheal aspirate (TA) from patients receiving invasive mechanical ventilation, 4) heat moisture exchanger (HME) filter fluid from patients receiving invasive mechanical ventilation, 5) non-bronchoscopic bronchoalveolar lavage (NBBAL) fluid among a subset of patients receiving invasive mechanical ventilation.

Description of the NBBAL procedure in this study:
The NBBAL procedure will be completed in a subset of participants undergoing invasive mechanical ventilation after a safety screen for the procedure (see below) and consent is obtained. The NBBAL will be performed as soon as possible (and no later than 96 hours) after initiation of invasive mechanical ventilation. The NBBAL procedure will entail a catheter being inserted into the endotracheal tube and the catheter tip being wedged into a distal airway. Serial aliquots of sterile saline are instilled and aspirated with gentle suction (maximum volume instilled, 120ml). The aspirated fluid will be collected and processed as a biospecimen for the study. As noted above, if bronchial samples are available from a clinically completed bronchoalveolar lavage procedure (either a bronchoscopic bronchoalveolar lavage or non-bronchoscopic bronchoalveolar lavage), those specimens may be used for this protocol in lieu of a research protocol NBBAL being performed.

Safety Screen for NBBAL in this study:
Participants meeting any of the following criteria will not undergo NBBAL for research purposes in this study:

1. Known pregnancy
2. FiO2 ≥ 0.80.
3. FiO2 increased by > 0.1 in the prior hour.

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4. PEEP ≥ 12 cm H2O.
5. PEEP increased by > 2 cm H2O in the prior hour.
6. Intracranial pressure > 15 or known labile intracranial pressure.
7. Open external ventricular drain (EVD).
8. Known pneumothorax.
9. Uncontrolled shock or rapidly escalating vasopressor requirements.
10. Unstable dysrhythmia.
11. Scheduled bronchoscopy.
12. Current therapeutic anticoagulation.
13. Known INR > 2.0.
14. Platelets < 50,000 /mcL.
15. Frank hemoptysis.
16. Partial lung resection or pneumonectomy.
17. Known pulmonary hypertension with pulmonary artery systolic pressure (PASP) >55 cm H2O.
18. Expected extubation attempt in the next 6 hours.
19. Tracheal or bronchial stent
20. Clinical provider or site investigator judgement that procedure would not be safe.

8.2.4 Other specimens during the index hospitalization

Participants will have rectal swabs and stool samples serially collected for the primary purpose of microbiome profiling and urine collected for future use.

8.3. Imaging studies during index hospitalization

During the index hospitalization, investigators will review chest imaging completed clinically during the observation window for clinical phenotyping (Study Day -2 through Day 7) and enter key findings into the study data collection forms. Additionally, as detailed in the schedule of events section, serial chest x-ray (CXRs) and CT scan images that were performed as part of the participant's clinical care will be collected and uploaded to a common, secure site, with eventual deposition into BioData Catalyst. The initial goal is to upload up to 3 CXRs and one CT scan (chest CT preferred, abdominal CT acceptable when no chest CT is available) completed during the index hospitalization as part of clinical care. The number of images uploaded per patient may change over time. These stored images will be used by the APS investigators and will be available in the APS databank for future work.

9. Study Procedures during Post-Hospital Assessments

9.1 Schedule of post-hospital study assessments

As specified in the Post-Hospital schedule of assessments, multiple study procedures will be used to collect data on participants following the hospitalization in which patients were enrolled in this study. Data collection will be at multiple timepoints out to approximately 12 months after enrollment. Data will

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be collected post-hospitalization remotely for all surviving patients, and a subset of patients will undergo additional study procedures via in-person visits at the site where they were enrolled, a nearby participating site, or at home.

Post-hospital procedures include:
1. Chart reviews
2. Short message service (SMS) ("text")/Email follow-up surveys
3. Telephone follow-up surveys (for patients who do not complete SMS/Email surveys)
4. In-person assessments/biospecimen collection/imaging in approximately 600 patients
5. At-home stool collection in approximately 600 patients

9.2 Data collection during post-hospital study visits

9.2.1 Post-hospital medical record reviews

Personnel will complete a medical record review that includes data generated after the hospitalization in which the patient was enrolled. These chart reviews will be collected at approximately 3, 6, and 12 months after enrollment. The chart review will include review of the site's electronic health record, as well as any other health records viewable. The 3-month chart reviews will be completed for all patients who were discharged alive. The 6-month and 12-month chart reviews will be completed for participants not known to be dead through the 3-month chart review and 6-month chart review, respectively. Key data collected by chart review will include:

1. Mortality (yes/no); if yes: date of death.
2. Participant location in ICU, long-term care facility (LTAC), or hospice care.

In addition to the chart review, personnel will conduct a Google search for public obituary information to confirm status for any patients not identified as deceased in the medical record and not reached for follow-up.

9.2.2 Email/SMS/Telephone Surveys

Following the chart review at each follow-up time point (3, 6, and 12 months), patients who have consent for study participation, are not known to be dead, and not in an ICU, LTAC or hospice care will be sent a link by email or SMS ("text") message to a REDCap survey. The survey may be completed by the participant or a surrogate if the participant is not able to complete the survey or is unavailable. The survey will be available in English and Spanish.

Patients who do not complete the online survey or who do not yet have consent for study participation will be contacted by telephone by study personnel. During this phone contact, patients who had not provided consent for study participation themselves (that is, who had been participating via surrogate consent or alteration of informed consent) will be given an opportunity to consent for the study themselves (see section 13.2.4). After consent procedures, surveys for data collection will be

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administered. If the patient lacks capacity or is unavailable at the time of follow-up, and a surrogate is available, a limited battery of questions may be administered to the surrogate. Survey phone calls will be completed in English or Spanish. In cases where the participant was enrolled via surrogate consent, the surrogate who initially consented for the participant to enroll in the study will be prioritized as the first surrogate option for answering the limited battery of questions. If the surrogate who consented for the participant is not available, other surrogates may be contacted, in any order compliant with state law and individual institutional policy. Similarly, if the participant consented for herself/himself initially for the study but lacks capacity at a follow-up time point, surrogates may be contacted to complete surveys, with the order of surrogate outreach compliant with state law and individual institutional policy.

For each survey completed, patients may be reimbursed by the local Clinical Center/site in compensation for their participation (suggested amount: $40). Reimbursement may be provided by gift card, electronic gift card, cash, or ClinCard, as determined by the local Clinical Center/site.

Surveys will include the following assessment domains/measures:

1. Reconfirm Contact Info: Email, Cell Number, Address
2. Pregnancy status (not known to be pregnant vs known to be pregnant)
3. Health related quality of life (EQ5D-5L)
4. Mental health, PTSD (IES-6)
5. Mental health, anxiety/depression (HADS)
6. Feedback questions related to ImproveLTO core outcome set surveys [www.improveLTO.com]
7. Recovery from acute illness (1-item Recovery Question from Long-COVID COS)
8. Dyspnea/pulmonary function (mMRC dyspnea scale)
9. Frailty (Clinical frailty scale)
10. Functional limitations: Abridged I/ADL limitations (toileting, transferring, feeding, telephone, med management, financial management)
11. Disability (WHODAS 2.0, 12-item)
12. Cognitive decline (PROMIS Cognitive Function Short Form 8a, 8-item)
13. Return to work (4 custom questions, used in prior research)
14. Financial toxicity (3 custom questions, used in prior research)
15. Health insurance (1 custom questions, used in prior research)
16. Healthcare utilization (Hospitalizations since last contact (capture admit date, discharge date, admitting diagnosis, ICU admission (yes/no), mechanical ventilation via endotracheal tube (yes/no) for each admission)
17. Housing instability (2 questions, from NHIS and All of Us Survey)

9.2.3 Mental health safety plan

Our mental health safety plan follows standard procedures used in prior cohort studies with longitudinal telephone follow-up. We will not directly ask patients about suicidality as part of the surveys, but there may be rare instances where patients express thoughts about suicide. In these instances, study personnel will direct participants to contact their psychiatrist or primary care provider (if available) or instruct them

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to call the National Suicide Line (988), which will connect them with local resources. Such instances will be discussed with the local investigator overseeing long-term follow-up. Additionally, the APS Consortium will have an on-call clinician who has experience with mental health emergencies. For a high-risk situation where there is uncertainty about the appropriate next steps, the local site investigator and study team can contact the on-call clinician for guidance. The on-call clinician can also do remote well checks.[10-14] Study personnel conducting phone surveys will be trained to assess suicide risk using the Columbia Suicide Severity Rating Scale.[15-19]

9.2.4 Post-hospital in-person visits

Expanded biospecimen, imaging, and data collection will be completed for a subset of enrolled participants. Post-hospital in-person visits may occur in a variety of settings, including but not limited to a hospital, clinic, or the participant's home. Participating in the in-person long-term outcome visits is limited to patients fluent in English or Spanish because information gathered during these visits will be paired with survey data and the participating sites have capacity to administer the surveys only in English or Spanish. Enrollment for in-person follow-up will close after approximately 600 patients have completed an in-person follow-up visit.

The first approximately 600 participants to complete an in-person study visit will constitute the in-person long-term outcome cohort. The 600-participant in-person long-term outcome cohort will be filled on a "first come" basis with eligible, consenting participants.

Eligibility for the in-person long-term outcome cohort:
To be included in the in-person long-term outcome cohort, participants must meet all the following inclusion criteria and none of the following exclusion criteria.

In-person long-term outcome cohort inclusion criteria:

1. Enrolled in the APS Consortium Study during the index hospitalization (all participants in the in-person long-term outcome cohort must also be in the primary APS study cohort).
2. Survived to hospital discharge.
3. Consent for the primary APS Study, including the long-term outcome surveys (informed consent document #1).
4. Consent for the in-person long-term outcome follow-up visits (informed consent document #2).

In-person long-term outcome cohort exclusion criteria:

1. Not fluent in English or Spanish.
2. Lives outside a reasonable distance of the in-person follow-up visit location to enable completion of in-person visits (the threshold to define a reasonable distance and how to operationalize this exclusion criterion will be determined at each site based on local context).
3. Discharged from the index hospitalization on hospice, palliative, or comfort care.
4. Known severe physical, neurological, cognitive, psychological, or other condition that existed prior to the participant's acute illness that would prevent completion of planned assessments at the in-person visits (note: impairments caused by the participant's acute illness should not result

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in the participant being excluded; only severe chronic conditions present before the participant's ARDS/pneumonia/sepsis event should be considered when assessing this exclusion criterion).

Study Procedures at in-person long-term outcome follow-up visits:

Prior to APS Consortium protocol version 6.0, in-person long-term outcome visits were scheduled at 3-months, 6-months, and 12-months. Beginning with protocol version 6.0, the 3-month and 6-month visits were collapsed into a single visit, resulting in in-person long-term outcome visits at two time points:

- Timepoint 1 (3 to 6 months) – the goal is to complete this visit as close to 3 months as possible
- Timepoint 2 (12 months) – the goal is to complete this visit as close to 12 months as possible

In-person follow-up will include the following assessments at timepoint 1 and timepoint 2:

1. Biospecimen collection
2. Short physical performance battery (SPPB), which includes balance test, timed 4-meter walk test, and chair stand test
3. Handgrip strength (dominant hand only; unless that hand is impaired)
4. CNS-Vital Sign (computerized test that assesses cognitive function)
5. Dominant hand side quadriceps muscle ultrasound for cross-sectional area and muscle thickness with banking of the DICOM images (at a subset of sites due to specialized equipment and training needs)
6. Dominant hand side quadriceps muscle strength using dynamometer (at a subset of sites due to specialized equipment and training needs)

Additionally, in-person follow-up will include the following assessments at timepoint 2 only:

1. Spirometry and DLCO (lung function tests) in patients who have not had clinically obtained spirometry and DLCO in the prior 6 weeks.
2. Chest CT scan (see section 9.4)

The local clinical center/site may contact participants to remind them about surveys and in-person study visits. These reminders may be delivered via a variety of methods, such as telephone, email, text message, mail, and communication via the electronic health record.

The local clinical center/site may provide reimbursement to patients for completing in-person visits (recommended amounts: $200 for timepoint 1 visit, and $250 for timepoint 2 visit). Reimbursement will be provided by the clinical center/site and may be dispensed as a gift card, electronic gift card, ClinCard, or cash, as determined by the clinical center/site.

9.3 Biospecimen collection during post-hospital study visits

Biospecimen collection will occur during in-person follow-up visits. Specimen collections are described below.

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9.3.1 Blood specimen collection during post-hospital study visits

Blood will be collected from patients in plasma EDTA tubes (for protein measurements) and PAXgene tubes (for RNA extraction and transcriptomics) at the timepoint 1 and timepoint 2 post-hospital study visits. Blood may also be collected in CPT tubes (for peripheral blood mononuclear cells) at the timepoint 1 follow-up visit.

9.3.2 Respiratory specimen collection during post-hospital study visits

Nasal and oral swabs will be collected at the timepoint 1 and timepoint 2 post-hospital study visits.

9.3.3 Other specimen collection during post-hospital follow-up

Patients participating in the in-person visits at timepoint 1 and timepoint 2 will be asked to provide a stool sample for each visit. These stool samples may be collected at the time of the in-person visit or collected by the patient at home and shipped to the study team. For patients who prefer to collect stool at home, the study team will provide a home collection kit and return mailer.

9.4 Imaging studies during post-hospital study visits

At the timepoint 2 (12-month) follow-up visit, patients will undergo a study-specified thin-cut chest CT scan. This CT scan will be obtained on study protocol and funded with study budget. (Patients who have had a similar CT scan completed in the prior 6 weeks will have the clinically obtained scan uploaded rather than undergoing an additional CT scan.) Patients who are pregnant at the time of the 12-month visit will not undergo the research CT scan. Pregnancy testing prior to the 12-month CT scan will be undertaken according to the relevant policies and/or practices of the radiology center performing the CT scan. This 12-month visit research CT scan may be completed on the same day as other 12-month visit study procedures or on another day within the 12-month visit time window. The CT scans will be interpreted by radiologists. Site investigators will be responsible for disclosing clinically important findings on these research-dedicated CT scans to participants.

Additionally, patients who complete the timepoint 1 (3 to 6 month) and/or timepoint 2 (12-month) follow-up visit will have up to 1 clinically obtained CT scan after hospital discharge uploaded into the study database. A chest CT scan is preferred. In the absence of a chest CT, an abdominal CT scan can be used. If multiple clinically obtained CT scans are available, the scan closest to 6 months will be used.

Images from both the study-specified and clinically obtained scans will be uploaded to the study database for banking and future use.

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10. Schedule of Events

Study procedures, including collection of data, biospecimens, and radiographic images, are detailed in tables within this section.

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Table 3. Schedule of Events: Data collection for in-hospital component of the study.

Event [a]	Time Point					
	Study Day (in-hospital only)				At Discharge	Hospital Summary
	-2	-1	0[b]	1 - 7		
A. Eligibility and Baseline Data						
Eligibility Criteria			X			
Informed consent (main study)			X[c]			
Informed consent (LTO in-person visits)					X[d]	
Baseline History			X			
Demographics			X			
Chronic Medications			X			
Admitting Diagnoses			X			
B. Participant/Surrogate Interview[e]						
Contact Information			X			
Skin Color			X			
Baseline Health Assessments			X			
Alcohol Use			X			
Smoking Status			X			
C. Data Collection						
Daily Data						
- Vital Signs	X	X	X	X		
- Laboratory Values	X	X	X	X	X	
- Ventilator Data	X	X	X	X		
- Select Medications	X	X	X	X	X	
- Fluid Balance	X	X	X	X		
- Cumulative IV Sedation[f]	X	X	X	X		
- Sedation/Delirium	X	X	X	X		
- Blood Products	X	X	X	X		
APS Classification	X	X	X	X		
Outcome Data						
- Mortality						X
- Discharge location					X	
- Organ Failures	X	X	X	X	X	X
- ECMO / Prone position						X

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Table 3 Footnotes

a. The data collection schedule of events displays the study days from which data will be collected. The collected data will reflect patient status on those study days. The data may be collected later in a respective fashion.

b. Study Day 0 is anchored on when the first study-specific biospecimen is collected. The time of first biospecimen collection is termed Time 0 and denotes the time a patient is enrolled into the study. Per study eligibility criteria, enrollment must occur within 48 hours of meeting inclusion criteria.

c. Informed consent for the main study is listed in this table as a Day 0 procedure because most participants are expected to have informed consent obtained on Day 0. However, patients who enter the protocol on Protocol B using alteration of informed consent procedures may have consent obtained on a later day.

d. Informed consent for the long-term outcome in-person visits is a separate consent process from consent for the main study. Informed consent for long-term outcome visits is listed as a hospital discharge procedure in this table because this consent is anticipated to be completed prior to hospital discharge for many patients. However, this consent may be completed at any time prior to initiation of in-person long-term outcome visit study procedures, including after hospital discharge. Long-term outcome surveys, which do not require in-person visits, may be completed without separate consent for the in-person long-term visits.

e. The participant/surrogate interview aims to obtain information about participants' pre-hospital/baseline health and can occur at any time during index hospitalization, with completion as soon as possible after enrollment preferred.

f. Cumulative amounts of continuous IV administration of select sedation agents will be collected from Study Day -2 to 7.

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Table 4. Scheule of Events: Timing of post-hospital study visits.

Event	Study Day window for completing Event, by time point		
	3-month	**6-month**	**12-month**
A. Events for all survivors			
Medical record review	Day 53-67	Day 143-157	Day 329-337
SMS/email survey invite	Day 68-70	Day 158-160	Day 338-340
SMS/email reminder 1	Day 75-77	Day 165-167	Day 345-347
SMS/email reminder 2	Day 82-84	Day 172-174	Day 352-354
Telephone survey	Day 85-135	Day 175-240	Day 360-450
B. Events for LTO in-person subset	**Timepoint 1 (3 to 6 months)**		**Timepoint 2 (12 months)**
In-person visit (details in Table 6)	Day 75-240		Day 330-450
Stool collection (mailer, if needed)[a]	Day 75-240		Day 330-450

Table 4 Footnotes

a. Stool will be collected from participants at in-person visits at timepoint 1 (3 to 6 months) and timepoint 2 (12 months). Stool may be collected at the visits or stool can be mailed by the participant to the consortium coordinating center.

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Table 5. Schedule of Events: Data collection during post-hospital long-term outcome assessments.

Event	Time Point (see Table 5 for windows for each event)		
	3-month	6-month	12-month
A. Chart review			
Mortality	X	X	X
Re-hospitalization	X	X	X
B. Participant survey (online or phone) [a]			
Pregnancy status	X	X	X
Health related quality of life	X	X	X
Mental health, PTSD	X	X	X
Mental health, anxiety/depression	X	X	X
Feedback questions related to ImproveLTO core outcome set surveys	X	X	X
Recovery from acute illness	X	X	X
Dyspnea/pulmonary function	X	X	X
Frailty	X	X	X
Functional limitations	X	X	X
Disability	X	X	X
Cognitive decline	X	X	X
Return to work	X	X	X
Financial toxicity	X	X	X
Health insurance	X	X	X
Healthcare utilization	X	X	X
C. In-person assessments [b]	**Timepoint 1 (3 to 6 months)**		**Timepoint 2 (12 months)**
Short physical performance battery	X		X
Handgrip strength	X		X
CNS-Vital Sign	X		X
Dominant hand side quadriceps muscle ultrasound [c]	X		X
Spirometry [d]			X
DLCO [d]			X
Chest CT scan [e]			X (not completed on pregnant participants)

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Table 5 Footnotes

a. All surviving patients will be sent a link to complete the survey online, patients who do not complete the online survey within 3 weeks of receiving the link will be contacted via telephone to complete the survey through a phone interview.

b. Enrollment for in-person follow-up will close after approximately 600 patients have completed an in-person follow-up visit.

c. Quadriceps ultrasound and strength testing will be conducted at only a subset of sites due to requirements for specialized equipment and in-person training.

d. Spirometry and DLCO will only be collected in patients who have not had clinically obtained spirometry and DLCO in the 6 weeks prior to the 12-month visit.

e. A chest CT scan will only be completed in patients who have not had a clinically obtained chest CT scan in the 6 weeks prior to the 12-month visit. CT scans will not be completed for research purposes on participants known to be pregnant.

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Table 6. Schedule of Events: Biospecimens collected during index hospitalization and long-term follow-up visits for participants who are not known to be pregnant.

This table shows the maximum number and volume of biospecimens collected per participant. All biospecimens will not be collected on all participants.

Participants Not Known to be Pregnant

Event	Time Point							
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)	
	Day 0[a]	NBBAL[b]	Day 2	Day 4[c]	Day 6	Day 14	Timepoint 1 (3 to 6 mons)	Timepoint 2 (12 mons)
Acceptable study days for collection	Day 0-1	+96 hrs from intu-bation	Day 2-3	Day 3-5[c]	Day 6-8	Day 14-17	Day 75-240	Day 330-450
Max Total Blood Volume (ml)[d]	33.2	16	15.2	6	27.2	8.5	28.5	12.5
- EDTA tubes (total mL)	2x 6ml (12)		1x 10ml (10)	1x 6ml (6)	1x 6ml (6)	1x 6ml (6)	1x 10ml (10)	1x 10ml (10)
- Extra EDTA tube if CPT not collected (total ml)[e]	1x6ml (6)				1x6ml (6)		1x6ml (6)	
- RNA Paxgene tubes (total mL)	1x 2.5ml (2.5)		1x 2.5ml (2.5)		1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)	1x 2.5ml (2.5)
- CPT tubes (total mL)[f]	2x 8ml (16)	2x 8ml (16)			2x 8ml (16)		2x 8ml (16)	
- Na citrate tubes (total mL)	1x 2.7ml (2.7)		1x 2.7ml (2.7)		1x 2.7ml (2.7)			
- Buffy coat from EDTA	From EDTA							
- RBCs	From EDTA						From EDTA	From EDTA
Urine tubes (total mL)	1x 5ml (5)							
Oral swab	x		x		x		x	x
Nasal swab	x		x		x		x	x
Rectal swab	x		x		x			
Stool	x		x		x		x	x
HME filter fluid[g]	x		x	x	x	x		
Tracheal aspirate[g]	x		x	x	x	x		
NBBAL[g]		x						

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Table 6 Footnotes

a. Study Day 0 is anchored as the calendar day when the first study-specified biospecimen is collected. By study eligibility criteria, this must occur within 48 hours of the patient meeting eligibility criteria. The first biospecimen collected will, by definition, be collected on Day 0. Other biospecimens scheduled for collection on Day 0 can be collected as late as Day 1.

b. The NBBAL time point is anchored on the time of completion of a research-dedicated NBBAL and may occur on any study day between Study Day 0 and Study Day 8. The NBBAL time point specimens should only be collected once and should be collected as soon as possible after intubation and the patient passes the NBBAL safety screen. Two specimen types are collected at the NBBAL time point: NBBAL fluid and CPT tubes (two 8 ml tubes). The window for collecting NBBAL fluid is between intubation and 96 hours later. Do not complete a NBBAL more than 96 hours after intubation. The CPT collection should occur as soon as possible after NBBAL fluid collection. For the NBBAL time point, only collect CPT tubes for patients who have NBBAL fluid collected. CPT collection for the NBBAL time point is in addition to the CPT tubes collected for other time points. The NBBAL time point may occur on the same study day as specimen collection for other time points. For example, if a patient has a NBBAL completed on Day 0, the specimens for both Day 0 and the NBBAL time point may be collected on Day 0, which would result in the collection of four 8ml CPT tubes on Study Day 0.

c. Day 4 specimens are only collected from participants on invasive mechanical ventilation (IMV). Day 4 specimen collection is preferred on Study Day 4 or 5 but may be collected on Day 3 if Day 2 specimens were collected on Day 2 (instead of Day 3).

d. The approach to blood volume collection is governed by 3 principles: collection of <50 mL combined on Day 0 and Day 2 to facilitate minimal risk blood collection volumes through day 2 in the alteration of informed consent protocol (Protocol B); collection of < 150 mL total blood volume during the index hospitalization; collection of <50 mL per timepoint at long-term outcome visits. The maximum blood volume listed in this table is the maximum volume any one participant will have taken. The volume of blood collected is variable among participants but will not exceed the volumes listed here.

e. Participants who do not have blood collected for cellular analyses (CPT tubes) will have an additional 6 ml tube of EDTA collected at the study visit on Day 0, Day 6, and 3 months. Participants who do have blood collected for cellular analyses (CPT) will not have this extra tube of EDTA collected.

f. During processing of CPT tubes, plasma will be saved and stored when possible.

g. HME filter fluid, tracheal aspirate fluid, and NBBAL only collected from participants on invasive mechanical ventilation (IMV). For additional information about NBBAL specimen collection, please see footnote b.

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Table 7. Schedule of Events: Biospecimens collected during index hospitalization and long-term follow-up visits for participants who are known to be pregnant.

Compared to the biospecimen collection schedule for non-pregnant participants, pregnant participants do not undergo the NBBAL procedure and do not have blood collected on Day 4, Day 6, or Day 14.

This table shows the maximum number and volume of biospecimens collected per participant. All biospecimens will not be collected on all participants.

Participants Known to be Pregnant

Event	Time Point						
	Index Hospitalization (Study Day)					Long-Term Visits (Study Month)	
	Day 0[a]	Day 2	Day 4[b]	Day 6	Day 14	Timepoint 1 (3 to 6 mons)	Timepoint 2 (12 mons)
Acceptable study days for collection	Day 0-1	Day 2-3	Day 3-5	Day 6-8	Day 14-17	Day 75-240	Day 330-450
Max Total Blood Volume (ml)[c]	33.2	15.2	0	0	0	28.5	12.5
- EDTA tubes (total mL)	2x 6ml (12)	1x 10ml (10)				1x 10ml (10)	1x 10ml (10)
- Extra EDTA tube if CPT not collected (total ml)[d]	1x6ml (6)					1x6ml (6)	
- RNA Paxgene tubes (total mL)	1x 2.5ml (2.5)	1x 2.5ml (2.5)				1x 2.5ml (2.5)	1x 2.5ml (2.5)
- CPT tubes (total mL)[e]	2x 8ml (16)					2x 8ml (16)	
- Na citrate tubes (total mL)	1x 2.7ml (2.7)	1x 2.7ml (2.7)					
- Buffy coat from EDTA	From EDTA						
- RBCs	From EDTA					From EDTA	From EDTA
Urine tubes (total mL)	1x 5ml (5)						
Oral swab	x	x		x		x	x
Nasal swab	x	x		x		x	x
Rectal swab	x	x		x			
Stool	x	x		x		x	x
HME filter fluid[f]	x	x	x	x	x		
Tracheal aspirate[f]	x	x	x	x	x		

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Table 7 Footnotes

a. Study Day 0 is anchored as the calendar day when the first study-specified biospecimen is collected. By study eligibility criteria, this must occur within 48 hours of the patient meeting eligibility criteria. The first biospecimen collected will, by definition, be collected on Day 0. Other biospecimens scheduled for collection on Day 0 can be collected as late as Day 1.

b. Day 4 specimens are only collected from participants on invasive mechanical ventilation (IMV). Day 4 specimen collection is preferred on Study Day 4 or 5 but may be collected on Day 3 if Day 2 specimens were collected on Day 2 (instead of Day 3).

c. For participants known to be pregnant, the approach to blood volume collection ensures less than 50 ml of blood collected in any 8-weeks and a maximum of 2 blood draws in any 1-week period.

d. Participants who do not have blood collected for cellular analyses (CPT tubes) will have an additional 6 ml tube of EDTA collected at the study visit on Day 0 and 3 months. Participants who do have blood collected for cellular analyses (CPT) will not have this extra tube of EDTA collected.

e. During processing of CPT tubes, plasma will be saved and stored when possible.

f. HME filter fluid and tracheal aspirate fluid only collected from participants on invasive mechanical ventilation (IMV).

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Table 8. Schedule of Events: Approximate number of participants who will have each biospecimen collected.

In-hospital biospecimen collection will only occur if the patient is alive and remains in the hospital on that day. Additionally, some samples will be collected only for a selected subset of participants. This table estimates the number of patients who will have samples collected based on these principles. The description of which patients will have specimen collection is shown in parentheses in each cell. These estimates are not exact. The final number of participants with each biospecimen type will depend on several factors that cannot be predicted exactly.

Event	Approximate number of patients (description of patients) with biospecimen collected at time point							
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)	
	Day 0	NBBAL[a]	Day 2	Day 4	Day 6	Day 14	Timepoint 1 (3 to 6 mons)	Timepoint 2 (12 mons)
EDTA blood	4,000 (all)		3700 (all still in hospital)	800 (on IMV)	1800 (all still in hospital)	500 (all still in hospital)	600 (all who attend visit)	500 (all who attend visit)
Extra EDTA tube if CPT not collected	3,000 (no CPT)				1350 (still in hospital, no CPT)		300 (attended visit, no CPT)	
RNA Paxgene blood	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)	500 (all still in hospital)	600 (all who attend visit)	500 (all who attend visit)
CPT blood	1000 (first 1000 patients)	700 (patients with NBBAL)			450 (first 1000 patients still in hospital)		300 (50% subset who attend visit)	
Na citrate tubes blood	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)			
Buffy coat from EDTA	4,000 (all)							
RBCs from EDTA	4,000 (all)						600 (all who attend visit)	500 (all who attend visit)

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Event	Approximate number of patients (description of patients) with biospecimen collected at time point							
	Index Hospitalization (Study Day)						Long-Term Visits (Study Month)	
	Day 0	NBBAL[a]	Day 2	Day 4	Day 6	Day 14	Timepoint 1 (3 to 6 mons)	Timepoint 2 (12 mons)
Urine	3500 (all who can produce urine)							
Oral swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	500 (all who attend visit)
Nasal swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	500 (all who attend visit)
Rectal swab	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)			
Stool	4,000 (all)		3700 (all still in hospital)		1800 (all still in hospital)		600 (all who attend visit)	500 (all who attend visit)
NBBAL		700 (intubated, passed safety screen, consented)						
HME filter fluid	1333 (on IMV)		1233 (on IMV)	800 (on IMV)	600 (on IMV)	200 (on IMV)		
Tracheal aspirate	1333 (on IMV)		1233 (on IMV)	800 (on IMV)	600 (on IMV)	200 (on IMV)		

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Table 8 Footnotes

a. The NBBAL time point is anchored on the time of completion of a research-dedicated NBBAL and may occur on any study day between Study Day 0 and Study Day 8. The NBBAL time point specimens should only be collected once and should be collected as soon as possible after intubation and the patient passes the NBBAL safety screen. Two specimen types are collected at the NBBAL time point: NBBAL fluid and CPT tubes (two 8 ml tubes). The window for collecting NBBAL fluid is between intubation and 96 hours later. Do not complete a NBBAL more than 96 hours after intubation. The CPT collection should occur as soon as possible after NBBAL fluid collection. For the NBBAL time point, only collect

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CPT tubes for patients who have NBBAL fluid collected. CPT collection for the NBBAL time point is in addition to the CPT tubes collected for other time points. The NBBAL time point may occur on the same study day as specimen collection for other time points. For example, if a patient has a NBBAL completed on Day 0, the specimens for both Day 0 and the NBBAL time point may be collected on Day 0, which would result in the collection of four 8ml CPT tubes on Study Day 0. NBBAL time point specimen collection may only occur for participants with consent for study procedures (that is, NBBAL time point specimen collection may not be performed under waiver of informed consent). Participants known to be pregnant do not undergo the NBBAL procedure.

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Table 9. Schedule of events: Radiology and pulmonary function tests during index hospitalization and long-term follow-up visits.

Images from the following radiographic studies will be collected by study personnel and uploaded into the study database. Results of pulmonary function tests will be recorded by study personnel into the study database.

Imaging type	Procedures	Estimated number of images
In-hospital clinically obtained CXRs	Upload up to 3 CXRs per participant. Details of which CXRs to upload will be outlined in study operating procedures. Initially, CXRs targeted for uploading will be CXRs completed closest to 8:00am on Day 0, Day 2, and Day 6.	3 per participant for all enrolled participants [number of x-rays =12,000 if all 4,000 patients each have 3 uploaded CXRs]
In-hospital clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan completed closest to 8:00am on Day 0. Chest CT preferred, abdominal CT acceptable if no chest CT performed during hospitalization.	1 per participant for all enrolled participants [number of scans = 4,000 if all 4,000 patients have a clinical CT scan in hospital]
Post-discharge clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan closest to 6 months. Chest CT preferred, abdominal CT acceptable if no chest CT performed between hospital discharge and 12 months.	1 per participant among those in the LTO cohort [number of scans = 600 if all 600 patients in the LTO cohort have a clinical CT scan after hospital discharge]
Study-specified Chest CT scan at 12-month visit (Not completed on pregnant participants)	Upload up to 1 scan per participant. Scheduled study-protocol thin-cut chest CT scan. Funded by study budget. If a similar scan has been completed clinically in the prior 6 weeks before 12-month visit, upload the clinical scan and do not repeat as a research procedure.	1 per participant among those who complete the 12-month follow-up visit [number of scans = 500]
Study-specified pulmonary function tests (spirometry and DLCO) at 12-month visit	Record results of up to 1 pulmonary function test battery per participant. Scheduled study specified spirometry and DLCO. Funded by study budget. If similar pulmonary function tests have been completed clinically in the prior 6 weeks before 12-month visit, record the results of those tests and do repeat as a research procedure.	1 per participant among those who complete the 12-month follow-up visit [number of PFT batteries = 500]

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11. Use of Data and Biospecimens

Data, biospecimens, and radiographic images collected in the APS Consortium will be used for 3 purposes:

1. Completion of APS Consortium-wide science as outlined in appendices to this protocol (funded by the APS Consortium budget).

2. Completion of APS Clinical Center-specific science as outlined in grants proposals from Clinical Centers investigators and selected for funding via the NIH peer review process (funded by the APS Consortium budget).

3. Banking for use in future ancillary studies conducted by investigators both inside and outside the APS Consortium (biobanking is funded by the APS Consortium budget but ancillary studies for use of bio-banked data and specimens is not funded by the APS Consortium budget).

During conduct of the APS Consortium study procedures outlined in this master protocol, data, biospecimens, and radiographic images will be collected by enrolling sites and transmitted to the APS Consortium Coordinating Center at Vanderbilt University Medical Center. Data and biospecimens will be organized and catalogued at the coordinating center. The coordinating center will distribute data and biospecimens as needed to complete Consortium-wide science and Clinical Center-specific science. Additionally, the coordinating center will periodically transmit data and biospecimens to repositories managed by the National Heart, Lung, and Blood Institute (NHLBI) -- BioData Catalyst (data repository) and BioLINCC (biospecimen repository).

During the period of performance for the APS Consortium, requests for data and/or biospecimens for use in ancillary studies will be reviewed for approval by the APS Steering Committee. After the completion of the APS Consortium period of performance, requests for data and/or biospecimens for use in ancillary studies will be governed by NHLBI through the BioData Catalyst and BioLINCC programs.

Table 10 outlines principles of governance for APS Consortium-wide science, APS Clinical Center-specific science, and ancillary studies.

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Table 10. Use of data and biospecimens.

Program Component	Consortium-Wide Study funded under APS Consortium	Clinical Center-Specific Projects funded under APS Clinical Center Grants	Investigator initiated ancillary study (from investigators inside or outside of APS)
Data	Data available includes all elements in the APS Consortium-wide CRF/EDC. The CCC oversees data management. Data will be deposited to BioData Catalyst by the Consortium Coordinating Center.	Data includes all elements from Consortium wide CRF/EDC plus any data collected independently by the Clinical Center. Data collected independently will be managed by the Clinical Center and deposited to BioData Catalyst as necessary by the Clinical Center.	Data available include the data approved for sharing by the APS Steering Committee (during the Consortium) or available in BioData Catalyst (after the Consortium). New data/results generated by an ancillary study are the responsibility of the ancillary study investigators, including transmission to BioData Catalyst if necessary.
Specimens	Specimen collection detailed in the Consortium-wide mast protocol. Eligible specimens will be deposited to BioLINCC by the Consortium Coordinating Center.	May use specimens captured via APS master protocol for the Consortium plus any biospecimens collected independently by the Clinical Center. Specimens not outlined in the APS Consortium master protocol are the responsibility of the Clinical Center investigators, including collecting specimens, shipping, and depositing in BioLINCC (as necessary).	Biospecimens available include the biospecimens approved for sharing by the APS Steering Committee (during the Consortium) or available in BioLINCC (after the Consortium). Once biospecimens are transferred to the ancillary study investigators, all biospecimen responsibilities lie with the ancillary investigators, including transmission to BioLINCC as necessary.
Protocol	Protocol written by APS Consortium steering committee and managed by the APS Consortium Coordinating Center.	Protocol written by and managed by Clinical Center investigators.	Protocol written by and managed by ancillary study investigators.
Consent Documents	Consent documents for the Consortium-wide protocol, which manages Consortium-wide science, is managed by the CCC and approved by the Consortium sIRB	If necessary, separate consent procedures and documents are the responsibility of the Clinical Center.	If necessary, separate consent procedures and documents are the responsibility of the ancillary study investigator.
IRB	Vanderbilt University Medical Center serves as the single sIRB. The Consortium Coordinating Center manages regulatory approvals.	As needed, the Clinical Center determines overseeing IRB and manages regulatory approvals.	As needed, the ancillary study investigator determines overseeing IRB and manages regulatory approvals.

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12. Terminology and Statistical Considerations

12.1 Phenotyping Terminology Considerations

Several categories of phenotypes will be evaluated in APS consortium analyses. This section outlines principles for how the different categories of phenotypes will be considered in the consortium's work (Table 11).

The first category of "phenotype" is the historical clinical syndromes that the APS consortium seeks to target – namely, ARDS, pneumonia, and sepsis. These syndromic phenotypes will be defined as previously stated (Section 8.1.3) using clinically established and published definitions.

The second category of "phenotype" to be considered is previously defined clinical, biologic, and/or physiologic phenotypes that were described in acute illness phenotyping literature prior to launch of the APS consortium. Examples of these types of phenotypes include: (i) the latent class analysis (LCA)-derived "hyperinflammatory" and "hypoinflammatory" phenotypes of ARDS and sepsis defined by clinical features and plasma protein biomarkers; (ii) the SRS1 and SRS2 phenotypes of sepsis defined by whole blood transcriptomic profiles, (iii) high/low elastance phenotypes of ARDS and pneumonia described by particular respiratory physiology parameters. Several of the consortium-wide scientific aims plan to incorporate these previously defined phenotypes in their analyses to place the Consortium's work in context with previous literature and/or as a contrast for the novel phenotypes to be identified within the Consortium (see protocol appendices, section 16).

The third category of "phenotype" to be considered is novel phenotypes identified within the APS Consortium using unsupervised and/or supervised clustering methodologies. Developing and validating these novel phenotypes will be key scientific goals of the Consortium as detailed in the appendices to this protocol (section 16).

Table 11. Phenotyping categories considered in the APS Consortium.

Phenotype category	**Examples**
1. Historical clinical syndromes	ARDS, pneumonia, sepsis
2. Previously defined phenotypes	i. hyper- vs hypo-inflammatory ii. SRS1 vs SRS2 iii. High elastance vs low elastance
3. Novel phenotypes	To be determined based on new findings in the APS Consortium

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12.2 Statistical Considerations

12.2.1 Global considerations

The APS Consortium has seven consortium-wide aims, within which there are a combination of discovery and hypothesis testing analyses proposed (see appendices, Section 16). The aims are developed at a time when computational power is increasing, analytical strategies are developing, and the field of data science is evolving more rapidly than ever before. Moreover, as data are accrued more will be learned about the data quality, completeness and fitness for purpose. To respect the emerging technologies that will be available by the time digital data are available for an analysis and with an understanding of data quality, availability, and completeness, we will fix the statistical analysis plan immediately prior to embarking on an analysis, as discussed below. A global statistical analysis plan (SAP) will be developed for each APS aim, and each manuscript or clinical question will also have a pre-specified SAP. The purpose of this section is to lay out general considerations and principles that will guide development of SAPs and conduct of analyses.

SAPs will be developed with close collaboration among biostatisticians, bio-informaticists, and APS subject matter experts, while keeping aims somewhat broad to encourage innovation and exploration of novel ideas and hypotheses. Biomedical knowledge will inform analysis strategies to the extent possible. Analytical methods will be chosen to make efficient use of the data; when a singular approach cannot be established due to competing methods, the alternative approaches should be discussed and opportunities to compare approaches considered for inclusion in the SAP. Any method selected ideally makes full use of available raw data, including raw longitudinal data. Explanatory and exploratory analyses will be distinguished in SAPs and reporting of results. Both unsupervised and supervised learning techniques are expected to play major roles. Strong emphasis will be given to validation of both pattern discoveries and estimation/predictive instruments.

12.2.2 Statistical Analysis Plans (SAPs)

All significant projects will develop and file SAPs before commencing the primary analyses. A central APS file structure will be used to hold all SAPs and to date-stamp changes to them as well as to name responsible analysis designers. SAPs should be specific enough to reproduce analyses with minor assistance from the project's statisticians/bio-informaticians in combination with reproducible analysis scripts. Unsupervised (to patient outcomes) preprocessing should be described and included in the ultimate SAP, recognizing the preprocessing steps may commence before SAPs are completed. SAPs may be changed after analyses commence but reasons for changes must be documented in the SAP document.

12.2.3 Reproducible research

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All analytical steps must be scripted in an accessible programming environment. The general approach will be to embed analysis code into a report document so that statistical reports can be recreated by issuing a single command, using software such as Quarto (which is programming language agnostic) or RMarkdown or equivalents in other languages. Program and package versions will be documented when running the final script and should match published results or discrepancies should be explained. Statistical code will be shared. More background information and some resources may be found here.[20]

12.2.4 Validation

When patterns or structures are discovered through data analysis, these identified patterns should be validated in the sense of demonstrating sturdiness to small changes in the dataset used to find the structures. Predictive and estimation instruments should also be validated. Split-sample validation (validating on a holdout sample) is often used and may be appropriate in some instances. Repeating the process of splitting into derivation and validation samples a few times can reveal volatility of the split-sample approach when the sample is of insufficient size, and splitting the sample reduces the derivation sample size. When the statistical approach allows, cross-validation and bootstrap validation should be considered in order to maximize use of information and to optimize the reliability of the validation. Carefully repeating, inside each resampling loop, all learning/data analysis steps can reduce overfitting.

Validation analyses should include an assessment of the stability of discovered patterns, feature selection, etc. In high-dimensional situations in which "winning" features are sought, it is important to validate the ability of the data to find such "winners." This can be done by, for example, computing bootstrap confidence intervals for importance rankings of competing features.[21,22] Human supervision of feature selection may be important and adopted in specific cases depending on individual project goals. When the goal is to make absolute predictions (outcome risks, life expectancy, etc.), validations should include information on the absolute predictive accuracy (e.g., overfitting-corrected smooth calibration curves).

When patient clustering or any method that categorizes patients is used, additional validation should be considered. For example, when the goal is to predict patient outcome or to estimate differential treatment effect, an approach to validating patient clustering might be:

- Start with cluster indicator variables as the only variables (other than background covariates that may be included in all models) in the model predicting patient outcome, or as main effect and interaction-with-treatment effects in an analysis of treatment effect heterogeneity. Measure the predictive information in the categorical cluster indicators.
- Verify that clusters are sufficiently homogeneous by adding distances from cluster centers (when cluster centers can be defined) in the second stage; if distance from centers provides additional predictive information over cluster indicators, then the clusters are not compact, and distances might be preferred predictors over categories.
- In the third stage, add pre-specified prognostic variables (including variables used to derive the clusters) to the cluster variables (indicators or distances). If the prognostic variables add more information than the cluster variables alone, then the use of clustering should be reconsidered.

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12.2.5 Considerations for particular analytic procedures

When there are missing data in variables being analyzed, case-wise deletion of partial patient records should be avoided. Multiple imputation or other missing data procedures should be considered. When using imputation procedures, leveraging longitudinal data should be considered. In some settings, for example estimation of correlation matrices and principal components, pairwise deletion of missing values may be a viable technique.

When fitting statistical models for longitudinal data it is often important to consider a realistic correlation structure. Where random intercepts and slopes fail to result in a well-fitting correlation structure, serial correlation structures (e.g., AR1 or Markov models) should be considered. When using generalized estimating equations (GEE) to correct for actual correlation patterns, the accuracy of GEE approximations and efficiency should be reported, and notice should be taken that GEE may not be robust to missing values that are not missing completely at random.

When considering cluster analysis outputs as explanatory variables, or effect modifiers, uncertainty in cluster membership should be carried forward through to final statistical inference. For example, if a cluster analysis also provides information on the probability of membership to each cluster for each individual, Bayesian regression models can incorporate this information in subsequent analyses.

Any special sampling or sub-sampling used in the study design phase should be considered in the analysis phase.

As APS is a multi-site study, adjusting for the multi-level structure of the data may be important when fitting statistical models. An exception occurs when performing single-site analyses or when patient-level characteristics already capture potential variation across sites. Accounting for multi-level structure may also be important in multiple imputation.

As a general principle, treatments received will be considered in analyses; treatments may affect biomarkers more than they affect patient outcomes. Adjusting biomarkers for treatments received ahouls be considered. Consideration will also be made of whether treatments alter cluster membership. Potential for confounding should be considered when estimating treatment effects on cluster membership or trajectory, as well as heterogeneity of treatment effect across clusters. When allowing for cluster membership to evolve over time, special consideration should be given to the potential for time-varying confounding if modeling cluster effects on outcomes.

Some consortium-wide science specific aims will use multiple-phase analytic approaches - needed because of the complexity of data and analytical methods. Although the statistical analysis procedures used in any one stage may be tried and tested in the statistical literature, the performance of all analytical phases in tandem may not have been validated for stability and accuracy. In those situations, simulation studies should be considered to check the performance of the multi-phase procedure; these will be considered on a case-by-case basis as SAPs are filed depending on complexity and review of relevant

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methods literature. The structure of such studies may be specific to the analytic approach but might generally include two key investigations. First, datasets that resemble the dimensions of the real data but for which the relationship/association/effect of interest is entirely noise, should be generated and the multi-phase procedure run on these random datasets to make sure that noise is not incorrectly identified to be statistical signals. Second, a simulation of a very large dataset in which real signals are present can be performed, making sure that the true signals are the ones found by the proposed analytic procedure. Then the sample size can be gradually reduced to find the breaking point where results are no longer reliable. Users of the procedure then make sure that the available sample size is higher than the identified breaking point.

12.2.6 Statistical considerations summary

As laid out above, we have developed a broad approach for each statistical analysis plan, and we have described robust methods to achieving reproducible findings. To demonstrate the range of statistical methods that might be used, we have described some analytical approaches within individual aims (described in the appendices, Section 16). Regardless of the details, every analysis will proceed as follows:

i) The analysis plan should be pre-specified, and a system to review, approve and file the SAPs will be implemented.
ii) When a preferred statistical approach cannot be easily identified or agreed upon, multiple approaches may be specified and comparisons among approaches will be incorporated into the SAP.
iii) The SAP should specify the approach to missingness.
iv) The SAP should specify if and how the multi-level data structure will be considered in the analysis.
v) The SAP should specify the approach to evaluating validity, including addressing the propagation of uncertainty through a complex or multi-phase analysis pipeline.
vi) Statistical code used to generate results will be made available, and deviations from the SAP should be transparently documented.

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13. Human Subjects

13.1 Risks and benefits

This master protocol for the APS Consortium describes an observational prospective cohort study with longitudinal collection of data and biospecimens with methods routinely used in current routine medical practice.

The risk to participants in the APS Consortium study are detailed in Table 12. The primary risks are related to the potential of disclosure of private health information and complications from biospecimen collection procedures. These risks will be minimized by using a secure REDCap data collection system for data entry and storage, maintaining good clinical practice procedures at all sites for handling of private health data, and training study teams on best practices for biospecimen collection.

Samples taken as part of this study may be used to evaluate human genetics in the future. Genetic testing results will not be linked to identifiable patients nor placed in the medical record. Inadvertent disclosure of genetic testing results could influence insurance policies or future employment.

Participants are not expected to receive direct personal benefits for participating in the APS Consortium study. On a societal level, benefits of participants joining the APS Consortium study include contributing to increased knowledge about ARDS, pneumonia, and sepsis, which could lead to medical advances that ultimately decrease morbidity and mortality from these syndromes.

Table 12. Potential risks to participants in the APS Consortium study by study procedure.

Study procedure	Potential risks	Study methods to minimize risks
Collection of personal data	Inappropriate disclosure of private health information (loss of confidentiality/privacy).	Only study personnel trained in good clinical practice for clinical research studies will be involved in data handling. Each enrolling site will maintain appropriate training and certification in protection of human participants for all study personnel involved in interacting with patients and/or handling data. Data will be entered into and stored within REDCap, a secure internet-based data collection tool. REDCap employs several layers of security, including authentication of end-users, automatic user logout after 30 minutes of inactivity, a time-stamped audit trail, encrypted web-based information transmission, and firewall protection of uploaded documents. Additional information on REDCap is available at: www.project-redcap.org.
Blood collection (phlebotomy)	Pain at phlebotomy site, infection at the phlebotomy site, bleeding, damage to surrounding nerves, transient light-headedness, fainting/syncope.	Only study or clinical personnel trained in phlebotomy will collect blood for this study. Patients will be positioned in a safe location for phlebotomy, such as supine in a bed or sitting in a chair. When possible, blood will be collected from pre-existing vascular catheters and timed with blood draws for clinical care to avoid additional phlebotomy.

Study procedure	Potential risks	Study methods to minimize risks
Urine collection	No plausible risks identified.	Only study or clinical personnel trained in the collection of urine will collect urine for this study. Urine will be collected via patient voiding or collection from pre-existing urinary catheters. No invasive procedures will be initiated in the study to collect urine.
Collection of oral, nasal, and rectal swabs	Localized transient irritation, pain, and/or bleeding may occur at the swab site.	Only study or clinical personnel trained in the collection of oral, nasal and rectal swabs will collect swabs for this study. Personnel will be trained to hold pressure on the swab site if it bleeds.
Stool collection	No plausible risks identified.	Stool will only be collected via participants freely stooling or via stool collection systems in place for clinical care (e.g., rectal tube, fecal incontinence bag). No invasive procedures will be initiated by the study to collect stool.
HME (ventilator) filter collection	Inadvertent disconnection of ventilator tubing for longer than necessary to collect the filter, which could rarely result in low oxygen levels, organ damage, and death if not recognized. The risk of patient injury from HME (ventilator) filter collection is considered very low.	Only study or clinical personnel trained in the collection of HME (ventilator) filters will collect the filters. Ventilators at all study sites have alarms that alert clinical teams when ventilator tubing is disconnected. HME filters are routinely used for clinical care. Patients receiving invasive mechanical ventilation are routinely disconnected from the ventilator in clinical care daily. When possible, HME filters will be collected during a disconnection of the ventilator that is occurring as part of clinical care.
Tracheal aspirate fluid collection	Bleeding, localized pain, drop in oxygen levels (hypoxemia), and dislodgement of the tracheal tube. A drop in oxygen levels from tracheal aspirate collection is very unlikely to cause patient harm.	Only study or clinical personnel trained in the collection of tracheal aspirate fluid will collect this specimen. Tracheal suctioning is a routine clinical procedure and tracheal aspirates for this protocol will be done at times that tracheal aspirates are being collected as part of clinical care, whenever possible.
NBBAL procedure	Bleeding, localized pain, dislodgement of the tracheal tube, and drop in oxygen levels (hypoxemia), which if severe and uncorrected, can lead to organ injury and death. Serious injury from a NBBAL is rare (<1%).[23]	Only study or clinical personnel trained in the NBBAL procedure will complete this procedure for the study. NBBAL is a common clinical procedure in some hospitals. Only patients who are intubated will undergo the NBBAL procure. Only patients who pass a pre-procedural safety screen under the supervision of the site investigator will undergo a NBBAL. Hypoxemia temporally related to a study NBBAL procedure will be collected as an AESI and reported to the sIRB and OSMB.
Long-term outcome surveys (via phone, SMS, or email)	No plausible physical risks. Participants might experience embarrassment or emotional discomfort.	Participants do not have to answer any questions that make them feel too uncomfortable. An on-call mental health specialist will be available to talk with patients that have severe emotional stress or suicidal thoughts.
Short physical performance battery (SPPB) [at in-person LTO visits]	SPPB assesses lower extremity functioning. It includes a balance test, a timed 4-meter walk test, and chair stand test. There is a small risk of falling. Activities may increase heart rate.	The testing will be performed by trained study staff. Movements are completed in a standard order, starting with easier movements. Participants will be able to skip any movement that they cannot do or feel would be unsafe to try.
CNS-Vital Signs [at in-person visits]	CNS vital signs is a computer based cognitive assessment. Participants might experience	Participants do not have to answer any questions that make them feel too uncomfortable.

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Study procedure	Potential risks	Study methods to minimize risks
	embarrassment or emotional discomfort from not being able to answer some questions.	
Handgrip strength [at in-person LTO visits]	No plausible risks identified.	Participants may skip this procedure if they do not feel comfortable completing it.
Muscle ultrasound and strength testing [at in-person LTO visits]	No plausible risks identified.	Participants may skip this procedure if they do not feel comfortable completing it.
Pulmonary function testing [at 12-month LTO visit]	Pulmonary function testing is a common procedure in clinical medicine and has very low risks. Participants may feel dizzy, lightheaded, or tired or cough during or shortly after the procedure. These symptoms should go away shortly after completion of the testing.	Pulmonary function testing, including spirometry and diffusing capacity of the lungs for carbon monoxide (DLCO), will be done using standard clinical procedures. Participants will be allowed to rest or stop the testing if they feel dizzy, lightheaded, or tired. Participants may skip any portion of testing they do not feel comfortable completing.
Chest CT scan [at 12-month LTO visit]	Chest CT involves exposure to a small dose of radiation. At doses much higher than participants will receive for the study CT scan, radiation is known to increase the risk of developing cancer after many years. At the doses participants will receive for the study CT scan, it is unlikely to cause any adverse effects.	The amount of radiation used in chest CTs is low (approximately 10 mSv); this amount of radiation from on chest CT scan is similar to the background radiation dose experienced by people in the United States over one year.[24,25] Participants with a recent chest CT scan obtained clinically will not undergo a research CT scan. Participants are able to skip any portion of testing they do not feel comfortable completing.
Storage of biospecimens for future genetic testing	Biospecimens will be placed in long-term storage, where they may be retrieved for future studies involving human genetic testing. Inadvertent disclosure of genetic testing results could cause loss of privacy and in severe cases affect insurance policies and employment opportunities.	Biospecimens for future genetic testing will be deidentified before they are stored. These biospecimens will be labeled with a study number and the link between that study number and the patient's identity will not be provided to long-term storage facilities. Results of genetic testing will not be placed in the medical record. We cannot guarantee that no one will ever be able to use genetic information to identify participants.

13.2 Informed Consent

13.2.1 Approach to informed consent for this study

Study procedures described in this protocol (APS Study Protocol A – full protocol) will be completed after written informed consent for the study has been obtained from the participant or a legally authorized representative (LAR)/surrogate decision maker. Consent may be obtained using a paper document or

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remotely with electronic/e-consent procedures. Remote consent with an LAR/surrogate is possible using electronic procedures as long as the risks and benefits of the study are discussed with the consenting study personnel and the LAR/surrogate has an opportunity to ask questions. In situations when the patient/LAR/surrogate does not speak English, a short-form consent document and qualified interpreter may be used to facilitate an informed consent discussion and documentation.

If a patient or LAR/surrogate declines study blood draws or research genetic testing during an informed consent discussion, the patient should not be enrolled.

13.2.2 Consent through a Legally Authorized Representative (LAR)/surrogate

Some patients who are eligible for this study are likely to not possess decision making capacity. In these cases, consent for study participation may be obtained through a legally authorized representative (LAR) or other surrogate decision maker.

When a patient is identified as eligible for study enrollment, the study team will assess the patient's capacity to make decisions about participation in research. This assessment is completed by a study team member trained in good clinical practice for the conduct of clinical research who is in direct contact with the patient. If the patient cannot meaningfully engage with the study team member (such as, a state of intubation, chemical sedation, and/or not responsive to verbal stimuli), the study team member may consider the patient to lack decision making capacity for consent. If the patient can meaningfully engage with the study team member, the study team member will explain the APS study to the patient and review the informed consent document with the patient. After this explanation, if the patient cannot describe the basic elements of the study, including risks and benefits of study participation, the study team member may consider the patient as not possessing decision making capacity for consent. The study team member will document the process and outcome of capacity assessment in the APS Study Electronic Data Capture instrument.

If an eligible patient lacks decision making capacity to consent for study participation, the study team may obtain consent for study participation through an alternative decision maker. In this study, we use the term "LAR/surrogate" to indicate the alternative decision maker who is making decisions for the patient while the patient lacks capacity. The US Code of Federal Regulations (CRF) section 46.102(i) defines a legally authorized representative (LAR) as: "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research. If there is no applicable law addressing this issue, *legally authorized representative* means an individual recognized by institutional policy as acceptable for providing consent in the nonresearch context on behalf of the prospective subject to the subject's participation in the procedure(s) involved in the research." The term "LAR/surrogate" is applied to the person making medical decisions for the patient, either as an explicit legally authorized representative or through local policy and custom. The study team should follow local laws and policies when identifying an alternative decision maker for consent for study participation.

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The study's informed consent document will be available in multiple languages. When an LAR/surrogate does not speak any of these languages, the study team may use a consent short form. When a short form is used, an IRB-approved translated Surrogate Rider will be attached as an addendum to the end of the translated Short Form and used to document the surrogate's written consent.

If consent for study participation is obtained via an LAR/surrogate, the study team will iteratively assess the patient to evaluate whether the patient has gained decision making capacity. These assessments will be made through hospital discharge or Study Day 14 (whichever occurs first). If the patient is identified as regaining capacity, the study team will obtain consent for study participation from the patient at that time by having the patient sign the consent form that has already been signed by an LAR/surrogate. At that time, the patient may decline to continue participation in future study procedures or withdraw informed consent; details of procedures for withdrawal of informed consent are outlined below.

13.2.3 Rationale for two study protocols with different approaches to informed consent

A key objective of this study is to rigorously phenotype ARDS, pneumonia, and sepsis, among severely and critically ill patients throughout their entire course of illness, beginning immediately when a patient meets eligibility criteria through 12 months later. To achieve this objective, patients must be enrolled as early as possible in their course of illness. Early biospecimens are crucial for the objectives of the APS Consortium. Up to 75% of patients in the ICU experience delirium or altered mental status prohibiting their ability to provide informed consent.[26] Frequently, LARs for critically ill adults are not available to provide written informed consent for research participation in a timely fashion. For example, prior work has shown that at a publicly-funded hospital, 18% of eligible patients for ARDS clinical trials are not enrolled due to the patient not having capacity for consent and no LAR/surrogate being available.[27]

An accompanying protocol (APS Study Protocol B – alteration protocol) will be used when consent for study participation cannot be obtained before study entry. These protocols are linked. Protocol B describes minimal risk study procedures that may be completed with alteration of informed consent and no written informed consent for study participation. The minimal risk study procedures in Protocol B are identical to study procedures in Protocol A. Protocol B contains a subset of study procedures from Protocol A that are minimal risk.

An approach of using two protocols to enroll participants – one that governs consented patients (Protocol A) and one that governs patients participating under alteration of informed consent (Protocol B) – is being utilized to minimize selection bias that would occur if only patients who were able to immediately provide written informed consent were enrolled. Outlining minimal risk procedures from the APS Study schedule of events in a separate protocol (Protocol B) enables participants without consent to begin minimal risk study procedures using an alteration of informed consent process. Meanwhile, participants with consent completed may engage in all study procedures, including both minimal risk procedures and greater than minimal risk procedures.

Goals of the study team include:

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- Early enrollment of a population that represents the patient population of those suffering from ARDS, pneumonia, and sepsis, including those who are critically ill and often unable to rapidly consent for research.
- Obtain informed consent for study participation prior to initiation of study procedures for as many participants as possible.
- For participants who enter the study with alteration of informed consent, obtain written informed consent as soon as possible.

13.2.4 Description of consent procedures for Protocol A and Protocol B

Protocol A (full protocol): This document contained herein is Protocol A. It describes all study procedures that a participant may complete during the course of the APS study and governs study procedures for participants who have completed informed consent for research participation (either directly or through an LAR/surrogate). Protocol A has two accompanying informed consent documents (ICDs):

- ICD #1 (primary study): Informed consent document for the primary APS study, including in-hospital study procedures and long-term outcome surveys. ICD #1 contains two parts--part 1, which includes information shared across all sites participating in the study and part 2, which contains local context information for a particular site and signature lines. Participants who entered the study with alteration of informed consent may consent for the full primary study at any time through the 3-month follow-up time point. While the participant is in the hospital, consent will be obtained via ICD #1. If a participant enters the study via alteration of informed consent (Protocol B) and is discharged from the hospital without informed consent being obtained, the study team will attempt to contact the participant through the 3-month study time point to obtain consent. If this contact is made via telephone, consent may be obtained with waiver of documentation of signature. Similarly, if a participant had previously been participating in the study via surrogate consent, the patient will be given the opportunity to consent for themselves ("reconsent") with waiver of documentation of signature. If participant does not possess capacity at the 3-month follow-up time point, surrogate consent according to state laws may be obtained, including with waiver of documentation of signature. Waiver of documentation of signature will use the following procedures:
  i. participant/surrogate receives information explaining the study;
  ii. participant/surrogate has the opportunity to review ICD #1;
  iii. participant/surrogate has the opportunity to ask questions and have those questions answered by study team members;
  iv. participant/surrogate provides confirmation of wanting to participate in the study;
  v. consent process is documented in the Study Electronic Data Capture instrument.

  If a participant attends a long-term outcome in-person visit without having previously provided informed consent for study participation, informed consent for study participation will be obtained at that time using ICD#1.

- ICD #2 (LTO in-person visits): Informed consent document for long-term outcome in-person visits. ICD #2 contains two parts--part 1, which includes information shared across all sites

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participating in the study and part 2, which contains local context information for a particular site and signature lines. Participants (either directly or through a surrogate) who attend in-person long-term follow-up visits will complete informed consent for the in-person long-term outcome study procedures prior to initiation of those procedures. This informed consent document for in-person long-term outcome visits (ICD #2) is separate from the informed consent document for the primary study (ICD #1).

Protocol B (alteration protocol): Protocol B is described separately in a dedicated protocol outside this document. It describes a procedure for participation in the APS Consortium study with alteration of informed consent. Protocol B will be used for participants for whom informed consent for the study participation cannot be obtained via the patient or LAR/surrogate prior to initiation of study procedures. Minimal risk procedures within the APS study may be completed using alteration of informed consent. Protocol B outlines the minimal risk procedures that may be completed without informed consent for research. Protocol B has no informed consent document but includes a patient information sheet to help notify patients and others about enrollment in the study.

Participants with informed consent will complete study procedures described in Protocol A (this document), and participants who have not provided informed consent will complete study procedures described in Protocol B (a separate document). Participants without informed consent for study participation will complete a subset of study procedures (described in Protocol B) completed by participants with informed consent for research (described in Protocol A). Participants who enter the study on the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A (Figure 2).

Greater than minimal risk procedures may be completed after informed consent is obtained among participants who started study participation under alteration of informed consent procedures. Patients who move from Protocol B to Protocol A will seamlessly continue on the APS Study schedule of events and maintain the same Study Day structure initiated at study entry. Study days continue through the transition from Protocol B to Protocol A; that is, if a patient provides informed consent on Day 4, study procedures completed on Protocol B for Day 0 through Day 3 will remain unchanged and Study Day 4 procedures will pick up on Protocol A.

For participants who enter the study with alteration of informed consent, study team members will attempt to obtain informed consent while the patient is in the hospital through Study Day 14 and at the 3-month time point.

Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

13.2.5 Withdrawal of informed consent

If a participant or LAR/surrogate indicates an intention to withdraw informed consent for study participation or forego future study procedures, the study team will clarify the wishes of the

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participant/LAR/surrogate and categorize the request into one of the following categories. Steps to honor wishes of the participant/LAR/surrogate are outlined for each category.

(i) Category 1 Request: Complete withdrawal of consent, including destruction of identifiable data and biospecimens and no participation in any future study procedures.

Action: The study team will note in the study data capture instrument that the participant or LAR/surrogate withdrew consent. This is termed, "study withdrawal." An attempt will be made to understand and document why the participant withdrew consent. No additional study procedures with the participant will be performed. Data and biospecimens that have not already been used will be destroyed, as possible. Identifiable participant data will be removed from the data capture instrument. Data and biospecimens that have already been used to generate results will not be retracted. Deidentified data and biospecimens that have been transferred to NIH repositories or to laboratories for analysis may not be able to be destroyed. Completed works, such as manuscripts, that used the participant's data and/or biospecimens will not be revised or retracted and data to support those works will be retained.

(ii) Category 2 Request: Previously collected data and biospecimens may be retained but desire expressed to not participate in future direct-contact study procedures.

Action: The study team will note in the study data capture instrument that the participant or LAR/surrogate maintains consent for the study but requested no additional direct contact study procedures. This is termed, "study discontinuation." The study team will attempt to understand and document why the participant requested to stop additional direct-contact study procedures. The study team will not perform any additional procedures that involve direct contact between study personnel and the participant, including interviews, biospecimen collection, long-term outcome surveys, and in-person long-term outcome visits. The participant or LAR/surrogate can choose to continue with study personnel accessing medical records for data capture in the future or not. All collected data and biospecimens will be used.

(iii) Category 3 Request: No participation in some specific study procedures but willingness to participate in other study procedures.

Action: The study team will work with the participant/LAR/surrogate to understand what specific procedures the participant/LAR/surrogate wants to avoid in the future. The study team will note in the data capture instrument which study procedures will not be completed and why the participant/LAR/surrogate requested to opt out of those procedures. The study team will avoid the specific study procedures as requested, such as future blood draws, future swabs, future interviews/surveys, or future in-person visits. All collected data and biospecimens will be used.

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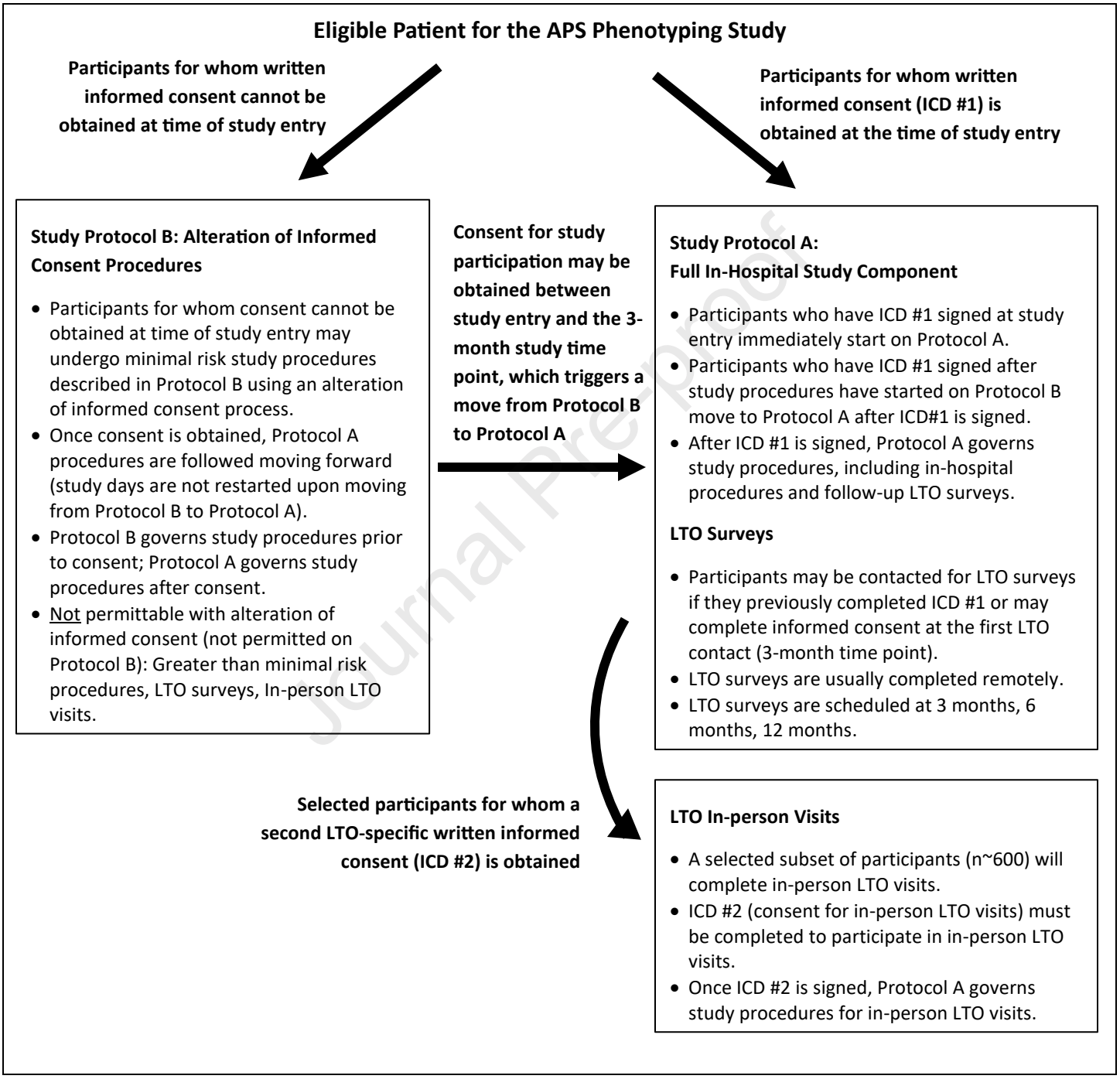
Figure 2. Flow diagram of informed consent procedures.



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13.3 Human subjects considerations for data and biospecimen banking and sharing

13.3.1 Disclosure of research results to participants

Results for CT scans completed at the 12-month visit for research purposes will be disclosed to participants via site investigators. The investigators do not intend to disclose the results of other research testing to participants.

13.3.2 Storage of data and biospecimens

Data collected as part of the Consortium-wide study, including those captured from the EHR and other hospital databases, will be transferred into the study database via standardized electronic case report forms (eCRFs), which will reside in a centralized database located on secure servers. Study data will be entered and accessed via a secure, password-protected REDCap database website wherein all web-based information is encrypted. REDCap was developed specifically around HIPAA Security guidelines and is recommended by both the Vanderbilt University Privacy Office and Institutional Review Board. REDCap is available to all sites participating in the APS Consortium.

Data transferred to the APS Coordinating Center will include dates (for example, hospital admission date, and date of birth) and contact information to facilitate post-hospital follow-up visits. Biospecimens will be labeled with a study identification number without patient name, medical record number, or date of birth. Access to personal health information in study databases will be limited to only those individuals requiring that level of access.

Study data and biospecimens will be stored for an indefinite period of time for future use. Deidentified data and biospecimens will be shared with researchers outside the APS Consortium. For long-term storage of data, personal identifiers will be removed once quality assurance has been confirmed and prior to data lock. All research records will be accessible for inspection by authorized representatives of the IRB, federal regulatory agency representatives, and NIH representatives.

13.3.3 Sharing data and biospecimens

The APS Consortium will develop data management and sharing plans consistent with NIH policies (https://sharing.nih.gov/).

APS Consortium investigators will be permitted to access and use data and biospecimens for the purpose of achieving the Consortium-wide and center-specific project aims directly from the Consortium Coordinating Center. Consortium-wide biospecimens and data will be sent to the central biorepository and study database housed at the Consortium Coordinating Center. Investigators seeking to perform approved ancillary studies with data and/or biospecimens collected by the APS Consortium may request data and biospecimens from the Consortium Coordinating Center before data and biospecimens are deposited in BioData Catalyst and BioLINCC.

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Ultimately, the Consortium Coordinating Center will deposit de-identified data and biospecimens in BioData Catalyst and BioLINCC. Once data and biospecimens reach BioData Catalyst and BioLINCC, they will be available to investigators through the governance of BioData Catalyst and BioLINCC without involvement of the Consortium Coordinating Center.

13.3.4 Co-enrollment with other studies

The APS Consortium steering committee, OSMB, and NHLBI will agree on co-enrollment procedures before participants in the APS study are co-enrolled with other studies. Principles for co-enrollment will include the following:

- Co-enrollment should not affect the scientific goals of the APS Consortium. Co-enrollment will not be permitted if it compromises the scientific integrity and/or statistical power of APS Consortium studies.
- Co-enrollment will be compliant with NIH and NHLBI guidelines and policies.
- Co-enrollment will only be allowed when study procedures for the APS Consortium studies can be achieved. Co-enrollment in the APS Consortium will not be permitted when study procedures in a co-enrolled study would prevent completion of data or biospecimen collection for the APS Consortium study.
- Safe blood collection procedures for critically ill patients will be followed as detailed by the PETAL Network Investigators.[28]

14. Adverse Events and Safety Monitoring

14.1 Overview of Safety Monitoring

In this observational study that uses routine techniques for data and biospecimen collection, substantial numbers of serious, study-related adverse events are not anticipated. The study will not be overseen by the US Food and Drug Administration (FDA). Safety monitoring will be performed by the investigators, the study's single IRB at Vanderbilt University Medical Center, and an NHLBI-appointed Observational Safety Monitoring Board (OSMB).

14.2 Adverse Events

14.2.1 Paradigm for collecting Adverse Events

In this study, investigators will collect and report adverse events (AEs) that are classified as serious and related to study procedures, those prespecified as adverse events of special interest (AESIs), and those that potentially change the risk: benefit balance for patient participation (Unanticipated Problem (UP)). AEs that meet at least one of the following three criteria will be collected in this study:

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1) both serious and study related;
2) AESI;
3) UP.

AEs that do not meet any of these criteria will not be collected.

Events that meet criteria for an AE in the population enrolled in this study will be numerous due to the severe medical conditions these patients have. The proportion of AEs experienced by the study population that are related to study procedures is anticipated to be extremely small. Thus, the paradigm of AE collection and reporting in this study was designed to capture all the events that could potentially represent a safety concern for the study while avoiding overly burdensome AE monitoring for participants, study teams, the IRB, and OSMB.

14.2.2 Definitions for Adverse Events

(i) Adverse Event

An AE is defined as any untoward medical occurrence.

(ii) Serious Adverse Event (SAE)

A serious AE (SAE) is an untoward medical occurrence that directly causes at least one of the following in the judgement of the study team:

- Death
- Life-threatening condition that places the participant at immediate risk of death
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or a congenital anomaly/birth defect.
- An important medical event not meeting the criteria for one of the outcomes above but, based on medical judgment, jeopardized participant safety or required medical or surgical intervention to prevent one of the outcomes listed in this definition.

(iii) Relatedness of SAEs

SAEs will be evaluated for relatedness to study procedures using the definitions below:

- Definitely Related: The adverse event meets all three of the following criteria: (a) a temporal sequence from study procedure to the adverse event suggests relatedness, (b) the event cannot be explained by the known characteristics of the participant's clinical state or therapies, and (c) evaluation of the participant's clinical state indicates to the study team the experience is definitely related to study procedures.

- Possibly Related: In the study team's opinion, the adverse event has a reasonable possibility of being related to study procedures but one or more of the above criteria for "Definitely Related" are not met.

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- Probably Not Related: The adverse event occurred at a time when it could have been caused by study procedures but, in the opinion of the study team, can reasonably be explained by the known characteristics of the participant's clinical state or therapies.

- Definitely Not Related: The adverse event was definitely produced by the participant's clinical state or therapies and not by the study procedures.

- Uncertain Relationship: The adverse event does not meet any of the criteria outlined above and the study team cannot ascertain enough information to classify relatedness of the event.

For the purposes of this study, an adverse event is considered related to study procedures if there is a "reasonable possibility" of a causal relationship between a study procedure and the adverse event or the relationship cannot be determined; this includes events that are classified as definitely related, possibly related, or of uncertain relationship.

(iv) Adverse events of special interest (AESIs)

An adverse event of special interest (AESI) is defined as a pre-specified event of scientific or medical concern that has the potential of being related to study procedures and is important to understand regardless of investigator classifications. In this trial, AESIs listed below will be recorded as an AE and will require a written narrative regardless of a site's study team classification of seriousness and relatedness. For this study, the following events are AESIs:

- **AESI #1**: New or worsening hypoxemia within 30 minutes of a study-specified NBBAL. For AESI consideration, new or worsening hypoxemia is defined by the event meeting both of the following criteria: (i) a decrease in SpO2 by at least 10 percentage points in the time window between the beginning of the procedure and 30 minutes after the procedure is completed; and (ii) a persistent absolute increase in FiO2 by at least 10% for at least 6 hours after the procedure compared to before the procedure.

(v) Unanticipated Problem (UP)

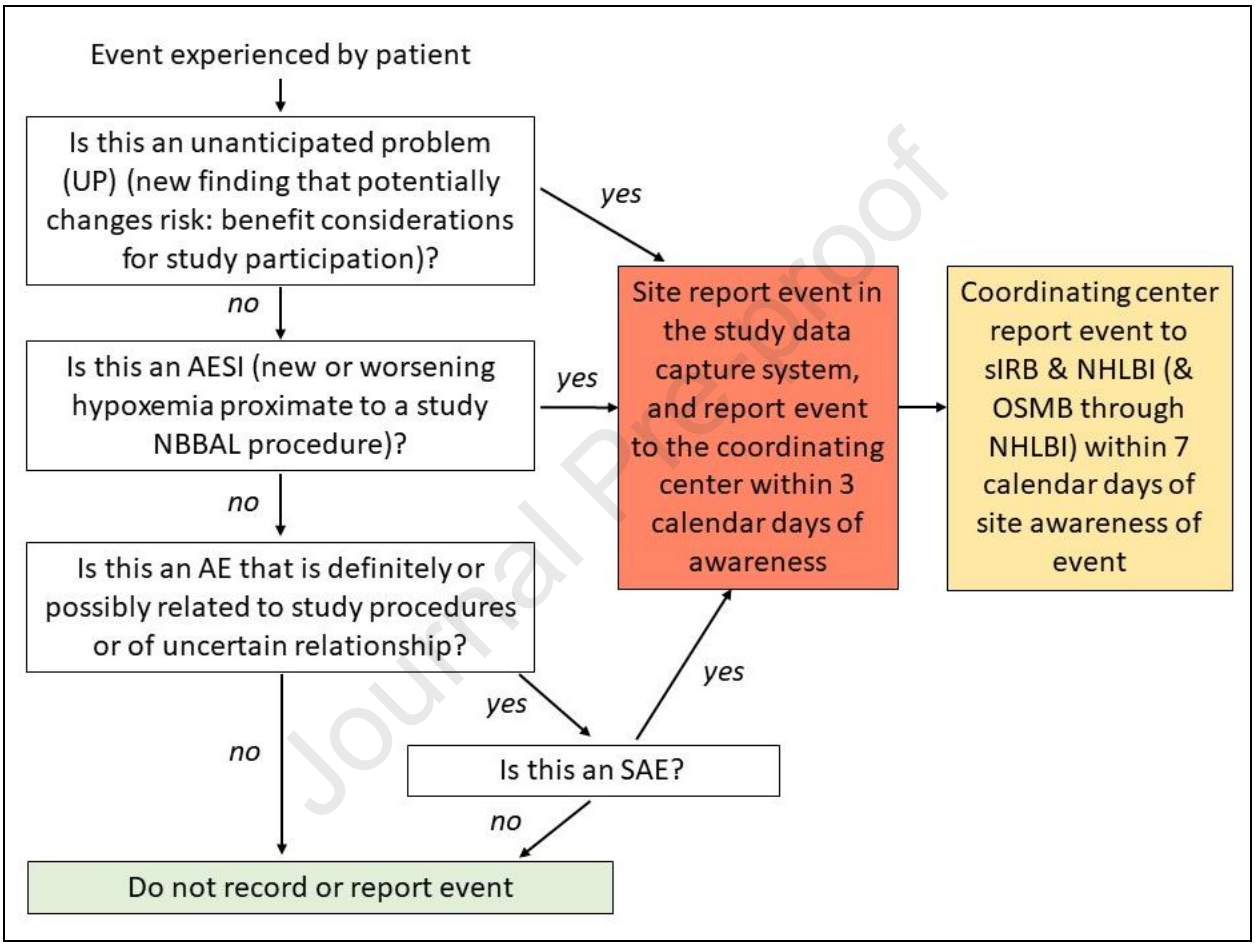
An unanticipated problem is a finding discovered during the conduct of the study that suggests participation in the study may have more risk than was anticipated at the time of study initiation. UPs have the potential to change the risk: benefit balance of the study compared to what was known at the time of study launch.

14.2.3 Reporting Adverse Events

AEs that meet criteria for reporting should be entered into the system's electronic data collection system within 3 calendar days of the study team becoming aware of the AE. Reporting an AE will include a clinical narrative explaining the context of the AE and rationale for the investigator's classification of the event as serious and related, an AESI, or an UP. The study team should also alert the Consortium Coordinating Center for reported events. The coordinating center will report the AE to the sIRB and NHLBI within 4 calendar days of receiving the report of an AE (thus, within 7 days of site awareness of the event). The OSMB will be alerted about AEs from NHLBI. Reported events will be followed until

resolution. Figure 3 is a summary flow diagram to assist study teams with deciding which adverse events to report in this study.

Figure 3. Flow diagram to assist with decisions about AE recording and reporting.



14.3 Observational Study Monitoring Board (OSMB)

The OSMB will be comprised of experts in APS syndromes and fields relevant for this study. The OSMB is appointed by NHLBI. The principal role of the OSMB is to evaluate the safety and integrity of the study. Full details of the OSMB structure and function will be provided in an OSMB charter, which will be reviewed with the OSBM at its first meeting with the study team.

Prior to initiation of study enrollment, the OSMB will review the study protocol and informed consent documents. OSMB meetings will be scheduled regularly in accordance with the OSMB charter. Additionally, the NIH, OSMB, and investigators may call ad hoc OSMB meetings.

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The OSMB will regularly monitor several aspects of the study, including the safety, enrollment rates, protocol compliance, cohort demographics and geographic distribution, and data quality and completeness.

Recommendations to end, modify, or continue aspects of the study will be communicated by the OSMB, through the OSMB executive secretary, to the Consortium Coordinating Center.

14.4 Institutional Review Boards (IRBs)

This is a multi-center cohort study for which a single IRB will be used for the ethical review of the proposed research per NIH policy (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-094.html). Vanderbilt University Medical Center will serve as the single IRB of record. Local context will be reviewed by local IRBs for each participating site.

This study utilizes a 2-part consent document. Part 1 of the informed consent document is the master consent document and contains information that applies to all study sites. Part 2 of the informed consent document is a site-specific document which outlines local, site-specific information.

Processes of obtaining informed consent should be reviewed during local context review to ensure study procedures are consistent with local laws and standards.

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16. Protocol Appendices

16.1 Appendix A (Aim 1): Determining and interrogating the role of microbiota (pathogen and microbiome) in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis, and their long-term outcomes.

16.1.1 Aim 1 Background and Rationale

Background: Efforts to date to understand clinical heterogeneity in ARDS, pneumonia, and sepsis have focused exclusively on variation in host-derived features.[1-5] These studies have overlooked two crucially important *microbial* sources of variation among critically ill patients: the *pathogen* and the *microbiome*.

Pathogens: All pneumonia and sepsis, and most ARDS, is initially provoked by infection with a pathogen: bacterial, fungal, or viral. Pathogens differ profoundly in their mechanisms of virulence and their effects on the host response and are frequently not identified using existing clinical microbiology practices[6,7]. Cutting-edge molecular techniques (metagenomics and metatranscriptomics) hold tremendous potential to (1) identify previously undetected pathogens and (2) advance our understanding of the pathogen's role in the biological and clinical heterogeneity of these syndromes.

Microbiome: The microbiome – the communities of microbiota living on and in the human body – represents a major source of biological variation that propels heterogeneity in diverse diseases and therapeutic responses (e.g., cancer[8,9] and cardiovascular disease[10,11]). Critically ill patients exhibit tremendous variation in the *density*, *diversity*, *composition*, and *function* of communities within the gut and lungs.[9,12-17] Via its production of systemically active metabolites, the gut microbiome is a major determinant of the serum metabolome in health and disease,[18,19] and gut microbiota play a crucial role in the maturation and calibration of systemic and alveolar immunity.[20-24]

Rationale: The pathogen and the microbiome are important yet incompletely understood sources of biologic heterogeneity in APS conditions, and both represent a highly promising treatment target. Yet we lack an actionable understanding of their role in ARDS, pneumonia, and sepsis. By determining and interrogating the role of the pathogen and microbiome in the *development*, *trajectory*, and *recovery* of these conditions, this Aim will both 1) facilitate the development of microbially-targeted therapeutics and 2) enhance our understanding of the role of varying pathogens and microbial environments on the variability in host response observed in critically ill patients with ARDS, pneumonia and sepsis.

16.1.2 Aim 1 Study Objectives

Primary Objective (Aim 1):

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Establish and understand the relationship between patients' microbiota (pathogen and microbiome) and their long-term physical, cognitive, and mental health outcomes. Specifically, we will evaluate the associations between:

Predictor 1: metagenomics- and clinically-identified etiologic pathogen
Predictor 2: final in-hospital gut microbiome diversity

and 3 to 6-month measurements of:

Outcome 1: Short Physical Performance Battery Protocol (*physical performance*)
Outcome 2: CNS-Vital Signs assessment (*neurocognitive performance*)
Outcome 3: Handgrip strength (*musculoskeletal function*)

These analyses for the primary objective will be adjusted for baseline comorbidity and health status.

Exploratory Objectives (Aim 1):

1. Determine how patient-associated microbiota (pathogen and microbiome) contribute to the heterogeneity of previously defined phenotypes of ARDS, pneumonia, and sepsis.
2. Determine how patient-associated microbiota (pathogen and microbiome) inform the heterogeneity of ARDS, sepsis, and pneumonia using unsupervised analyses (development of novel phenotypes).
3. Evaluate the extent to which recovery of microbiome at timepoint 1 (3 to 6 months) (vs. final in-hospital time point) is associated with physical, cognitive and mental health outcomes, adjusting for baseline comorbidity and health status.

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16.1.3 Aim 1 Study Population
The study population will be the complete cohort of approximately 4000 hospitalized patients as described in the study protocol.

16.1.4 Aim 1 Study Design
Prospective observational cohort study.

16.1.5 Aim 1 Study Procedures
We will characterize the *pathogen* (and its corresponding host response) using metagenomic and metatranscriptomic next-generation sequencing using previously described methods.[25,26] We will characterize the *microbiome* using the following approach: we will assess *bacterial density* in rectal swabs and oral and nasal swabs using droplet digital PCR (Bio-Rad) using universal 16S primers. *Bacterial communities* of all specimens will be characterized using 16S rRNA amplicon sequencing (Illumina MiSeq).

Additionally, plasma protein biomarkers needed to identify the previously defined LCA-derived "hyperinflammatory" and "hypoinflammatory" phenotypes will be measured in all 4000 patients, using

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previously described multiplex assays. The "hyperinflammatory" and "hypoinflammatory" phenotypes will then be identified using previously published parsimonious biomarker models.[27] As a secondary analysis, latent class models will be fit to the entire cohort using previously described approaches[28] to confirm concordance with the parsimonious classifier model and test for variance according to clinical syndrome (i.e., ARDS, pneumonia, sepsis).

16.1.6 Aim 1 Data, Images, and Biospecimens Used from the APS Consortium

The primary measurements performed in this Aim will be made using: plasma from all patients (day 0: mNGS), endotracheal aspirates from all mechanically ventilated patients (day of intubation: mNGS), rectal swabs (day 0, 2, 6; 3 to 6-month, 12-month: 16S rRNA gene amplicon sequencing), and oral and nasal swabs from all mechanically ventilated patients (day of intubation: 16S rRNA gene amplicon sequencing). As indicated in Sub-Aims, these measurements will be compared with plasma biomarker-adjudicated phenotypes as well as clinical and functional outcomes.

16.1.7 Aim 1 Pre-processing and Statistical Analysis

Methods for analysis to be used in this Aim continue to be developed at a rapid pace, and the approach should be optimized based on knowledge at the time of analysis. To that end, the SAP will be fixed prior to analyzing the digital data. The general approach to rigor and reproducibility described in the protocol will be followed, with the aim-specific details incorporated into the SAP. To provide an example of the kinds of analyses we may pursue for this aim, we outline one possible approach here.

To analyze the pathogen data, samples will be processed using the open-source CZ-ID pipeline,[25] which performs reference-based alignment at both the nucleotide and amino acid level against sequences in the National Center for Biotechnology Information (NCBI) NT and NR databases, respectively, followed by assembly of reads matching each detected taxon. The resulting matrix of taxon counts in the patient samples and water controls will be used for downstream analyses, as detailed below.[25] We will first fit a negative binomial background model of taxon-specific counts, normalized to the total number of ERCC spike-in reverse-transcribed cDNA counts in the water control samples. We will use this model to filter out taxa whose abundance does not significantly exceed the background model. Background-corrected taxonomic count matrices will be used for downstream analyses. From here, analyses will diverge for plasma and respiratory samples. In plasma, a generally sterile compartment, we expect to identify only a few taxa above background, even in cases of bacteremia. We will employ a previously developed rules-based model[25] that ranks species within each sample by abundance and identifies those above the maximum drop-off in abundance, i.e. the dominant taxa. These taxa will then be intersected with a literature-curated list of established causal agents of bacteremia, resulting in a per-sample determination of mNGS-identified bacteremia and likely pathogen.

We will use the R package metagenomeSeq to perform differential abundance testing at the individual genus level. We will identify probable pathogens using a validated rules-based model[25] that identifies respiratory viruses and established bacterial and fungal respiratory pathogens present at disproportionate abundance. We will then compare the distributions of detected pathogens between the phenotypes, as

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described for plasma. For both plasma and respiratory sample data, metagenomics-identified pathogens will be compared with clinical microbiologic data and identified clinical pathogens.

For microbiome analysis, pre-processing steps will use *mothur*, *R*, and *vegan*, or comparable packages. Informed by published studies and preliminary data, for each specimen, we will derive key community features for analysis, such as: 1) bacterial density (quantified via ddPCR); 2) bacterial diversity (calculated using the Shannon Diversity index); and 3) community composition (using PERMANOVA analysis of beta-diversity and relative abundance of key bacterial taxa).

Comparative analyses for Aim 1a will compare features between previously derived LCA-defined phenotypes to test for enrichment of bacteremia, specific pathogens, or categories of pathogens (e.g., gram-positive or gram-negative bacteria) in a particular phenotype. In respiratory samples, we expect a more complex microbial community, including both airway commensals and potential pathogens so phenotypes may be compared using more global assessments of the microbiome, such as Bray-Curtis dissimilarity at the genus level as well as the Shannon diversity index.

For Aim 1b, we will incorporate data derived from whole blood host gene expression (Aim 2) in addition to the data described in this Aim. We will apply unsupervised analysis to the high-dimensional data (including whole-blood host gene expression, ETA host gene expression, ETA and plasma microbial composition) to characterize and interpret biological variation in the cohort. For host gene expression, we may perform principal component analysis (PCA) of gene counts following variance stabilizing transformation and selection of the top 1000 (or top 5000) most variable genes. Principal components representing >5% of total variance then enter the permutation-based jackstraw method to assign statistical significance to the association of individual genes with each principal component (or combinations thereof). The resulting gene loadings and p-values can then serve as input to functional/pathway enrichment methods, such as gene set enrichment analysis and IPA, in a manner analogous to traditional differential expression analysis, to facilitate biological interpretation. Canonical correlation analysis can then be used to characterize co-variation of biological features between the assayed compartments, namely blood and the lower airway.

To harness all the high-dimensional data types for generating novel phenotypes, one approach we may use is to apply variance stabilizing transformation to count-based features, or log transformation for other continuous measurements, such as abundance of pathogens detected in mNGS. Then, reduced representations of high-dimensional data can be generated using linear combinations of the most variable original features derived from PCA. Principal components representing >5% of total data type variance might then be included in the reduced representation. Reduced datasets are then concatenated and can be clustered using the K-means algorithm. The optimal number of clusters can be determined using metrics of clustering quality, such as within cluster sums of squares, silhouette score, and gap statistics.

Once novel clusters have been established, we can compare the new clusters and LCA classes based on the maximum overlap and assess the similarity between novel cluster labels and LCA labels using the adjusted Rand index. The prognostic value of the new cluster labels and LCA labels can then be assessed for various outcomes, including mortality. Of particular interest when comparing methods is samples

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allocated to different classes by different methods. Data types and features driving class discordance can be highly informative. Once novel clusters are established and validated, associations of clusters with various pathogen and microbiome features will be assessed similarly to Aim 1a.

16.1.8 Aim 1 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

Given (1) the pathophysiological centrality of the pathogen and microbiome in APS conditions, (2) the inadequacy of current clinical practices to identify both pathogenic and non-pathogenic host-associated microbiota, and (3) the dynamic nature of the microbiome in response to critical illness and ICU exposures (e.g., antibiotics), we plan on generating microbe-related measurements at the following timepoints:

- plasma mNGS on 1,500 APS participants at day 0
- ETA mNGS on all mechanically ventilated APS participants on the day of intubation
- rectal swab microbiota on all APS participants with rectal swabs collected at day 0, 2, 6 and the timepoint 1 and timepoint 2 in-person long-term outcome visits
- oral and nasal swab microbiota on 300 mechanically ventilated APS participants on the day of intubation

Based on preliminary and published data, these measurements will (1) meaningfully increase our ability to identify etiological pathogens in most APS patients, (2) provide rich and dynamic characterization of changes in lower gastrointestinal microbiota across our cohort both during hospitalization and in the months following discharge, and (3) provide sufficient time-matched measurements to compare pharyngeal microbiota with lower respiratory tract microbiota and determine if noninvasive respiratory sampling can improve our ability to predict and prevent ventilator-associated pneumonia.

Specifically, for Aim 1a (determine how patient-associated microbiota contribute to the heterogeneity of previously derived LCA-defined phenotypes of ARDS, sepsis, and pneumonia), we anticipate that roughly 25-30% of the cohort will be in the “hyper-inflammatory” phenotype and 70-75% will be in the “hypo-inflammatory” phenotype. With a total sample size of 1500 participants, we should therefore have approximately 450 patients in the hyperinflammatory phenotype, with 50-60% of those being mechanically ventilated.(Sinha LRM 2023) In preliminary analyses of blood and plasma mNGS data from 156 sepsis patients, we identified 44% higher prevalence of mNGS-defined bacteremia in hyper-inflammatory compared to hypo-inflammatory patients (74% vs 30%; $p<0.001$), reflecting the large magnitude of effect sizes that might be observed. As noted in the rationale for cohort sample size, for this ratio of group sizes, a sample size of just 400, and a modest effect (odds ratio of 0.5), the half width of the confidence interval is about 0.25. If the group size is 1500, the half width of the odds ratio is 0.13, assuming a 50% effect rate. Analyses involving all 4,000 persons would result in a half width of 0.08, demonstrating an ability to estimate even small effects with precision. This suggests that the sample is

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sufficient to detect meaningful differences in pathogens and microbiota between hyper and hypo classes both within and across the two LCA-defined phenotypes.

Aim 1b (Determine how patient-associated microbiota inform the heterogeneity of ARDS, sepsis, and pneumonia using unsupervised analyses) will follow the approaches to cluster analysis as laid out in the main protocol. We anticipate that at least 500 features can be evaluated for the purposes of unsupervised (or supervised) analyses. The number of features can increase with employment of data reduction techniques.

16.1.9 Aim 1 Expected Output

Via these measurement, analyses, and integration with host-directed measurements acquired on the same 4,000 patients, we anticipate the following outputs:

- Discovery of specific microbe-host interactions (pathogen-host, gut-systemic, respiratory-respiratory) that contribute to immune calibration and clinical heterogeneity, confirming the significance of the microbiome as an "upstream" driver of dysregulated immunity and providing specific targets for coordinated microbe/host modulation.
- Earlier and more informative recognition of pathogens, improving our ability to provide tailored antimicrobial treatment with fewer off-target effects.
- Identification and interrogation of the gut as a reservoir of secondary pathogens, facilitating development of real-time tools for characterizing the microbiome, improving our ability to provide selective, narrow gut and pharyngeal decontamination.
- We anticipate that the hyper-inflammatory phenotype will be characterized by increased expression of IFN-stimulated genes and activation of innate immune and integrated stress responses in the lower respiratory tract, and increased expression of genes related to neutrophil function/degranulation in the blood, in comparison to the hypo-inflammatory phenotype (Aim 1a). We also anticipate that the pathogens and microbiota characterizing each phenotype will differ (Aim 1a). These results would have important implications for identification of future targeted therapies for testing in each phenotype.
- We anticipate that unsupervised analysis of mNGS data will identify novel phenotypes that partially overlap but will not be fully concordant with prevously derived LCA-defined phenotypes (Aim 1b). We anticipate that these analyses will identify host response patterns associated with particular pathogens or pathogen types, indicating the importance of incorporation of pathogen data into critical illness phenotypes.

16.1.10 Aim 1 References

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16.2 Appendix B (Aim 2): Determine host response phenotypes that most strongly associate with organ failure and 3, 6 and 12-month health status, and test the incremental utility of markers of vascular dysfunction, plasma DAMPs, and leukocyte expression for APS short- and long-term outcomes.

16.2.1 Aim 2 Background and Rationale

APS syndromes overlap physiologically and display similar sequelae, yet pathways from mechanism to outcomes are unclear. Dysregulation in both vascular and damage-associated molecular pattern (DAMP) signaling may explain pharmacologically targetable clinical heterogeneity in APS critical illness. Vascular injury is a central pathogenic mechanism in APS that may explain progression from limited pneumonia to multi-organ involvement. Dysfunctional endothelium is leaky, attracts leukocytes and platelets, forms neutrophil-endothelial traps (NETs), and sheds glycocalyx.[1–3] Rarely, vessel injury manifests as overt or threatened disseminated intravascular coagulopathy with fibrinolysis.[4] However, each of these pathologies requires unique treatment strategies, so deconvoluting vascular injury using proven markers has value. A more precise recognition of which features of endothelial dysfunction drive organ injury and persistent health impairments will clarify the best therapeutic strategy for APS. Each marker (ANGPT2, sTM, sICAM, Syndecan-1) is selected based on our preliminary data in our large sepsis population and in published literature.[1,2,5]

Dysregulated damage-associated molecular pattern (DAMP) signaling is another central mechanism in APS that may explain heterogeneity in pauci- versus multi-organ failure.[6,7] The DAMP axis may interact with vascular injury and host inflammation, and drugs to neutralize specific features of DAMP signaling (including markers such as cell free nucleic acids, alarmins, and soluble RAGE), are increasingly available. Thus, understanding the contribution of aberrant or excessive DAMP signaling, and which specific features are most prognostic for specific organ failure, APS state, and clinical outcomes, is important.

Survivors of APS experience varied impairments in their physical, psychological, cognitive, and socioeconomic health that persist for months to years following discharge.[8–10] These multiple impairments are often collectively termed post-intensive care syndrome (PICS)[11,12] and experts recommend serial sequential assessments post-discharge for its diagnosis.[13] Improved prediction of impairments might allow testing of targeted interventions yet our ability to predict these impairments is limited. No externally validated models exist. Better predictive tools are needed for long term outcomes, and simple ADL metrics may have value both for predicting these impairments and should be accounted for in analyses testing associations with new markers. Furthermore, the links between acute biologic events and post-ICU health are poorly understood. A longitudinal cohort with repeated batteries and molecular sampling is needed to understand the relationships between host premorbid status, acute illness, and long-term impairments. We hypothesize that integrating both pre-ICU health and molecular features of illness response will result in more accurate prediction of long-term health. If we can validate a predictive tool for specific health states, then we could aid patients and families in decision-making during acute illness, better anticipate the workforce needed for long-term recovery, and perform more efficient trials of interventions to improve post-APS outcomes via prognostic enrichment. Specific markers for excessive

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vascular injury, DAMP signaling, circulating markers for sarcopenia (GDF-15), and markers for neuronal injury (neurofilament light chain, tau protein) will be tested as candidate markers to predict long term health status. Post-APS health will be assessed using patient-reported long term outcome recovery questions and WHODAS, EQ-5D utility score and clinical frailty score, adjusted for pre-APS comorbidity and retrospective baseline status, at 3, 6, and 12-month follow-up.

16.2.2 Aim 2 Study Objectives

Primary Objective (Aim 2):

To utilize day 0 plasma measurements of vascular injury (permeability, vascular activation, coagulopathy/fibrinolysis, and glycocalyx degradation), altered damaged associated molecular pattern (DAMP) signaling, and indicators of sarcopenia and/or neuronal injury to test association with 60-day mortality (primary objective), organ failure free days (secondary objective), and individual organ-specific outcomes (ventilator free days, vasopressor-free days, dialysis-free days; secondary).

Exploratory Objectives (Aim 2):

1. To determine reproducible multi-marker plasma phenotypes of immune activation, vascular injury, DAMP activation, and sarcopenia/neuronal injury and test for association with 60-day survival (primary), organ failure free days (secondary), and individual organ-specific outcomes (VFD, vasopressor-free days, dialysis-free days)
2. To test whether the association between multi-marker plasma phenotypes and 60-day mortality is modified by classically defined syndromes (ARDS, pneumonia, and sepsis).
3. To test whether day 0 plasma measurements of vascular injury (permeability, vascular activation, coagulopathy/fibrinolysis, and glycocalyx degradation), altered damaged associated molecular pattern (DAMP) signaling, and indicators of sarcopenia and/or neuronal injury associate with for patient-reported health states (cognitive, emotional, functional, or respiratory) at 3, 6, and 12 months.
4. To test whether multi-marker plasma phenotypes early during the APS illness have predictive utility for patient-reported health states at 3, 6, and 12 months post-APS, specifically in cognitive, emotional, physical function, and respiratory domains.
5. To test whether a change in multi-marker plasma phenotypes between acute illness and 90 days associates with improved patient-reported health states at 3, 6, and 12 months.

16.2.3 Aim 2 Study Population

All participants enrolled in the APS Consortium will be eligible to participate in this aim. Our analytic plan, as detailed below, calls for a sample size of 1500 individuals at day 0 to test associations of both individual markers and patterns of co-expression of multiple plasma analytes while retaining adequate power to test for effect modification by APS state (ARDS, pneumonia, and sepsis). The 1500 subset for this profiling will be drawn from the first 2200 participants enrolled to allow for balance of demographic features, geographic areas, and APS states.

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16.2.4 Aim 2 Study Design

Prospective observational cohort study with scheduled blood sampling, and longitudinal follow-up at 3, 6, and 12 months after critical illness.

16.2.5 Aim 2 Study Procedures

We will quantify the following proteins in plasma: Syndecan-1, angiopoietin-2, soluble ICAM, soluble thrombomodulin, growth differentiation factor 15, soluble RAGE, S100A12, neurofilament light chain, and tau protein using multiplex electrochemiluminescence, conventional enzyme-linked immunosorbent assay (ELISA), or other automated multiplex immunoassay platforms (Simoa and/or ELLA). We will also quantify cell free DNA and mitochrondrial DNA in plasma using Qiagen DNeasy miniprep kits and then quantitative polymerase chain reaction (qPCR) to quantify ND-1 and COX-IV genes to represent mtDNA and nuclear DNA, respectively.

16.2.6 Aim 2 Data, Images, and Biospecimens Used from the APS Consortium

Clinical data including syndromic phenotype (ARDS, pneumonia, sepsis), presence/type of infection, risk factors for ARDS, demographic information, comorbidities, severity of illness, physiologic data, and outcomes (survival, duration of organ support) will be utilized from each participant's inpatient admission. Long term outcomes including measures of functional status, quality of life, emotional health, cognitive performance, and respiratory symptoms at 3, 6, and 12 months will also be tested outcomes, and will be adjusted for metrics of pre-illness health. Images from the inpatient stay or the longitudinal 12-month visit will be utilized, and from longitudinal muscle ultrasound, will be used.

Biological samples will include the plasma drawn at 0, 2, and 6 days, as well as 90 days. Depending on findings, additional timepoints including d180 and d360 may be assayed for leading candidate markers. In addition, whole blood gene expression from the PAXgene RNA-stabilizing tubes will be utilized for the clustering aim.

16.2.7 Aim 2 Statistical Analysis

It is recognized that methods for analysis continue to be developed. A detailed SAP will be generated before analysis begins, in which we will specify the method considered optimal at the time or, if an optimal method cannot be easily determined, competing methods will be compared. The following outlines a general approach that might be considered for this aim.

Unadjusted tests for association between individual markers and outcomes (mortality, organ failure, or post-ICU health state) might be conducted as a t-test or Wilcoxon rank sum test as appropriate to the data distribution. Marker concentrations can be transformed for normality as needed and then used in multivariable regression analysis controlling for factors that might act as confounders (including but not exclusive to age, sex, pulmonary versus non-pulmonary source of infection, presence of sepsis, and risk factors or comorbidities). A directed acyclic graph (i.e., DAG) will be derived by the study team to guide the analysis.

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Longitudinal data might use a 'joint longitudinal and time-to-event' model, with a longitudinal model appropriate for the outcome distribution (e.g., logistic, linear, negative binomial), and a competing risk accelerated failure time model for dropout (e.g., death, moribund, lost). The shared parameter specification can be selected for each outcome based on model fit.

Multiple methods can be enacted for clustering, and consistency across methodologies will be assessed. For example, we may employ agglomerative hierarchical clustering, Wald's clustering, and uniform manifold approximation and projection (UMAP), and other potentially informative clustering approaches to visually inspect data.[18–20] Within the UMAP space, we can visualize the overall gradient, or contribution, of specific biologic features using a feature-weighted kernel density weighting as we have previously published.[18] Latent class modeling can also be accomplished,[16] and the number of classes tested by Vuong-Lo-Mendell-Rubin test. Longitudinal data can be incorporated into longitudinal clustering by growth mixture modeling or a 2-step approach with growth curve modeling and K-means.[21] Once we have established discernable and reproducible clusters, we can then use regression modeling to test the association between cluster and organ failure, mortality, and disability scores (CNS Vital Signs, WHODAS II, and HADS).

As discussed in the protocol, the SAP will be written and filed centrally a priori. If there are competing, plausible approaches, theses will be compared. Adjustments to pre-specified plan will be documented with rationale. The SAP will follow the principles outlined in the protocol.

16.2.8 Aim 2 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

With continuous traits such as plasma protein or nucleic acid quantification, we will have ample power to test for associations with mortality (estimated 30%), specific organ failure (estimated 10 – 40%), or specific post-ICU health impairment (estimated 30%) with the targeted deep phenotyping sample size of 1000-1500 participants at inpatient timepoints and approximately 600 participants at day 90 (3 months). This sample size will also provide adequate power to test for statistical interaction with APS state or other clinical or biological states such as sex, or race/ethnicity. This sample size will also facilitate the ability to preserve cohort participants in the remaining n=2500 of the APS cohort as a validation set for findings identified in the first n=1500 (as a secondary validation procedure to the cross-validation described in Section 12).

For clustering analyses, prior published experience in critical illness phenotypes has successfully identified between 2 and 4 biologic clusters using sample sizes ranging from n=265 to n=1200.[14–17] With 1500 we expect adequate power for clustering with up to 150 features (more if dimension reduction is employed) as described in the rationale for the cohort sample size.

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16.2.9 Aim 2 Expected Output

With this careful characterization of vascular, DAMP, and immune plasma protein and whole blood gene expression profiling across 1500 participants from the APS cohort, we anticipate:

- the generation of multivariable models including both clinically-derived and lab-derived variables that display predictive value for ARDS, AKI, 28-day mortality, and overall change in SOFA score
- the identification of plasma or gene expression features that will enhance prediction of post-APS cognitive or functional health impairments
- that better classification of the specific vascular health or DAMP signaling dysregulation across APS states, and a careful mapping of their trajectories, will suggest new therapeutic strategies
- that specific vascular, DAMP, or whole blood gene expression features will be informative to integrated subphenotype classification, and that such features can be leveraged to test for heterogeneous treatment effect in future trials

16.2.10 Aim 2 References

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16.3 Appendix C (Aim 3): Test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, pneumonia and sepsis will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term outcomes, including pulmonary dysfunction.

16.3.1 Aim 3 Background and Rationale

ARDS and pneumonia are diseases of the lungs. Similarly, up to 50% of severe sepsis cases result from lower respiratory tract infections. Compared to the bloodstream, the luminal compartment of the lungs has been largely understudied. Moreover, despite the fact that studies of airspace biology in ARDS have yielded novel mechanistic insights,[1] phenotypes identified in ARDS patients are predominantly driven by markers of systemic inflammation and coagulation rather than respiratory variables, and airspace biomarkers were not included in the models.[2] There are few published studies that have sought to determine whether the previously derived hyper- and hypoinflammatory phenotypes are mirrored in airspace biology and available evidence suggests that airspace phenotypes are discordant with systemic phenotypes.[3,4] Taken together, these factors have limited our ability to identify APS phenotypes and represent a major barrier in the field.

A new method for serially sampling the airspace in mechanically ventilated patients using fluid that condenses on the heat moisture exchanger (HME) filter has recently been described. This method is completely non-invasive, inexpensive, requires minimal investigator time and can be done serially and safely over the course of mechanical ventilation.[5,6] We have prospectively enrolled over 400 mechanically ventilated patients at Vanderbilt in HME filter fluid studies and we have implemented this method in NHLBI-funded studies at multiple sites including University of Colorado, University of Washington, Stanford and UCSF. Moreover, we have demonstrated that protein biomarkers can reliably be measured in HME filter fluid collected from mechanically ventilated patients with ARDS and other causes of acute respiratory failure.[5,6]

Non-bronchoscopic bronchoalveolar lavage (NBBAL) is a well-recognized approach that enables sampling of the distal airways and airspaces of individuals that are on mechanical ventilation. The technique involves instillation of saline into the distal airspaces, followed by gentle aspiration. Accordingly, cells, bacteria, lipids and proteins from the distal airspaces can be sampled. NBBAL is minimally invasive and has been demonstrated to be safe in critically ill participants.[7]

The overarching goal of this scientific aim is to test the hypothesis that sampling the airspaces of mechanically ventilated patients with ARDS, pneumonia and sepsis using HME filter fluid and NBBAL will uncover novel APS phenotypes and endotypes and inform our understanding of central mechanisms that drive short- and long-term clinical outcomes, including pulmonary dysfunction.

16.3.2 Aim 3 Study Objectives

Primary objectives (Aim 3):

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1. Identify acute phenotypes of APS in mechanically ventilated patients using bulk RNA sequencing of mononuclear cells isolated from NBBAL and identify their association with short-term and long-term clinical outcomes.
2. Identify acute phenotypes of APS in mechanically ventilated patients using a panel of protein biomarkers measured in exhaled breath condensate obtained from HME filters on day 1 of mechanical ventilation along with clinical respiratory variables and identify their association with short-term and long-term clinical outcomes.

Secondary objectives (Aim 3):

1. Use longitudinal collection of HME filter fluid to determine whether phenotypes identified at the onset of acute respiratory failure are stable over time and whether new respiratory phenotypes emerge.
2. Determine which biologic processes in the airspaces are reflected in the bloodstream and which processes are lung-specific by comparing bulk RNA sequencing of mononuclear cells from blood and lungs and proteins measured in HME fluid and plasma.
3. Determine whether phenotypes derived from lung compartment biomarkers improve the ability to predict clinical outcomes versus currently accepted phenotypes defined from blood markers alone.

16.3.3 Aim 3 Study Population

All mechanically ventilated patients enrolled in APS will be eligible for inclusion if mechanical ventilation is initiated during the period of in-hospital biospecimen collection. We estimate that 1500 of the 4000 patients enrolled in the APS study will be mechanically ventilated during the first four study days.

16.3.4 Aim 3 Study Design

Prospective observational cohort study

16.3.5 Aim 3 Study Procedures

HME filter fluid studies:

After passing a safety screen to rule out severe hypoxemia (FiO2 > 80%) or high PEEP (>15 cm H2O), HME fluid will be collected on days 0, 2, 4, 6 and 14 if patient is mechanically ventilated by placing a fresh Airlife HME filter in the ventilator circuit for 4 hours. Patients who are intubated prior to enrollment will have the first (day 0) sample collected on the day of enrollment. The Airlife HME filter is optimal for these studies, with a small dead space volume and a reliable HME fluid yield of ~1-2 ml after a 4-hour dwell time.[5] At study sites where heated ventilator circuits are routinely used, the heated circuit will be removed or turned off for the 4-hour HME dwell time. Once removed, HME filters will be centrifuged to collect exhaled breath condensate which will be frozen in small aliquots.

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A panel of protein biomarkers representing key facets of lung pathology during acute respiratory failure will be measured in duplicate in HME samples including IL-6, IL-8, TNFR1, MCP1, RAGE, SP-D, Angiopoietin-2, ICAM-1, procollagen peptide-3, protein C, PAI-1 and total protein.

NBBAL studies:
All patients that are initiated on mechanical ventilation will be considered for NBBAL. Ideally, NBBAL will be performed within 24 hours of initiation of mechanical ventilation. However, we recognize that this might not be feasible for all participants. Therefore, NBBAL may be performed as late as 96-hours after initiation of mechanical ventilation. Safety criteria for NBBAL include FiO2 $\leq$ 75%, PEEP < 15, INR $\leq$ 3.0, and platelets > 50,000 / ul. All must be met at the time of the procedure. Prior to performing NBBAL, the patient is placed on 100% FiO2 for 10 minutes. BAL is performed using a flexible 16 gauge catheter that is gently advanced through the endotracheal tube to "wedge" the distal tip of the catheter. Serial aliquots of saline are instilled into the catheter and then aspirated. The maximum instillation volume is 120 ml.

Aspirated BAL fluid will be pooled and placed in a sterile collection container and kept on ice until processing. BAL fluid will be run through a 70 micron strainer to remove mucus and debris. Fluid will be centrifuged to pellet cells. Cell-free fluid is gently aspirated and aliquoted for banking. Cells will be washed once, and then preserved for RNA sequencing studies.

16.3.6 Aim 3 Data, Images, and Biospecimens Used from the APS Consortium

To identify acute lung-specific APS phenotypes and endotypes using airspace biospecimens (Objective 1) we will utilize clinical data, chest radiograph images and HME fluid that are collected for the APS study. To determine relationships between acute lung-specific APS phenotypes and short- and long-term pulmonary dysfunction (Objective 2) we will utilize clinical data including duration of mechanical ventilation and mortality from APS and long-term outcome data including pulmonary function testing (spirometry, DLCO) and CT fibrosis scoring that will be obtained in approximately 500 survivors at 12 months. In addition, we will utilize plasma biomarker levels and LCA phenotyping results that are planned for the entire APS cohort for comparison to lung-specific phenotypes derived from respiratory variables and biomarkers measured in HME fluid (Objective 3).

16.3.7 Aim 3 Statistical Analysis

As with all aims, the final statistical analysis plan will be fixed and filed prior to conducting the analysis. Here, we describe one potential approach for this aim, which will either be confirmed and fleshed out or updated with new methods available at the time of analysis. The primary analysis will include all enrollees to determine if there are latent respiratory phenotypes that are common to all critically ill patients; mechanically ventilated patients enrolled in the APS study will include patients intubated for pneumonia, sepsis, ARDS, and other causes of acute respiratory failure. Secondary analyses will focus within subgroups of patients who have been clinically phenotyped as having sepsis (both pulmonary and non-pulmonary), ARDS, and pneumonia. Some patients will be classified into more than one subgroup.

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To approach this aim, a *de novo* latent class analysis (LCA) using previously described methods might be proposed.[2] For the primary analysis, clinical and respiratory variables along with HME biomarkers measured on the first day of mechanical ventilation would be considered. These variables include protein biomarkers (IL-6, IL-8, TNFR1, MCP1, RAGE, SP-D, Angiopoietin-2, ICAM-1, procollagen peptide-3, protein C, PAI-1 and total protein) along with age, sex, etiology of acute respiratory failure, PaO2/FiO2, PEEP, static compliance of the respiratory system, ventilatory ratio,[10] and RALE score.[11] Respiratory and HME biomarker data available at a later timepoints for patients who are still intubated may be included in the analysis to determine whether phenotypes identified at the onset of ARF are stable over time and whether new respiratory phenotypes emerge in those who remain in ARF. Once we have determined whether latent respiratory phenotypes are present, we will investigate phenotype generalizability and stability, and test associations between respiratory phenotypes and pre-illness factors such as air pollutant exposure and socioeconomic factors.

For long-term outcomes, we will focus first on two key hypotheses. First, we hypothesize that acute lung epithelial injury as measured by HME fluid RAGE and SP-D will be associated with development of fibrotic lung changes (increased lung fibrosis scoring on CT, decreased FVC). Second, we hypothesize that the severity of lung endothelial injury as measured by HME fluid Ang-2 and ICAM-1 will be associated with long-term destruction of the lung vascular bed as measured by DLCO. The dependent variables of interest are continuous variables and will be DLCO, FVC, lung fibrosis score. Independent variables include age, sex, HME biomarker data from both day 1 and latest day available, ventilator settings, RALE score, duration of mechanical ventilation, underlying cause of ARF (pneumonia, ARDS, sepsis, other). The initial proposal is for use of linear regression models; dependent variables may be log-transformed to improve fit. Analyses done are expected to include the entire cohort, as well as in subgroups divided by clinical syndrome (pneumonia, ARDS, sepsis). To determine whether long term respiratory outcomes differ by any *de novo* respiratory phenotypes, we can include the phenotype variable and its interaction terms with the main independent variables of interest.

We plan to check for concordance and discordance between novel respiratory phenotypes identified in this Aim and previously derived phenotypes based on clinical and biomarker data and the LCA approach. We will then assess reasons for concordance and discordance. If the same number of clusters are identified with each analysis, standard metrics including Cohen's Kappa will be used to assess the degree of concordance. Identifying a different number of clusters for systemic vs. respiratory phenotypes, or the same number with lack of concordance, may indicate that respiratory phenotypes differ from systemic inflammatory phenotypes. When different numbers of clusters are identified between aims, correlation between cluster variables will be assessed, as well as evaluating whether some clusters function as sub-clusters of those identified in other aims.

Finally, a key question is whether the biologic mechanisms that form the basis for NBBAL endotypes are reflected in the blood. This can be achieved by exploring targeted gene expression profiles and weighted gene cluster network analysis (WGCNA) in bulk RNA seq between peripheral blood PBMCs and NBBAL.

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16.3.8 Aim 3 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

Among the estimated 1500 ventilated patients with HME fluid collected, we estimate that 1050 will have sepsis (both pulmonary and non-pulmonary), 600 will have ARDS, and 600 will have pneumonia. All subgroups are larger than n=500, which is sufficient for the proposed analyses as shown in the rationale for the cohort sample size, and is true even if feature quality is suboptimal.[8] For example, Hsieh et al,[9] showed that with n=600 (the smallest subgroup) a multivariable logistic regression to detect an association between a covariate of interest (COI) and the response variable, has 90% power to detect an odds ratio of 1.55 to 1.60, for each standardized unit increment in the COI when the COI is moderately correlated with other covariates in the model (multiple correlation of 0.2 to 0.5).

For analysis of long-term respiratory outcomes, approximately 500 hospital survivors will have follow-up testing (PFT, CT) collected and among these we anticipate that the majority will have been mechanically ventilated during the acute hospitalization. Enrollment of mechanically ventilated patients in the in person LTO cohort will be monitored over time and sampling strategy can be adjusted to further enrich for mechanically ventilated patients if needed. Assuming just 200 mechanically ventilated patients, the sample size is sufficient for complex regression models with interactions and regression splines; Cohen's F^2 will be 0.08 for 90% power and 5% type I error rate when the base model has 10 degrees of freedom and the new model adds 4 degrees of freedom (e.g., adding a three-level categorical variable and its interaction with two-level categorical variable). Thus, power is sufficient to detect a medium effect in terms of improvement in model fit by including new respiratory phenotypes and their interactions, represented by relative increase in R^2 values.

Finally, of the 1500 subjects that we expect will be on mechanical ventilation in the study, we anticipate that 700 will have NBBAL. We can assess the association between NBBAL and blood expression of 500 candidate genes and up to 50 WGCNA networks. We estimate an FDR adjusted significance threshold of 0.0047 assuming 10% of genes are truly associated in blood and BAL, 80% power and a 5% FDR. Using this adjusted significance threshold, 700 participants will provide 80% power to detect correlations of 0.14 between blood and NBBAL expression measures.

16.3.9 Aim 3 Expected Output

We anticipate that we will find lung-specific phenotypes, and that sepsis, ARDS and pneumonia will share respiratory phenotypes that will differ from systemic hyper- and hypo-inflammatory phenotypes. In addition, we expect to definitively answer the longstanding question as to how well the biological processes in the airspace can be reflected in the plasma. This study will allow direct comparison between plasma and airspace biomarkers from contemporaneous samples in a large and clinically diverse cohort allowing us to directly address that question.

With regard to long-term outcomes, we anticipate that we will identify key biological signatures in the airspace that will correlate with different long-term outcomes. Specifically, lung epithelial injury biomarkers will correlate with long term restrictive/fibrotic lung dysfunction, and lung endothelial injury markers will correlate with reduced DLCO. It may be that our selected biomarkers do not correlate with these outcomes, in which case we will explore associations with biomarkers of other mechanistic pathways that will be measured for the respiratory LCA.

Lastly, we expect that results from this analysis will shed light on relationships between the alveolar environment and systemic disease. As an example, it has been suggested that COVID-19 pneumonia is driven by self-sustaining inflammatory circuits. However, it is unclear how this mechanism contributes to systemic inflammation and extra-pulmonary organ dysfunction. Similarly, it is unclear how severe pulmonary infections set the stage for impaired systemic immune function. Head-to-head assessment of gene expression pathways and network modules between lung and blood will help address these questions and will define central biologic mechanisms that contribute to disease pathogenesis in the lung versus the periphery.

16.3.10 Aim 3 References

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16.4 Appendix D (Aim 4): Determine trajectories of established phenotypes over time, and utilize longitudinal data to identify novel phenotypes of APS during the acute phase and during recovery.

16.4.1 Aim 4 Background and Rationale

Molecularly-defined phenotypes with different predicted survival and differential response to multiple randomized therapies have been validated in multiple critical illness populations with APS.[1,2,3] However, the vast majority of existing work has been performed using plasma protein and clinical phenotyping from only the earliest timepoint, and not considering the dynamic nature of class-defining markers over time. We hypothesize that the trajectory of molecular phenotypes have important prognostic information about patients' condition, and that the resolution of 'hyperinflammatory' phenotype will associate with improved survival. Furthermore, by considering new plasma proteomic features, transcriptomic features, and microbial data over time, we hypothesize that novel phenotypes with distinct biological dysregulation can be identified, which in turn may yield novel precision treatment paradigms for APS.

16.4.2 Aim 4 Study Objectives

Primary Objective (Aim 4):

To utilize longitudinal molecular (plasma and HME), transcriptomic, and microbial data over the first seven days of critical illness to identify novel longitudinal, trajectories-based phenotypes, to determine the prognostic value for short-term clinical outcomes (primary: in-hospital mortality; secondary: organ-failure free days).

Exploratory Objectives (Aim 4):

1. To utilize longitudinal molecular, transcriptomic, and microbial data over the course of critical illness to identify novel longitudinal, trajectories-based phenotypes, to determine the prognostic value for and long-term patient outcomes assessed through months via physical, cognitive, and mental health outcome measures.
2. To determine the biologic trajectories of novel molecular and transcriptional phenotypes of APS over time and whether phenotype resolution is associated with recovery (Recovery = survival; 1-item recovery question; WHODAS ≤ baseline WHODAS), and compare their natural course to previously described phenotypes.

16.4.3 Aim 4 Study Population

All participants enrolled in the APS Consortium will be eligible to participate in Substudy D.

16.4.4 Aim 4 Study Design

Prospective molecular cohort study with scheduled blood and microbiome sampling during the index hospitalization and with longitudinal follow-up through 12 months after critical illness.

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16.4.5 Aim 4 Study Procedures
Clinical and molecular data generated in the previously described in Aims 1, 2, and 3 will be collated and standardized.

16.4.6 Aim 4 Data, Images, and Biospecimens Used from the APS Consortium
Clinical data including syndromic phenotype (ARDS, pneumonia, sepsis), presence/type of infection, risk factors for ARDS, demographic information, comorbidities, severity of illness, physiologic data, and outcomes (survival, duration of organ support) will be utilized from each participant's inpatient admission. Clinical laboratory values and treatments will also be collected. Long term outcomes including measures of functional status, quality of life, emotional health, cognitive performance, and respiratory symptoms through 12 months will also be tested, and will be adjusted for metrics of pre-illness health. Images from the inpatient stay or the longitudinal 12 month visit will be utilized, and from longitudinal muscle ultrasound, will be used.

Biological samples will include the results from all assays performed on the plasma, host whole blood RNA expression, and pathogen and microbiome assessment from samples obtained on days 0, 2, 4, and 6, along with respiratory sample profiling (HME filter fluid proteins) obtained at intubation and day 6.

16.4.7 Aim 4 Statistical Analysis
It is recognized that methods for analysis continue to be developed and evaluated. A detailed SAP will be generated before analysis begins, in which we will specify the method considered optimal at the time or, if an optimal method cannot be easily determined, competing methods will be compared. The following sections outlines a general approach that might be expected undertaken for this aim.

16.4.7.1 Evaluate existing phenotypes
Initially, we propose that for each cohort, at each time point, we will take two complementary modelling approaches to identify classes and/or phenotypes: 1) latent class analysis (LCA); 2) parsimonious classifier model (or these will be substituted or augmented if comparable of better preforming models are identified). To date, LCA has been the gold-standard algorithm to identify the molecular phenotypes at cross-sectional timepoints. However, LCA uses variables standardized and scaled to the distribution of each cohort / timepoint. Therefore, at later timepoints, as disease states resolve and/or therapies take effect, physiology will likely normalize, leading to a divergence in the statistical solution and biological signature captured by LCA. It is conceivable, that while LCA at later time points may identify two classes, their biological characteristics may differ from those identified at baseline, as overall distributions in the population changes. Thus, we currently propose to evaluate the temporal kinetics of the biological signature of the previously derived "molecular phenotypes", defined as Hypoinflammatory and Hyperinflammatory phenotypes identified using LCA at enrollment. At each time point, we will fit a parsimonious logistic regression classifier model which, unlike LCA which uses z-score standardized variables, uses the original scales of protein biomarkers and clinical variables;[7] therefore, it is more likely to capture the biological signatures that define the phenotypes.

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The variables used to perform LCA will be similar to those used in our prior studies and include clinical variables and protein biomarkers. Non-normally distributed data will be log-transformed and continuous variables will be scaled using z-scoring using values specific to the study day. Outcome data and severity scores (e.g., APACHE and SOFA scores) will be excluded from the modelling. We will build a minimum of five models, comprising of 1 to 5 classes respectively. We will use fit statistics and the Vuong-Lo-Mendall-Rubin test to determine the best fitting model in each cohort at each timepoint. Once identified, we will use the highest probability generated by the best fitting model to assign class membership. We will use latent transition modelling to evaluate the temporal stability of the classes at sequential time points. To establish overlap with the baseline molecular phenotypes, we will use the parsimonious classifier models comprising of IL-8, protein C, and serum bicarbonate. We plan to use a probability of ≥ 0.5 to assign molecular phenotypes. We will perform mixed effect modeling assessing for overall group differences in parsimonious model probabilities over time between survivorship groups in each phenotype independently. We will perform unadjusted (main) and confounder-adjusted analysis of probabilities trajectories, to ascertain cluster association with outcomes.

For gene-based existing phenotypes, we will use repeated RNA-sequencing data to classify patients to the various phenotypes (SRS, MARS, etc.). We will use the probabilities of belonging to the phenotypes on each day to model for trajectories and use outcome data to evaluate the prognostic value of the changing probabilities and switching of phenotypes, adjusting the models for treatment such as corticosteroids.

16.4.7.2 Discovery of novel phenotypes

For discovery of novel phenotypes that are based on trajectories of patient characteristics, we will use two approaches: 1) trajectories-based latent class analysis; and 2) group-based trajectories modelling (or these will be substituted or augmented if comparable of better preforming models are identified). Given the computational demands of trajectories-based LCA, we may only perform this modelling on clinical data and protein biomarkers and not on transcriptomics data; if methods for transcriptomic data become feasible they will be considered. The time-dependent LCA will be performed using the procedures and features described in the section above, with the addition of repeated measures to identify novel phenotypes. The group-based trajectories modeling (GBTM) will be performed on the repeated measures of the most important variables identified in the time-dependent latent class analyses, and we will seek overlap between this single variable derived cluster and its corresponding latent class. GBTM, like LCA, is a finite mixture modelling approach, and the rationale for its use to interrogate trajectories of a single variable is that it may be more clinically implementable than a multivariate trajectories LCA. The GBTM trajectory monitoring algorithm computes unique equations of the variable of interest as a function of time, which is used to define a subgroup. Individual patients are then classified into the trajectory subgroups using the equation and how closely their measurements match the function defined by the equation. Depending on the variable of interest in the model, we will adjust the GBTM for the effects of treatments such as corticosteroids.

Both for novel and existing phenotypes, we will leverage the breadth of physiological and biological data to better understand the underlying biology of the phenotypes. We will use differential gene-expression analysis and pathway analyses of RNA-sequencing data to study within group and between group

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differences in longitudinal gene-expression patterns. Within groups we will use time as variable stratified by survivorship to further study distinct temporal pathways associated with recovery and death.

Longitudinal models will be fit using mixed-effects models that will include a random effect for individual participants. Similar to Aim 2, we will examine the results of joint longitudinal and time-to-event models to account for various dropout mechanisms.

16.4.8 Aim 4 Adequacy of sample size

While the statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan, we provide an assessment of the adequacy of the sample size, recognizing that the final methods will be highly optimized using rapidly developing methodology. For the LCA, one of many potential clustering methods, a sample size of 300-500 observations is generally considered sufficient, and we have shown in the cohort sample size rationale that a sample size of 1500 is sufficient for a cluster analysis with 150 variables or more. In our prior work, we identified well separated classes and demonstrated that the proposed variables in our LCA are high quality. The sample sizes proposed here (n=1500-4000 depending on analysis) for the deeply phenotyped subset, and n=1500 for the mechanically ventilated subset, should be sufficient to interpret fit statistics from the LCA overall, and for each component syndrome of APS. These estimates also include later time points where we anticipate at least 1000-1800 patients having molecular data assayed at Day 6 (depending on the variable), with the sample size expected to be slightly larger in sepsis and pneumonia than for ARDS.[2-7]

16.4.9 Aim 4 Expected Output

The completion of these described study objectives will generate the following expected output:

- Evidence to accept or refute a state change from hyperinflammatory subphenotype to a less inflamed phenotype as a prognostic indicator, which can then be tested as a theragnostic marker in future trials
- Novel biologic subphenotypes that incorporate longitudinal data and more diverse data elements than currently exist, which may yield better tools for prediction of long-term health states
- Novel biologic subphenotypes that may demonstrate unique biology that is both identifiable and pharmacologically targetable

16.4.10 Aim 4 References

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16.5 Appendix E (Aim 5): Determine and interrogate the role of patient comorbidities, exposures, and biophysical constitution in the clinical and biological heterogeneity of ARDS, pneumonia, and sepsis.

16.5.1 Aim 5 Background and Rationale

Background: Efforts to date to understand clinical heterogeneity in ARDS, pneumonia, and sepsis have focused on biological measurements (e.g., plasma biomarkers and vital signs) obtained at the time of acute illness. Yet chronic and constitutional elements of patient biology are important in APS trajectories, both in acute and long-term outcomes. *Rationale*: By performing granular measurements of patients' comorbidities, exposures, and biophysical constitution, we will determine their relative influence and significance in the heterogeneity of APS conditions. This knowledge will equip us to design and trial tailored therapies, informed by each patient's underlying clinical substrate.

16.5.2 Aim 5 Study Objectives

Primary Objective (Aim 5):

We will assess the association between patients' comorbidities, environmental exposures, and biophysical constitution with membership in both previously described and newly identified phenotypes of ARDS, pneumonia, and sepsis. This approach will allow us to identify the contribution of pre-acute illness factors to the pathophysiology of ARDS, pneumonia, and sepsis. There is no intent to develop novel phenotypes under this aim. We will assess the association between exposure history and clinical syndromes, existing molecular phenotypes (e.g., hypo/hyperinflammatory, SRS classes, MARS classes), and phenotypes newly developed under the other aims of this protocol.

The comorbidities of interest are cancer, diabetes mellitus, and cirrhosis; current state and history of these conditions will be considered. The Functional Comorbidity index will also be assessed for contribution to phenotype assignment.

The environmental exposures of interest are cigarette smoking, alcohol use measured using the AUDIT and via blood phosphatidylethanol (PeTH) concentration, air quality index based on 9-digit zip code of residence, and drug use based on urine toxicology screen.

Measures of biophysical constitution are biological sex, age, and body composition (BMI, CT scan imaging), as well as disability assessed using the WHODAS II, cognition assessed using the IQCODE, and frailty assessed using the Clinical Frailty Score.

Exploratory Objectives (Aim 5):

We will assess whether patients' comorbidities, environmental exposures, and biophysical constitution contribute to short-term and long-term outcomes of ARDS, pneumonia, and sepsis, and whether these factors modify the association between phenotype membership and outcomes. Short-term outcomes

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include mortality, hospital length of stay, and ICU length of stay. Long-term outcomes include 3-, 6-, and 12-month assessments of physical, respiratory, cognitive, and mental health status.

16.5.3 Aim 5 Study Population
The study population will be the complete cohort of 4000 hospitalized patients as described in the study protocol.

16.5.4 Aim 5 Study Design
Prospective observational cohort study.

16.5.5 Aim 5 Study Procedures
As detailed in the protocol, for each patient we will use surveys to assess the following:

Biophysical constitution: Comorbidities (Functional Comorbidity Index), disability (WHODAS II), cognition (IQCODE), frailty (Clinical Frailty Score), and obesity (BMI). We will also obtain DICOM images from clinically acquired chest and abdominal CT scans to derive indices of body composition.

Psychosocial factors: Social vulnerability (social vulnerability index), social isolation (NHATS 6-item)

Exposures: Air pollution (AQI), tobacco, alcohol (AUDIT). We will also obtain phosphatidylethanol measurements from plasma specimens to estimate cumulative alcohol consumption.

16.5.6 Aim 5 Data, Images, and Biospecimens Used from the APS Consortium
In addition to the listed surveys, we will collect DICOM images from clinically acquired chest and abdominal CT scans to derive indices of body composition and will measure phosphatidylethanol in plasma to estimate alcohol consumption.

16.5.7 Aim 5 Statistical Analysis
As noted for other aims, analyses will either compare outcomes or characteristics between classes, or will identify *de novo* classes. Following the principles laid out in the protocol, each analysis will be pre-specified in a statistical analysis plan. When an optimal approach is not clear, different methods will be compared; comparison of different approaches will be a strong contribution to the methods literature. We also note that discordance between methods can identify informative features and help to further interrogate individual approaches for biological meaning. we have offered multiple approaches for identifying *de novo* classes, for example in Aims 1, 3 and 6. We will follow one of these approaches (or another approach that is considered optimal at the time), replacing the data used in that aim with patient comorbidity, exposure, and biophysical constitution related features.

16.5.8 Aim 5 Adequacy of sample size
Aim 5 includes all 4000 participants. As described in the rationale for the cohort size, this is sufficient for both supervised (e.g., outcome prediction) and unsupervised (e.g. clustering) analyses considering 500

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features or more. Also, when n=4000 it is possible to estimate odds ratios of about 0.8 or 1.2 with the half width of the confidence intervals being 0.1 or smaller. This aim has robust statistical power for understanding the role of comorbidities and biophysical constitution on both APS phenotypes and outcomes from ARDS, pneumonia and sepsis.

16.5.9 Aim 5 Expected Output

These analyses will provide us with the following new insights into APS conditions:

- How patient comorbidities differ across previously described subphenotypes and newly identified subphenotypes
- How patient exposures (tobacco, alcohol, air pollution) differ across previously-described subphenotypes and newly identified subphenotypes; whether patient exposures represent a "treatable trait" that module patient risk for clinically unfavorable heterogeneity
- How biophysical constitution (as assessed using body composition and integrative functional assesssments) contributes to acute and long-term trajectories of APS conditions
- How these contributing factors interact with common ICU interventions (corticosteroids, neuromuscular blockade, early mobilization and antibiotics) as related to acute and long-term outcomes

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16.6 Appendix F (Aim 6): Develop approaches for translating phenotypes to the bedside to enable follow-on precision clinical trials.

16.6.1 Aim 6 Background and Rationale

ARDS, sepsis, and pneumonia cause severe morbidity and mortality globally. While supportive care has improved outcomes over the last decades, persistently high morbidity and mortality indicate that new treatments are needed. Unfortunately, the history of trials in ARDS, sepsis, and pneumonia has been disappointing. At most 5% of Phase 3 trials in critical care demonstrate efficacy,[1–3] even in settings where pre-clinical data has been promising.[4,5] This finding is likely due to improperly specified target populations, inadequate predictive enrichment, and inappropriate trial endpoints.[6–9]

Robust statistical/machine-learning techniques hold promise for the definition of relevant subgroups of patients within complex syndromes. Such subgroups may include modifiable biological pathways (e.g., fibrosis and repair), an influential polymorphism (e.g., IL-6 promoter),[10] a comorbidity (e.g., diabetes mellitus), or an important social context (e.g., economic deprivation). Identification of such subgroups holds promise as the foundation of a subsequent generation of targeted therapeutics.

The focus in this appendix is on phenotypes as actionable target populations—i.e., homogenous, treatment-responsive subgroups of patients identifiable at the time a treatment decision must be made. We do so recognizing that such phenotype identification has improved clinical care in, e.g., pulmonary hypertension[11] and asthma.[12] Multiple analytic aspects are important to accomplishing the ultimate goal in this appendix of designing high-quality trials in the right target populations within ARDS, pneumonia, and sepsis, with higher probability of success.

Logistically, the use of phenotypes as actionable target populations includes two critical components.

The first component is the ability to identify phenotype membership at the time a treatment decision must be made. Usual phenotyping methods are based on high-dimensional data in the derivation and validation cohorts and cannot be straightforwardly applied in clinical practice. In order to be used in clinical practice, simpler, parsimonious definitions are required. These parsimonious models utilize a restricted set of variables—all of which can be obtained clinically—to identify phenotype membership in real time. Treatment decisions can then be made based upon the phenotype assignments of the parsimonious models. Substantial progress in machine learning, combined with the richness of the APS Consortium cohort dataset, makes possible the development and validation of such classification rules for application in future trials.

The second component is to identify the most promising future trial candidates in ARDS, pneumonia, and sepsis, whether repurposed agents (i.e., those already in clinical use among patients enrolled in the APS Phenotypes Consortium cohort) or hypothetical novel agents (i.e., the expected effect of modulating a protein biomarker or other biological signal among Consortium patients). Fortunately, statistical methods for causal inference and observational data methods allow for the performance of simulated clinical trials in a way that has been demonstrated to closely approximate inferences drawn from prospective randomized trials.[13] These methods of "target trial emulation" provide the opportunity to make key observations relevant to the design and conduct of future clinical trials.[14]

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16.6.2 Aim 6 Study Objectives

Primary Objective (Aim 6):

Aim 6a. Perform target trial emulations of observed or potential novel therapies, with prespecified assessment of possible treatment effect heterogeneity. As an illustrative example of one such emulation, we will estimate the effect of a potential DAMP-blocking agent administered to lower DAMP to the below the median level versus usual care alone on all-cause mortality at 60 days. The baseline historic syndrome (ARDS, pneumonia, or sepsis) will be the primary subgroups for treatment effect heterogeneity.

Aim 6b. Derive and validate parsimonious classification rules using clinically available baseline data to assign patients to APS-identified phenotypes.

Exploratory Objectives (Aim 6):

Aim 6a:

- Assess treatment effect heterogeneity within target trial emulations based on (a) the hyper- vs. hypo-inflammatory phenotypes identified by Calfee and colleagues and (b) novel phenotypes identified within the APS Consortium
- Assess secondary endpoints for the target trial emulation, including mortality at 30- and 90-days, ICU-free days, organ-support-free days, WHO ordinal outcome scale, and the 12-month outcomes measured among the long-term outcome cohort within the APS Consortium.

Aim 6b:

- Compare the accuracy of (a) classification rules restricted to currently available clinical values with (b) classification rules that incorporate results that could feasibly be reduced to clinical application (e.g., cytokine results that could be performed at the point of care).

16.6.3 Aim 6 Study Population

All enrolled APS Phenotypes Consortium cohort patients

16.6.4 Aim 6 Study Design

No additional laboratory procedures will be performed for this Aim.

Objective 1 will be approached using statistical learning classification models based on clinically available data and/or parsimonious biological data, we will derive and validate classification models, with an intraclass correlation coefficient (ICC) ≥ 0.75.

Objective 2 will be approached using treatment normalization, causal inference, and design of simulated trials using structured design deliberations and target trial emulation techniques. Using these methods, we

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will define and perform 5 high-priority trial simulations. Results of those simulated trials will be reported back to the Steering Committee and NIH.

16.6.5 Aim 6 Study Procedures

No additional non-statistical procedures will be performed for this aim beyond those in the main protocol and the Appendices that generate biomarker data.

16.6.6 Aim 6 Data, Images, and Biospecimens Used from the APS Consortium

All data, with the exception of biomarkers or other biological assays (e.g., microbiome) that are not expected to be reducible to a clinically relevant turnaround time (i.e., biomarkers or other biological assays that would take >24h to return results). These data centrally include (1) medications and other therapies (e.g., respiratory support modes) for simulated trials of repurposed medications and (2) protein biomarkers and other biological assays (e.g., transcriptomic results given recent progress in turn-around times for peripheral blood RNA sequencing) for simulated trials of potential novel agents that modulate a given biological signal. The biomarkers and other bioassays proposed in the APS Phenotypes Consortium cohort Aims have been chosen on the basis of their expected causal roles in ARDS, pneumonia and sepsis pathophysiology. It is therefore anticipated that the output of simulated trials of potential novel agents will be highly relevant to the development and design of future clinical trials.

16.6.7 Aim 6 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on reasonable assumptions, recognizing that the final methods will be highly optimized using rapidly developing methodology.

For Objective 1, historically, formal sample size calculations have not been performed for classification problems such as these, with general reliance on heuristics (e.g., 5–7 observations per variable in the classification problem). Nevertheless, Walter's method[15] makes possible a power estimate based on comparisons against a null hypothesis for ICC. In this case, an overall sample size of 460 provides approximately 90% power to detect an ICC difference of 0.05 against a null hypothesis of 0.70, 80% with a null of 0.60, and 70% with a null of 0.50. (Note that this sample size refers to the cohort size for all phenotypes for a given phenotyping problem, rather than the size of any individual phenotype within the overall cohort.)

For Objective 2, the overall sample size of 4,000 is 2.5–10 times larger than other prospective phenotyping efforts in this disease area. Recognizing the complexities of the data and associated analyses, power estimates by simulation would be heavily assumption-laden. We focus here on power associated with trial emulation. A conservative assessment of power can be determined by considering inference about the weighted average causal effect obtained using matching weights.[16]

This approach mimics 1:1 propensity score matching and emphasizes trials that enroll participants whose propensity scores are close to 0.5.[16] This is consistent with the goal of trial emulation, as patients with propensities close to 0.5 represent patients for whom greater equipoise exists. Using the analogue to 1:1 matching, we can estimate the minimum detectable treatment effects, based on the sample size of the

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smaller of the two comparison groups (Table 14). The table rows apply either to the full cohort or to a subgroup of interest when evaluating treatment effect heterogeneity.

16.6.7 Aim 6 Statistical Analysis

As noted for other aims, following the principles laid out in the protocol, each analysis will be pre-specified in an SAP. When an optimal approach is not clear, different methods may be compared; comparison of different approaches will be a strong contribution to the methods literature. Below, we outline the general direction that we plan to undertake to accomplish this aim.

For Objective 1, we plan to first pursue a dimension reduction exercise (e.g., penalized regression) to identify the variables most likely to identify class membership. The next step will be use of a machine learning technique (e.g., classification and regression trees) to derive the classification rule. Validation can be performed by assessment of the ICC, comparing the underlying phenotype assignment with the classification generated by the classification rule.

For Objective 2, a rigorous, multi-stage method will be employed to minimize bias and maximize the probability of accurate inference. The key features of the multi-stage method are treatment normalization, phenotype identification, phenotype assignment (e.g., using the methods of Objective 1), definition and prioritization of simulated trials, and the actual performance of the simulated trials. The phases of analysis are displayed in Table 13.

Table 13. Aim 6: Flow of analysis for Objective 2.

Step	Task	Methods	Output
A1	Account for the role of treatment in characterization of phenotypes	Normalize disease trajectories via dynamic treatment regime and inverse probability weighting	A normalized pseudo-cohort for phenotyping
A2	Identify disease phenotypes using longitudinal data	Hidden Markov Models and machine-learning techniques	3–5 distinct disease phenotypes
A3	Predict disease phenotypes for individual patients	Elastic net multinomial regression	Patient-level probabilities of phenotype membership
A4	Define and prioritize trial emulations	Structured Steering Committee review of phenotype attributes and potential treatments	Prioritized list of ≤5 trial emulations
A5	Identify efficacy signals for treatments among phenotypes	Target trial emulations with exploration of treatment effect heterogeneity by phenotype	Results of trial emulations, by phenotype

Treatment normalization is a crucial initial step, given the risk that variations in treatment may shape the measured features for phenotyping, independent of the patient's acute biology and premorbid status. Treatments may change over time, and their effect may similarly vary over time. Thus, for example, glucocorticoids decrease inflammatory biomarkers,[17-19] while low-tidal volume ventilation decreases

inflammatory markers, including interleukin-6.[20] Failure to control for the dynamic effect of treatment would thus introduce severe bias, motivating techniques to control for treatment over time. Treatment normalization generates a reweighted cohort on which the relevant phenotyping (and phenotype assignment; steps A2–A3) are performed. After the results of these initial steps, a structured deliberation at the Steering Committee level (step A4) before performance of simulated trials will minimize the risk of alpha inflation (i.e., false positive inferences from simulated trials). The performance of simulated trials (step A5) will follow valid techniques for causal inference, including appropriate cohort definition and simulated treatment plus adjustment for propensity to receive the treatment. For repurposed medications, the treatment definition is simple and familiar—the treatment actually received by the patient in the cohort. In addition to repurposed medications, the simulated trials are able to include assessment of potential novel treatments. For simulated trials of potential novel treatments, levels of measured biomarkers are used as surrogates for treatments that could modulate a biomarker level by a certain level (e.g., assess a hypothetical agent that would lower plasma levels of sRAGE by 50%). Continuous-variable approaches to treatment and treatment adjustment are used to support these analyses for hypothetical novel treatments.[21,22] Note that where a given phenotype has a valid classification rule (as developed in Objective 1), that classification rule will be used for the simulated trials to improve generalizability.

16.6.8 Aim 6 Adequacy of sample size

The statistical approaches that will be used in the ultimate analysis will be specified *a priori* in a Statistical Analysis Plan. Here, we provide an assessment of the adequacy of the sample size based on the approach described above, recognizing that the final methods will be highly optimized using rapidly developing methodology.

As described in the rationale for the cohort sample size, formal sample size calculations have traditionally not been performed for classification problems such as these, with general reliance on heuristics (e.g., 5–7 observations per variable in the classification problem). We have shown that with the overall sample size of 4,000, cluster analyses with as many as 500 features are feasible. As well as the approach described in the sample size justification, Walter's method[15] makes possible a power estimate based on comparisons against a null hypothesis for ICC – the ICC is used as a measure of concordance between actual and predicted class membership with a high ICC reflecting good concordance. Sample sizes calculated in this way are consistent with sample sizes based on the correlation coefficient or detectable difference approach; an overall sample size of 460 provides approximately 90% power to detect an ICC difference of 0.05 against a null hypothesis of 0.70, 80% with a null of 0.60, and 70% with a null of 0.50.

For objective 2, we focus on power associated with trial emulation. A conservative assessment of power can be determined by considering inference about the weighted average causal effect obtained using matching weights.[16] This approach mimics 1:1 propensity score matching and emphasizes trials that enroll participants whose propensity scores are close to 0.5.[16] This is consistent with the goal of trial emulation, as patients with propensities close to 0.5 represent patients for whom greater equipoise exists. Using the analogue to 1:1 matching, we can estimate the minimum detectable treatment effects, based on the sample size of the smaller of the two comparison groups (Table 14). The table rows apply either to the full cohort or to a subgroup of interest when evaluating treatment effect heterogeneity, and show that small to modest differences can be observed even in the smaller subgroups.

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Table 14. Aim 6: Minimum detectable average treatment effect (90% power, 2-sided α = 0.05)

Evaluable patients in smaller group (N)	Detectable mean difference in continuous outcome (% SD)	Detectable % reduction in hazard ratio		
		10% of pts with events	20% of pts with events	40% of pts with events
200	32.5%	64.1%	51.5%	40.1%
400	22.9%	51.5%	40.1%	30.4%
800	16.2%	40.1%	30.4%	22.6%
1200	13.2%	34.2%	25.6%	18.9%

16.6.9 Aim 6 Expected Output

The expected output from Objective 1 is a classification rule for each major phenotyping task for which ≥ 460 overall patients are available. These classification rules can be immediately applied to future patient populations for future trials.

The expected output from Objective 2 is a suite of simulated trials of repurposed and/or hypothetical novel therapies. These trials would be used to motivate and to design next-generation trials in ARDS, pneumonia, and sepsis.

16.6.10 Aim 6 References

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16.7 Appendix G (Aim 7): Determine whether APS phenotypes identify differences in multiple organ dysfunction syndrome (MODS).

16.7.1 Aim 7 Background and Rationale

Multiple organ dysfunction syndrome (MODS) is a life-threatening disruption of organismal homeostasis. MODS is the hallmark of sepsis, is extremely common in ARDS, and is frequently present in pneumonia treated with respiratory support. In health, organs maintain their own homeostasis and communicate with other organs to maintain organismal homeostasis, a phenomenon termed crosstalk. The physiology of MODS is highly complex, in terms of the range of initial organs affected, disruptions in crosstalk pathways, and the pattern of organ dysfunction. In its terminal stages, MODS appears to be a final common pathway of a fatal insult; prior to those terminal stages, however, MODS may be a diverse syndrome. By convention, MODS in critically ill populations has been graded in terms of severity, using one of several scoring systems, e.g., the Sequential Organ Failure Assessment (SOFA).[1] Prior work in sepsis-related organ dysfunction has suggested various phenotypes that differ in clinical presentation and possible treatment effect heterogeneity in prior sepsis trials.[2] While these clinical phenotyping efforts provide preliminary evidence supporting the existence of phenotyping, deep phenotyping of MODS has not yet occurred. This Appendix seeks to determine whether deep phenotyping will identify distinct phenotypes within MODS.

16.7.2 Aim 7 Study Objectives

Primary Objective (Aim 7):

To determine whether the pattern of multiple organ dysfunction syndrome (MODS) differs by APS-identified phenotypes, using dimension reduction techniques to summarize the baseline Sequential Organ Failure Assessment (SOFA) scores, the most common clinical measure of MODS.

Exploratory Objectives (Aim 7):

1. Explore associations among constituent biomarkers (i.e., those used to define the APS phenotypes) and SOFA sub-scores at baseline.
2. Explore evolution of MODS over time, including its interaction with longitudinal phenotypes.
3. Explore the implications of alternative definitions of organ dysfunction (e.g., incorporation of lactate levels into cardiovascular sub-score, alternative methods of central nervous system dysfunction, relevance of specific biomarkers to organ failure definition)

16.7.3 Aim 7 Study Population

All APS Phenotypes Consortium Cohort Patients (N=4000)

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16.7.4 Aim 7 Study Design

Broadly, dimension reduction techniques will be used to compare the distribution of organ dysfunction among the phenotypes identified within the APS Cohort.

16.7.5 Aim 7 Study Procedures

No additional laboratory procedures will be performed for this Aim. Procedures for this Aim are statistical and are outlined below. SOFA subscores will be calculated from clinically available results.

16.7.6 Aim 7 Data, Images, and Biospecimens Used from the APS Consortium

All available data results will be available for the underlying phenotyping. For the definition of MODS, daily SOFA scores will be utilized. (Based on previous analyses, last observation carried forward (LOCF) imputation will be employed for SOFA subscore constituents that are not obtained clinically.)

16.7.7 Aim 7 Statistical Analysis

The analysis for Aim 7 will follow principles of reproducibility and validation laid out on the protocol. There are two aspects of the proposed analysis: first is to use a dimension reduction technique such as PCA on the components of the SOFA score. This is expected to generate interpretable principal components reflecting combinations of organ dysfunction. Additional variables reflecting organ-specific functionality may be included in the analysis to fully capture dysfunction. Then, the principal components can be compared among *de novo* phenotypes to determine whether MODS differs among both previously derived and novel phenotypes. Comparison among phenotypes can utilize multiple group rank-based statistics (e.g., Wilcoxon rank sum) or regression modeling.

16.7.8 Aim 7 Adequacy of sample size

As indicated in the master protocol, there is no clear method for establishing precise sample sizes for unsupervised analyses such as clustering. We have shown in the rationale for the cohort sample size that group sizes of 2000 or more sufficient for identifying clusters when using 500 features or more. For comparison among phenotypes, we have shown that a small effect such as an odds ratio of 0.8 can be estimated with a half width of the confidence interval ranging from about 0.1 to 0.2 under the full range of assumptions.

16.7.9 Aim 7 Expected Output

It is expected that this Appendix will identify relevant differences in the distribution of organ failure among the APS phenotypes. It is anticipated that this will further illuminate the possibility of phenotypes that span the historic syndromes of ARDS, pneumonia, and sepsis.

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16.7.10 Aim 7 References

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The APS Phenotyping Study Protocol B (alteration protocol)

Protocol Full Title:	The ARDS, Pneumonia, and Sepsis (APS) Consortium: A Prospective Observational Study to Evaluate Phenotypes (Protocol B – alteration protocol)
Protocol Short Title:	The APS Phenotyping Study Protocol B (alteration protocol)
Protocol Version:	5.0
Protocol Date	August 10, 2025
Network:	The ARDS, Pneumonia, and Sepsis (APS) Consortium
Funders:	(1) National Heart, Lung, and Blood Institute (NHLBI) (2) National Institute for General Medical Sciences (NIGMS)
Clinical Center Hub Institutions:	(1) University of California, San Francisco (2) Intermountain Medical Center (3) University of Colorado (4) Vanderbilt University Medical Center (5) University of Michigan (6) University of Pennsylvania
Coordinating Center & Single IRB:	Vanderbilt University Medical Center
Investigators:	See section 1
Corresponding Investigator:	Wesley H. Self, MD, MPH wesley.self@vumc.org
Purpose:	This protocol outlines study procedures for participants entering the APS Consortium Study with alteration of informed consent procedures. It describes the alteration of informed consent process and the minimal risk procedures that can be completed with a patient participating under alteration of informed consent. This protocol (APS Protocol B) is a companion protocol accompanying APS Consortium Study Protocol A (full protocol), which fully describes the APS Consortium Study. Protocol B will only be run at sites that are also conducting Protocol A. Participants entering the study with alteration of informed consent on Protocol B will be converted to Protocol A when informed consent for study participation is obtained.

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1. Investigators

Table 1. APS Consortium investigators

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NHLBI / NIH		Ingrid Espinoza, PhD

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2. Protocol Notes

2.1 Protocol Revision History

Protocol Version Number	Protocol Version Date	Summary of Revisions
1.0	March 18, 2024	Original protocol submitted to sIRB
1.1	March 18, 2024	(1) Revised protocol language to make it clear that participants without informed consent will not undergo an interview for collection of study data. (2) Revised section 12.2.3 to change the time frame of reporting adverse events to the sIRB from within 14 days to within 7 days of site awareness of event.
2.0	April 13, 2024	Protocol version used to initiate study enrollment (approved by single IRB at Vanderbilt University Medical Center).
2.1	June 6, 2024	Throughout the document, especially section 11, language was revised to more precisely outline the use of identifiable information. Enrolling sites will enter identifiable information, such as date of hospital admission and date of birth, into the study's database. These data will be visible by APS coordinating center personnel. De-identified datasets will be created for long-term storage of data outside the APS Consortium.
2.2	July 13, 2024	- Section 1: Added Xiaoli Zhao as a consortium investigator. - Section 6.1.3: Section added outlining rationale for eligibility criteria, including the inclusion of pregnant people.
3.0	August 5, 2024	- Section 7.2: Language added to clarify that aliquots of biospecimens collected on this research protocol may be used for clinical laboratory testing if the local team judges such tests to be potentially beneficial for the patient.
4.0	October 23, 2024	- Section 6.1.2: Exclusion criterion #4 changed to: The clinical team has initiated transfer of the patient to a lower level of care that does not meet inclusion criterion #2 (such as placement of a transfer order out of the ICU). - Section 7.2.1: This sentence was added: Additionally, if residual volumes of biospecimens collected clinically meet the volume and collection time needed to fulfill biospecimens outlined in protocol schedule of events of this protocol, those residual samples may be used for research purposes in this protocol.
5.0	August 10, 2025	- Section 1 (Investigators): updated the investigators list. - Phrasing updated throughout for clarity.

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2.2 Abbreviations

Table 2. Abbreviations

Abbreviation	Full term
AE	Adverse Event
AESI	Adverse events of special interest
APS	ARDS, Pneumonia, and Sepsis
ARDS	Acute Respiratory Distress Syndrome
AUDIT-C	Alcohol Use Disorder Identification Test – Concise
CC	Clinical Center
CCC	Consortium Coordinating Center
COPD	Chronic Obstructive Pulmonary Disease
CPT	Cell Preparation Tube
CT	Computed Tomography
CXR	Chest X-Ray
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EHR	Electronic Health Record
EQ-5D-5L	EuroQol 5 Dimensions 5 Level
HME	Heat Moisture Exchanger
ICAP-Revised	Inventory for Client and Agency Planning-Revised
ICU	Intensive Care Unit
IQ-CODE	Informant Questionnaire on Cognitive Decline in the Elderly
IRB	Institutional Review Board
Katz ADL	Katz Index of Independence in Activities of Daily Living
LAR	Legally Authorized Representative
Lawton IADL	Lawton Instrumental Activities of Daily Living scale
lpm	liters per minute
NBBAL	Non-Bronchoscopic Bronchoalveolar Lavage
NHATS	National Health and Aging Trends Study
NHLBI	National Heart, Lung, and Blood Institute
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
OSMB	Observational Study Monitoring Board

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Abbreviation	Full term
P:F ratio	Partial pressure of oxygen: Fraction of inspired oxygen ratio
PFT	Pulmonary function testing
PRC	Protocol Review Committee
RALE score	Radiographic Assessment of Lung Edema score
RNA	Ribonucleic acid
SAE	Serious Adverse Event
sIRB	Single IRB
SIRS criteria	Systemic Inflammatory Response criteria
SOFA score	Sequential Organ Failure Assessment score
UP	Unanticipated Problem
WHODAS-12	World Health Organization Disability Assessment Schedule 12-item version

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2.3 List of Tables and Figures

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3. Purpose of this Protocol / Program Overview

3.1 Scope of Study Protocol B

This study protocol – APS Consortium Study Protocol B (alteration protocol) – describes the process by which participants for whom informed consent for study participation cannot be obtained before study entry may enter the study with an alteration of informed consent, and the minimal risk study procedures that may be completed under alteration of informed consent. This is a companion protocol to APS Consortium Study Protocol A (full protocol), which details the APS Consortium Study at length, including the rationale for the study and how data and biospecimens will be used.

Only sites that are running Protocol A will be eligible to open Protocol B. The goal is to convert participants from Protocol B to Protocol A as soon as completion of written informed consent is possible. Protocol B will only govern study procedures for participants who enter the study using alteration of informed consent until informed consent for study participation is obtained.

3.2 Protocol Documents

The APS Consortium study is governed by two study protocols:

- Protocol A (full protocol): Protocol A is a separate document that describes all study procedures that a participant may complete during the course of the APS study and governs study procedures for participants who have completed informed consent for research participation. Protocol A contains two parts:
    - Master protocol: Study procedures that govern enrollment, data collection, and biospecimen collection. The master protocol is described in the main text of this document.
    - Consortium-wide science: specific analyses that will use data and biospecimens collected in this study and will be undertaken by the APS Consortium investigators collaboratively across the entire consortium. Consortium-wide science is described in appendices to this document.

- Protocol B (alteration protocol): Protocol B is this document. It describes a procedure for participation in the APS Consortium study with alteration of informed consent. Protocol B will be used for participants for whom informed consent for research cannot be obtained via the participant or a legally authorized representative prior to initiation of study procedures. Minimal risk procedures within the APS study may be completed using alteration of informed consent. Protocol B outlines the minimal risk procedures that may be completed without informed consent for research. Identical minimal risk procedures are also included in Protocol A.

Participants who have provided informed consent will complete study procedures described in Protocol A (a separate document), and participants who have not provided informed consent will complete study procedures described in Protocol B (this document). Protocol B includes a subset of study procedures in

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Protocol A and does not include any study procedures not in Protocol A. Hence, participants without informed consent for research will complete a subset of study procedures (Protocol B) completed by participants with informed consent for research (Protocol A). Participants who enter the study on the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A. Greater than minimal risk procedures may be completed after informed consent is obtained and are governed by Protocol A. Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

4. Introduction

Please see APS Consortium Study Protocol A (full protocol) for a description of study background, rationale, and objectives. In brief, the overarching goal of the APS Consortium is to support the development of deeper mechanistic understandings of critical illness syndromes to facilitate precision-based therapies that will curtail the devastating morbidity and mortality caused by ARDS, pneumonia and sepsis. The goals of the APS Consortium were established by the NIH/NHLBI in the request for applications (RFA) soliciting applications for the clinical centers and are described in detail at (https://grants.nih.gov/grants/guide/rfa-files/RFA-HL-23-001.html).[1] The Consortium seeks to understand the heterogeneity and underlying mechanisms of critical illness syndromes and recovery in adults with ARDS, pneumonia, and/or sepsis, as well as the relationship and biological overlap among these syndromes, through a prospective, longitudinal observational study with common data and biospecimen collection. The scientific focus of the consortium is on identifying novel phenotypes of critical illness, describing the clinical and biological features that define these phenotypes, establishing their prognostic and clinical value, and identifying their fundamental mechanisms; in addition, as set out in the RFA, the Consortium will generate a richly characterized clinical dataset and biobank for future investigations.

The Consortium will conduct a cohort study of approximately 4,000 adults hospitalized in the United States with ARDS, pneumonia and/or sepsis, and collect multidimensional data and biospecimens for up to one year from the time of index hospitalization. These data and biospecimens will be used both within the Consortium and by others to enhance our understanding of the mechanistic underpinnings of ARDS, pneumonia and sepsis.

5. Study Design

Please see APS Consortium Study Protocol A (full protocol) for a full description of study design. In brief, the APS Consortium study is a multicenter observational cohort study enrolling participants with acute cardiovascular or pulmonary organ dysfunction in the context of ARDS, pneumonia, sepsis, or a condition at high risk to progress to one of these syndromes. Targeted sample size for enrollment is 4,000 participants. Biospecimens including blood, respiratory samples, circulating blood cells, urine, stool, and

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oral, nasal, and rectal swabs will be collected. Rich clinical data will be collected from the medical record. These data will be used to phenotype multiple domains of health.

6. Participant Enrollment

6.1 Eligibility Criteria

Eligibility criteria for Protocol B (alteration protocol) are identical to Protocol A (full protocol). These eligibility criteria are shown below.

6.1.1 Inclusion Criteria

To be eligible for enrollment, a patient must meet all the following inclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Age ≥ 18 years old.

2. Admitted (or planned to be admitted) to an ICU or other in-patient hospital location where IV vasopressors or advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula) are routinely provided (referred to as an "eligible unit.")

3. Acute cardiovascular or pulmonary organ dysfunction defined by meeting at least one of the two criteria below:
    i) New receipt of invasive mechanical ventilation, non-invasive ventilation, high flow nasal cannula, or supplemental oxygen at a flow rate of ≥ 6 lpm for acute hypoxemia.
        → Patients who use chronic oxygen therapy are eligible to participate if they are receiving at least 6 lpm higher than their baseline oxygen requirement (e.g., a patient on 3 lpm O2 at baseline is eligible if they require ≥9 lpm for hypoxemia) or are started on advanced respiratory support (invasive mechanical ventilation, non-invasive ventilation, or high flow nasal cannula).
    ii) Receipt of intravenous infusion of a vasopressor medication for at least one hour.

4. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) is attributed to an acute inflammatory condition, including but not limited to any of the following:
    i) Any infection including pneumonia.
    ii) Aspiration pneumonitis.
    iii) Pancreatitis.
    iv) Auto-inflammatory condition such as:
        a. Hemophagocytic lymphohistiocytosis.
        b. Suspected acute rheumatologic or auto-immune disease with pulmonary or cardiovascular manifestations.
        c. Suspected cryptogenic organizing pneumonia presenting acutely.
        d. Suspected diffuse alveolar hemorrhage.
        e. Suspected acute anaphylaxis.
        f. Suspected acute pulmonary drug toxicity.

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6.1.2 Exclusion Criteria

To be eligible for enrollment, a patient must not meet any of the following exclusion criteria at the time of the first study-specified biospecimen collection (Time 0):

1. Patient/LAR declines participation.

2. Acute cardiovascular or pulmonary organ dysfunction (inclusion criterion #3) has been present for > 48 hours.

3. Patient has been in an eligible unit (inclusion criterion #2) for more than 120 hours (five days).

4. The clinical team has initiated transfer of the patient to a lower level of care that does not meet inclusion criterion #2 (such as placement of a transfer order out of the ICU).

5. Patient desires comfort measures only.

6. Patient is a prisoner.

7. Patient had out-of-hospital cardiac arrest leading to this hospitalization.

8. Residence immediately before this hospitalization in a long-term acute care facility.

9. Presence of tracheostomy for respiratory failure.

10. Home invasive mechanical ventilation or non-invasive ventilation (except patients with non-invasive ventilation prescribed as a treatment for a sleep disorder may participate).

11. Suspected cause of the patient's acute cardiovascular and/or pulmonary dysfunction (inclusion criterion #3) is an alternative condition (not ARDS, pneumonia, or sepsis), including but not limited to the list below:

    i) Drug overdose (without aspiration, lung injury, pneumonia, or infection).
    ii) Trauma (without aspiration, pneumonia, or infection).
    iii) Chronic lung disease without suspected infection, aspiration, or inflammation.
    iv) Asthma, COPD, sarcoidosis, interstitial lung disease, neuromuscular respiratory failure.
    v) Status epilepticus.
    vi) Acute pulmonary embolism.
    vii) Acute decompensated heart failure.
    viii) Diabetic ketoacidosis.
    ix) Acute stroke or intracranial hemorrhage.
    x) Acute bleeding (GI bleeding, post-procedural bleeding, hemolysis).
    xi) Cytokine release syndrome due to chemotherapy.

12. Inability or unwillingness to complete study-specified blood draws, for example, due to local policies about hemoglobin thresholds for research blood draws.

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6.1.3 Rationale for eligibility criteria

The goal is to enroll patients with acute cardiopulmonary dysfunction that is due to either (a) ARDS, pneumonia, or sepsis, or (b) an acute inflammatory condition that places patients at high risk for short-term development of ARDS, pneumonia, or sepsis. Patients with common critical care conditions that may result in acute cardiopulmonary dysfunction, but which have established pathophysiologic mechanisms distinct from ARDS, pneumonia, and sepsis will be excluded. This approach will optimize the ability to understand the pathophysiology of ARDS, pneumonia, and sepsis, whereas an approach that limited enrollment to patients who meet the historical syndrome definitions would limit our ability to identify new, meaningful phenotypes. In addition, this approach recognizes the difficulty in making definitive clinical diagnosis of historical syndromes (ARDS, pneumonia, and sepsis) at the time of enrollment among critically ill patients. By taking this approach to inclusion criteria, the APS Consortium is expected to further improve our ability to make diagnoses in real time.

Potential participants of all sexes, races, ethnicities, language proficiencies, and nationalities are invited to participate. Enrollment in the cohort will not be limited by the language(s) spoken by the participant. All adult patients across the age span are invited to participate; pediatric participants are not the focus of this project and will not be enrolled.

Pregnant individuals are eligible to participate in the APS cohort because pregnant patients experience APS syndromes at rates higher than the general population of similar age, and there is no strong rationale to exclude such participants. The risks of the procedures in this study are not greater for pregnant participants than for other participants.

Prisoners are excluded because there is concern that their participation may not be fully voluntary.

Pediatric participants <18 years old are excluded because they are frequently admitted to different, specialized pediatric hospitals, the tools for assessing organ dysfunction and molecular phenotyping are different for children, and the focus of this study is on adults.

6.2 Definition of Enrollment Time Zero

The calendar day of the first study-specified biospecimen collection is defined as “Day 0.” The study schedule of events follows “study day” nomenclature, with enrollment day (the day of the first biospecimen collection) identified as Day 0. Study Day 1 is the calendar day following enrollment. Study Day -1 is the calendar day before enrollment. In some circumstances, consent for participation may be obtained after Day 0 (such as on Day 1) or prior to Day 0 (such as Day -1).

Time zero for enrollment is defined as the time of the first biospecimen collection for the study, regardless of the timing of hospital admission, and development of ARDS, pneumonia, or sepsis. A patient is considered enrolled when the first study-specified biospecimen collection occurs. Time stamps will be collected for key events, such as hospital admission, meeting study eligibility criteria, and collection of biospecimens, so that analyses can evaluate the time between these events.

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7. Study Procedures During Index Hospitalization

This section describes study procedures during the index hospitalization for patients participating in the study under alteration of informed consent.

7.1 Data collection during index hospitalization

7.1.1 Data collected by direct observation or medical record review

Patients participating in the study under alteration of informed consent (Protocol B) are unlikely to be able to answer interview questions for collection of study data. For participants unable to answer interview questions, below are domains of data that may be collected by direct observation from study personnel or medical record review. If a patient later consents for study participation and converts to study Protocol A, the research team may collect these data at that time via participant and/or surrogate interview.

- Contact information of patient and family members
- Skin color (Monk skin tone scale)
- Smoking status
- Alcohol use
- Employment status
- Education
- Current/former opioid misuse

7.1.2 Medical record data abstraction

Medical record data abstraction may occur at varying times during the hospitalization based on operationalization at a given site. These data, whether extracted at the time of a given study visit or near or after hospital discharge, will be tied to the schedule of events. Features of data abstraction will be extracted to correspond with the baseline visit as well as subsequent visits. Domains of data collection via medical record review include:

- Contact information of patient and family members, including home address and type of residence
- 9-digit zip code. This allows for linkage to other datasets to capture measures of social vulnerability (e.g., Social Vulnerability Index, Social Deprivation Index, Area Deprivation Index), as well as healthcare availability and rurality. Note: to avoid risk of re-identification of study subjects, zip code will be stripped from shared datasets; data on social vulnerability, healthcare availability, rurality, and other factors obtained by linkage to 9-digit zip code will be retained as categorial variables in shared datasets.
- Demographics

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- Home medications
- Biometrics (height, weight)
- Language fluency
- Place of residence
- Chronic health conditions (including tobacco use, alcohol misuse, and opioid misuse)
- Health insurance status
- Prior hospitalizations
- Clinical laboratory results, medications, and vital signs, including SOFA score elements
- Therapies administered (including respiratory support therapies)
- Discharge disposition

7.1.3 Acute Respiratory Distress, Pneumonia, and Sepsis Syndrome Classification

Enrolled participants will be evaluated for ARDS, pneumonia, and sepsis utilizing detailed, investigator driven review and application of published and accepted clinical criteria for each syndrome. To evaluate for ARDS, pneumonia, and sepsis, investigators will evaluate data and the clinical record for each participant for Study Day -2 through Day 7 while the patient is hospitalized (the phenotyping observation window). Individual criteria for each syndrome will be collected and recorded separately allowing for patients to be classified as ARDS, Sepsis, and/or Pneumonia using multiple published criteria (e.g., for sepsis using both "sepsis-2" and "sepsis-3" criteria). These data will then be used to code variables in our dataset that classify patients according to syndrome definitions. Standard operating procedures will be provided to each site to ensure accuracy and consistency of syndrome adjudication.

ARDS will be identified based on the Berlin Definition with the additional modifications proposed in the New Global Definition of ARDS.[2,3] Trained physician investigators will determine the presence of potential precipitating factors for ARDS (e.g., sepsis, aspiration, pancreatitis). Chest radiographs and chest computed tomography scans conducted for clinical purposes during the observation window will be reviewed by trained physician investigators for bilateral infiltrates consistent with ARDS. The type of chest imaging, time and date of imaging acquisition, and bilateral infiltrates present, absent, or equivocal will be recorded in case report forms. The presence or absence of pleural effusions will also be recorded. Arterial blood gas (ABG) values drawn for clinical purposes and corresponding fraction inspired oxygen percentage (FiO2) will also be recorded in case report forms. If an ABG is unavailable on a given day, SpO2 and corresponding FiO2 will be recorded to calculate the S/F ratio. Invasive and non-invasive oxygenation and ventilation parameters will also be extracted from the medical record. We will apply strict physiologic criteria in ARDS definitions to determine the presence or absence of ARDS daily, timing when ARDS criteria are met, and the severity of ARDS based the level of hypoxemia (P:F and S:F ratios).

Participants will be assessed for the presence of pneumonia based on radiographic, clinical, and laboratory criteria, including those published by CDC and the Infectious Disease Society of America/American Thoracic Society.[4,5] Chest imaging conducted for clinical purposes during the phenotyping observation window will be reviewed by trained physician investigators for new/persistent/progressive infiltrates, consolidation, or cavitation consistent with a pneumonia event.

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Clinical signs and symptoms potentially indicating a respiratory infection will be identified, including vital signs, laboratory results, and clinical status. Assessment of clinical status will include: 1) new onset purulent sputum or change in character of sputum, increasing respiratory sections, or increasing suctioning requirements, 2) new onset or worsening cough, dyspnea, or tachypnea, 3) rales or bronchial breath sounds, and 4) worsening gas exchange. These data will form the core dataset for classifying the presence and absence of pneumonia.

Sepsis will be identified based on the Sepsis-2 and Sepsis-3 definitions of sepsis, severe sepsis and septic shock.[6,7] Trained physician investigators will review the medical record to determine if an infection is confirmed, suspected, not suspected, or unknown daily for the observation window. The presence of positive culture will not be required to identify the confirmed or suspected presence of an infection; however, positive cultures can be used to help determine the presence of sepsis. Suspected sources of infection will be adjudicated by the physician investigators. The physiology of sepsis and septic shock will be classified based on the change in Sequential Organ Function Assessment (SOFA) scores, the presence or absence of the systemic inflammatory response (SIRS) criteria, the presence or absence of vasopressors, and values for lactate. These data will be used to facilitate sepsis classification by the investigator adjudicators.

7.2. Biospecimen collection during index hospitalization

7.2.1 Interplay between clinical and research procedures for biospecimen collection

Aliquots of biospecimens collected on this research protocol may be sent for clinical laboratory testing if such testing is judged to be of potential benefit to the patient. Clinical laboratory testing is optional and may be completed at the discretion of the local site team. If clinical laboratory testing is completed, results may be delivered to clinicians caring for the patient according to local practice patterns.

Additionally, if residual volumes of biospecimens collected clinically meet the volume and collection time needed to fulfill biospecimens outlined in protocol schedule of events of this protocol, those residual samples may be used for research purposes in this protocol.

7.2.2 Blood specimen collection during index hospitalization

During the index hospitalization, blood will be collected from patients at multiple timepoints, collected in EDTA or sodium citrate tubes (for protein or metabolite measurements), PAXgene tubes (for nucleic acids), CPT tubes (for peripheral blood mononuclear cells), and sodium heparin tubes (for whole blood CyTOF and flow cytometry). Blood will be collected at the timepoints indicated in the schedule of events for biospecimen collection and will be coordinated with blood draws for clinical purposes as much as possible.

7.2.3 Respiratory specimen collection during index hospitalization

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During the index hospitalization, the following respiratory specimens will be collected: 1) nasal swabs to facilitate both microbiome profiling and pathogen detection, 2) oral swabs, 3) tracheal aspirate (TA) from patients receiving invasive mechanical ventilation, 4) heat moisture exchanger (HME) filter fluid from patients receiving invasive mechanical ventilation.

7.2.4 Other specimens during the index hospitalization

Participants will have rectal swabs and stool samples serially collected for the primary purpose of microbiome profiling and urine collected for future use.

7.3. Imaging studies during index hospitalization

During the index hospitalization, investigators will review chest imaging completed clinically during the observation window for clinical phenotyping (Study Day -2 through Day 7) and enter key findings into the study data collection forms. Additionally, as detailed in the schedule of events section, serial chest x-ray (CXRs) and CT scan images that were performed as part of the participant's clinical care will be collected and uploaded to a common, secure site, with eventual deposition into BioData Catalyst. The initial goal is to upload up to 3 CXRs and one CT scan (chest CT preferred, abdominal CT acceptable when no chest CT is available) completed during the index hospitalization as part of clinical care. The number of images uploaded per patient may change over time. These stored images will be used by the APS investigators and will be available in the APS databank for future work.

8. Schedule of Events

Study procedures, including collection of data, biospecimens, and radiographic images, are detailed in tables within this section.

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Table 3. Schedule of Events: Data collection (Protocol B - alteration protocol)

Event [a]	Time Point							
	Study Day (in-hospital only)					At Discharge	Hospital Summary	3-months by telephone (Study Day window: Day 85-135)
	-2	-1	0[b]	1 - 7	8-14			
A. Eligibility and Baseline Data								
Eligibility Criteria			X					
Attempts to obtain informed consent				X	X			X
Baseline History			X					
Demographics			X					
Chronic Medications			X					
Admitting Diagnoses			X					
B. Observation or Medical Record Review [c]								
Contact Information			X					
Skin Color			X					
Alcohol Use			X					
Smoking Status			X					
C. Data Collection								
Daily Data								
- Vital Signs	X	X	X	X				
- Laboratory Values	X	X	X	X		X		
- Ventilator Data	X	X	X	X				
- Select Medications	X	X	X	X		X		
- Fluid Balance	X	X	X	X				
- Cumulative IV Sedation[d]	X	X	X	X				
- Sedation/Delirium	X	X	X	X				
- Blood Products	X	X	X	X				
APS Classification	X	X	X	X				
Outcome Data								
- Mortality							X	
- Discharge location						X		
- Organ Failures	X	X	X	X		X	X	
- ECMO / Prone position							X	

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Table 3 Footnotes

a. The data collection schedule of events displays the study days from which data will be collected. The collected data will reflect patient status on those study days. The data may be collected later in a respective fashion.

b. Study Day 0 is anchored on when the first study-specific biospecimen is collected. The time of first biospecimen collection is termed Time 0 and denotes the time a patient is enrolled into the study. Per study eligibility criteria, enrollment must occur within 48 hours of meeting inclusion criteria.

c. Due to the patient's status, an interview will not be possible while a patient is participating in the study under alteration of informed consent. Contact information, skin color, smoking status, and alcohol use status will be collected as possible through direct observation and medical record review.

d. Cumulative amounts of continuous IV administration of select sedation agents will be collected from Study Day -2 to 7.

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Table 4. Schedule of Events: Biospecimens collected during index hospitalization (Protocol B – alteration protocol)

Event	Index Hospitalization (Study Day)				
	Day 0[a]	**Day 2**	**Day 4[b,c]**	**Day 6[c]**	**Day 14[c]**
Acceptable study days for collection	Day 0-1	Day 2-3	Day 3-5	Day 6-8	Day 14-17
Max Total Blood Volume (ml)[d]	33.2	15.2			
- EDTA tubes (total mL)	2x 6ml (12)	1x 10ml (10)			
- Extra EDTA tube if CPT not collected (total ml)[d]	1x6ml (6)				
- RNA Paxgene tubes (total mL)	1x 2.5ml (2.5)	1x 2.5ml (2.5)			
- CPT tubes (total mL)[e]	2x 8ml (16)				
- Na citrate tubes (total mL)	1x 2.7ml (2.7)	1x 2.7ml (2.7)			
- Buffy coat from EDTA	From EDTA				
- RBCs	From EDTA				
Urine tubes (total mL)	1x 5ml (5)				
Oral swab	x	x		x	
Nasal swab	x	x		x	
Rectal swab	x	x		x	
Stool	x	x		x	
HME filter fluid[f]	x	x	x	x	x
Tracheal aspirate[f]	x	x	x	x	x

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Table 4 Footnotes

a. Study Day 0 is anchored as the calendar day when the first study-specified biospecimen is collected. By study eligibility criteria, this must occur within 48 hours of the patient meeting eligibility criteria. The first biospecimen collected will, by definition, be collected on Day 0. Other biospecimens scheduled for collection on Day 0 can be collected as late as Day 1.

b. Day 4 specimens are only collected from participants on invasive mechanical ventilation (IMV). Day 4 specimen collection is preferred on Study Day 4 or 5 but may be collected on Day 3 if Day 2 specimens were collected on Day 2 (instead of Day 3).

c. Patient participating in the study under alteration of informed consent will not have blood collected for study Day 4, Day 6, or Day 14. This ensures that research blood draws for participants without informed consent for study participation will not be completed more than 2 times in a week and that total blood volume for this research study will not exceed 50 ml in 8 weeks (criteria used by the Office of Human Research Protections to define minimal risk for adults who are not healthy).[8]

d. Participants who do not have blood collected for cellular analyses (CPT tubes) will have an additional 6 ml tube of EDTA collected at the study visit on Day 0. Participants who do have blood collected for cellular analyses (CPT) will not have this extra tube of EDTA collected.

e. During processing of CPT tubes, plasma will be saved and stored when possible.

f. HME filter fluid and tracheal aspirate fluid only collected from participants on invasive mechanical ventilation (IMV).

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Table 5. Schedule of events: Storage of clinically-obtained radiology images (Protocol B – alteration protocol)

Images from the following radiographic studies performed as part of clinical care will be collected by study personnel and uploaded into the study database.

Imaging type	Procedures
In-hospital clinically obtained CXRs	Upload up to 3 CXRs per participant. Details of which CXRs to upload will be outlined in study operating procedures. Initially, CXRs targeted for uploading will be CXRs completed closest to 8:00am on Day 0, Day 2, and Day 6.
In-hospital clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan completed closest to 8:00am on Day 0. Chest CT preferred, abdominal CT acceptable if no chest CT performed during hospitalization.
Post-discharge clinically obtained CT scans	Upload up to 1 scan per participant. If multiple scans are available, select the scan closest to 6 months. Chest CT preferred, abdominal CT acceptable if no chest CT performed between hospital discharge and 12 months.

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Table 6. Study procedures in Protocol A (full study completed with informed consent for study participation) that will not be performed on Protocol B (this protocol with alteration of informed consent).

Study procedures that will <u>not</u> be completed with alteration of informed consent. **These study procedures may only be initiated after informed consent for study participation and transition to Study Protocol A.**
Day 4 blood collection
Day 6 blood collection
Day 14 blood collection
Non-bronchoscopic bronchioalveolar lavage (NBBAL)
Long-term outcome surveys, including: - 3-month surveys - 6-month surveys - 12-month surveys
Long-term outcome in-person visits, including: - 3-month visit - 6-month visit - 12-month visit (which includes pulmonary function tests and chest CT)

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9. Use of Data and Biospecimens

Data, biospecimens, and radiographic images collected in the APS Consortium will be used for 3 purposes:

1. Completion of APS Consortium-wide science as outlined in appendices to Protocol A (funded by the APS Consortium budget).

2. Completion of APS Clinical Center-specific science as outlined in grants proposals from Clinical Centers investigators and selected for funding via the NIH peer review process (funded by the APS Consortium budget).

3. Banking for use in future ancillary studies conducted by investigators both inside and outside the APS Consortium (biobanking is funded by the APS Consortium budget but ancillary studies for use of bio-banked data and specimens is not funded by the APS Consortium budget).

During conduct of the APS Consortium study procedures, data, biospecimens, and radiographic images will be collected by enrolling sites and transmitted to the APS Consortium Coordinating Center at Vanderbilt University Medical Center. Data and biospecimens will be organized and catalogued at the coordinating center. The coordinating center will distribute data and biospecimens as needed to complete Consortium-wide science and Clinical Center-specific science. Additionally, the coordinating center will periodically transmit data and biospecimens to repositories managed by the National Heart, Lung, and Blood Institute (NHLBI) -- BioData Catalyst (data repository) and BioLINCC (biospecimen repository).

During the period of performance for the APS Consortium, requests for data and/or biospecimens for use in ancillary studies will be reviewed for approval by the APS Steering Committee. After the completion of the APS Consortium period of performance, requests for data and/or biospecimens for use in ancillary studies will be governed by NHLBI through the BioData Catalyst and BioLINCC programs.

10. Terminology and Statistical Considerations

A complete description of the terminology the APS Consortium will use to describe phenotypes and the statistical approaches is available in APS Consortium Study Protocol A (full protocol). In brief, the Consortium will be evaluating historical syndromes (e.g., ARDS, pneumonia, sepsis), previously defined phenotypes (e.g., hyper- vs hypo-inflammatory, SRS1 vs SRS2, high elastance vs low elastance), and novel phenotypes discovered within the science of the Consortium itself. Analyses of Consortium-wide science will be led by the APS Consortium Coordinating Center. Analyses of Clinical Center Specific studies will be led by the Clinical Center driving that study. Ancillary studies will be analyzed by the investigative team running the ancillary study.

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11. Human Subjects

11.1 Risks and benefits

The APS Consortium Study is an observational prospective cohort study with collection of data and biospecimens with methods routinely used in current routine medical practice. This protocol (Protocol B – alteration protocol) describes the study procedures that will be completed while a patient is participating under alteration of informed consent. While participating under alteration of informed consent, patients will undergo study procedures that are minimal risk. The US Code of Federal Regulations (CRF 46.102(j)) defines minimal risk as "the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests" (https://www.ecfr.gov/on/2018-07-19/title-45/subtitle-A/subchapter-A/part-46#46.102).[9]

Table 7 details each study procedure for patients participating with alteration of informed consent and the rationale for each being minimal risk. These study procedures are consistent with guidance from the Office of Human Research Protections on research procedures that present no more than minimal risk to human participants (https://www.hhs.gov/ohrp/regulations-and-policy/guidance/categories-of-research-expedited-review-procedure-1998/index.html).[8]

The primary risks of study participation are related to the potential of disclosure of private health information and complications from biospecimen collection procedures. These risks will be minimized by using a secure REDCap data collection system for data entry and storage, maintaining good clinical practice procedures at all sites for handling of private health data, de-identifying data and specimens prior to long-term storage outside the consortium, training study teams on best practices for biospecimen collection, and collecting biospecimens at times that clinical labs are already being collected when possible.

Samples taken as part of this study may be used to evaluate human genetics in the future. Genetic testing results will not be linked to identifiable patients nor placed in the medical record. Inadvertent disclosure of genetic testing results could influence insurance policies or future employment. Genetic research that produces data that could result in identification of the patient will not be pursued with biospecimens collected under alteration of informed consent without consent ever being obtained except for patients known to have died prior to the end of the 3-month study time window (Study Day 85-135).

Participants are not expected to receive direct personal benefits for participating in the APS Consortium study. On a societal level, benefits of participants joining the APS Consortium study include contributing to increased knowledge about ARDS, pneumonia, and sepsis, which could lead to medical advances that ultimately decrease morbidity and mortality from these syndromes.

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Table 7. Potential risks to participants enrolled in the APS Consortium study by study procedure that will be completed with alteration of informed consent (Protocol B – alteration protocol).

Study procedure	Potential risks	Study methods to minimize risks	Rationale for completing study procedure with alteration of informed consent
Collection of personal data	Inappropriate disclosure of private health information (loss of confidentiality/privacy).	Only study personnel trained in good clinical practice for clinical research studies will be involved in data handling. Each enrolling site will maintain appropriate training and certification in protection of human participants for all study personnel involved in interacting with patients and/or handling data. Data will be entered into and stored within REDCap, a secure internet-based data collection tool with multiple layers of security, including authentication of end-users, automatic user logout after 30 minutes of inactivity, a time-stamped audit trail, encrypted information transmission, and firewall protection of documents. Additional information on REDCap is available at: www.project-redcap.org.	Data collection will be noninvasive and rely on extraction from the medical record. Data will be stored in secure REDCap tools. Only deidentified data will be placed in long-term storage outside the consortium. The probability of harm anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults hospitalized with ARDS, pneumonia, or sepsis.
Blood collection (phlebotomy)	Pain at phlebotomy site, infection at the phlebotomy site, bleeding, damage to surrounding nerves, transient light-headedness, fainting/syncope.	Only study or clinical personnel trained in phlebotomy will collect blood for this study. Patients will be positioned in a safe location for phlebotomy, such as supine in a bed. When possible, blood will be collected from pre-existing vascular catheters and timed with blood draws for clinical care to avoid additional phlebotomy.	Patients participating under alteration of informed consent will have a maximum of 2 blood draws and <50 ml blood collected, consistent with guidance from the Office of Human Research Protections for minimal risk blood collection research procedures.[8] Patients in this population typically have pre-existing vascular catheters in place, providing a convenient and clean point of collection. Blood for research will be collected at the same time as blood collection for clinical purposes whenever possible.
Urine collection	No plausible risks identified.	Only study or clinical personnel trained in the collection of urine will collect urine for this study. Urine will be collected via patient voiding or collection from pre-existing urinary catheters. No invasive procedures will be initiated in the study to collect urine.	No plausible risk identified. Patients within the study population routinely have urine collected while they are in the hospital. Invasive procedures will not be used to collect urine in this study. The probability of harm or discomfort anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults hospitalized with ARDS, pneumonia, or sepsis.
Collection of oral, nasal, and rectal swabs	Localized transient irritation, pain, and/or bleeding may occur at the swab site.	Only study or clinical personnel trained in the collection of oral, nasal and rectal swabs will collect swabs for this study. Personnel will be trained to hold pressure on the swab site if it bleeds.	These types of swabs are routinely collected from hospitalized adults. The probability of harm or discomfort anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults hospitalized with ARDS, pneumonia, or sepsis.

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Study procedure	Potential risks	Study methods to minimize risks	Rationale for completing study procedure with alteration of informed consent
Stool collection	No plausible risks identified.	Stool will only be collected via participants freely stooling or via stool collection systems in place for clinical care (e.g., rectal tube, fecal incontinence bag). No invasive procedures will be initiated by the study to collect stool.	No plausible risk identified. The probability of harm or discomfort anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults hospitalized with ARDS, pneumonia, or sepsis.
HME (ventilator) filter collection	Inadvertent disconnection of ventilator tubing for longer than necessary to collect the filter, which could rarely result in low oxygen levels, organ damage, and death if not recognized. The risk of patient injury from HME (ventilator) filter collection is considered very low.	Only study or clinical personnel trained in the collection of HME (ventilator) filters will collect the filters. Ventilators at all study sites have alarms that alert clinical teams when ventilator tubing is disconnected. HME filters are routinely used for clinical care. Patients receiving invasive mechanical ventilation are routinely disconnected from the ventilator in clinical care daily. When possible, HME filters will be collected during a disconnection of the ventilator that is occurring as part of clinical care.	Ventilator filters are routinely changed as part of clinical care of patients on invasive mechanical ventilation. The collection of HME filter fluid is a non-invasive procedure that entails capturing filters that would otherwise be discarded. The probability of harm or discomfort anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults on invasive mechanical ventilation.
Tracheal aspirate fluid collection	Bleeding, localized pain, drop in oxygen levels (hypoxemia), and dislodgement of the tracheal tube. A drop in oxygen levels from tracheal aspirate collection is very unlikely to cause patient harm.	Only study or clinical personnel trained in the collection of tracheal aspirate fluid will collect this specimen. Tracheal suctioning is a routine clinical procedure.	Tracheal aspirate collection is a routine clinical procedure for adults on invasive mechanical ventilation. Tracheal aspirate collection for this protocol will be done at times that tracheal aspirates are being collected as part of clinical care, whenever possible. The probability of harm or discomfort anticipated from this research procedure is not greater than that from the routine in-hospital experience of adults on invasive mechanical ventilation.
Storage of biospecimens for future testing	Biospecimens will be placed in long-term storage, where they may be retrieved for future studies. Inadvertent disclosure of testing results could cause loss of privacy.	Biospecimens for future testing will be deidentified before they are stored. These biospecimens will be labeled with a study number and the link between that study number and the patient's identity will not be provided to long-term storage facilities. Thus, the risk of inadvertent disclosure of identifiable information is judged to be very low. Results of testing will not be placed in the medical record.	Stored specimens will not have any patient identifiers. Thus, the probability of harm is judged to be very low and not greater than the normal experience of adults hospitalized with ARDS, pneumonia, or sepsis. Genetic research that produces data that could result in identification of the patient will not be pursued with biospecimens collected under alteration of informed consent without consent ever being obtained except for patients known to have died prior to the 3-month study time point.

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11.2 Alteration of Informed Consent

11.2.1 Approach to informed consent for this study

This protocol (APS Study Protocol B – alteration protocol) describes study procedures that will be completed by patients participating in the study through an alteration of informed consent procedure and without written informed consent for study participation. This protocol will only govern study conduct for patients participating under alteration of informed consent.

A companion protocol (APS Study Protocol A – full protocol) governs study conduct for patients participating with written informed consent for study participation.

11.2.2 Description of and Rationale for two study protocols (Protocol A and Protocol B)

A key objective of this study is to rigorously phenotype ARDS, pneumonia, and sepsis, among severely and critically ill patients as early as possible after onset of these diseases. To achieve this objective, patients must be enrolled as early as possible in their course of illness. Early biospecimens are crucial for the objectives of the APS Consortium. Up to 75% of patients in the ICU experience delirium or altered mental status prohibiting their ability to provide informed consent.[10] Frequently, LARs/surrogates for critically ill adults are not available to provide written informed consent for research participation in a timely fashion. For example, prior work has shown that at a publicly funded hospital, 18% of eligible patients for ARDS clinical trials were not enrolled due to the patient not having capacity for consent and no LAR/surrogate being available.[11]

We have an option for participants to enter the study with alteration of informed consent for a subset of study procedures that involve minimal incremental risk above ongoing clinical care, because obtaining written informed consent at the time of enrollment would be impracticable in a significant subset of the population eligible for the study, and conducting the study without that subset would result in detrimental selection bias. Requiring written informed consent prior to study enrollment would compromise the integrity of the study by biasing the enrolled sample toward a less severely ill population (those who can consent for themselves). For example, in the Etiology of Pneumonia in the Community (EPIC) Study, eligible patients not enrolled were twice as likely to receive invasive mechanical ventilation compared to eligible patients who were enrolled.[12] Furthermore, requiring informed consent prior to enrollment could result in systemic exclusion of patients from minoritized groups. For example, in an ARDS Network trial that enrolled patients with ARDS, non-enrollment due to lack of an available LAR/surrogate was 18-times more likely at a publicly-funded hospital compared to a nearby academic medical center.[11]

When a study team identifies a patient as eligible for the study, if the patient can consent for research, or, in the case of the patient not having capacity for consent, if an LAR/surrogate is available, the study team will pursue informed consent for research participation before initiation of study procedures. If consent is obtained, study conduct will be governed by Study Protocol A (separate document).

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Alternatively, when a study team identifies a patient as potentially eligible for the study, if the patient lacks capacity for consent and no LAR/surrogate is available, the patient may enter the study under Study Protocol B described in this document using an alteration of informed consent. This protocol includes only minimal risk procedures. The minimal risk study procedures in Protocol B correspond to identical study procedures in Protocol A. Protocol B contains a subset of study procedures from Protocol A that are minimal risk. Protocol B has no informed consent document.

Participants who enter the study using the alteration of informed consent protocol (Protocol B) will be iteratively approached for consent; if and when informed consent for participation in the APS Study is obtained, the participant will be moved from Protocol B to Protocol A (Figure 1). Greater than minimal risk procedures may be completed after informed consent is obtained among participants who started study participation under alteration of informed consent procedures. Patients who move from Protocol B to Protocol A will seamlessly continue on the APS Study schedule of events and maintain the same "study day" structure initiated at study entry. Study days continue through the transition from Protocol B to Protocol A; that is, if a patient provides informed consent on Day 4, study procedures completed on Protocol B for Day 0 through Day 3 will remain unchanged and Study Day 4 procedures will pick up on Protocol A.

For participants who enter the study with alteration of informed consent, study team members will attempt to obtain informed consent while the patient is in the hospital through Study Day 14 and at the 3-month time point.

Data and biospecimens collected under Protocol A and Protocol B will be pooled for storage and analysis.

An approach of using two protocols to enroll participants – one that governs consented patients (Protocol A) and one that governs patients participating under alteration of informed consent (Protocol B) – is being utilized to minimize selection bias that would occur if only patients who were able to immediately provide written informed consent were enrolled. Outlining minimal risk procedures from the APS Study schedule of events in a separate protocol (Protocol B) enables participants without consent to begin minimal risk study procedures using an alteration of informed consent process. Meanwhile, participants with consent completed may engage in all study procedures, including both minimal risk procedures and greater than minimal risk procedures. Completion of greater than minimal risk procedures enables more rigorous scientific evaluation.

Goals of the study team include:

- Early enrollment of a population that represents the patient population of those suffering from ARDS, pneumonia, and sepsis, including those who are critically ill and often unable to rapidly consent for research.
- Obtain informed consent for study participation prior to initiation of study procedures for as many participants as possible.
- For participants who enter the study with alteration of informed consent, obtain subsequent written informed consent as soon as possible.

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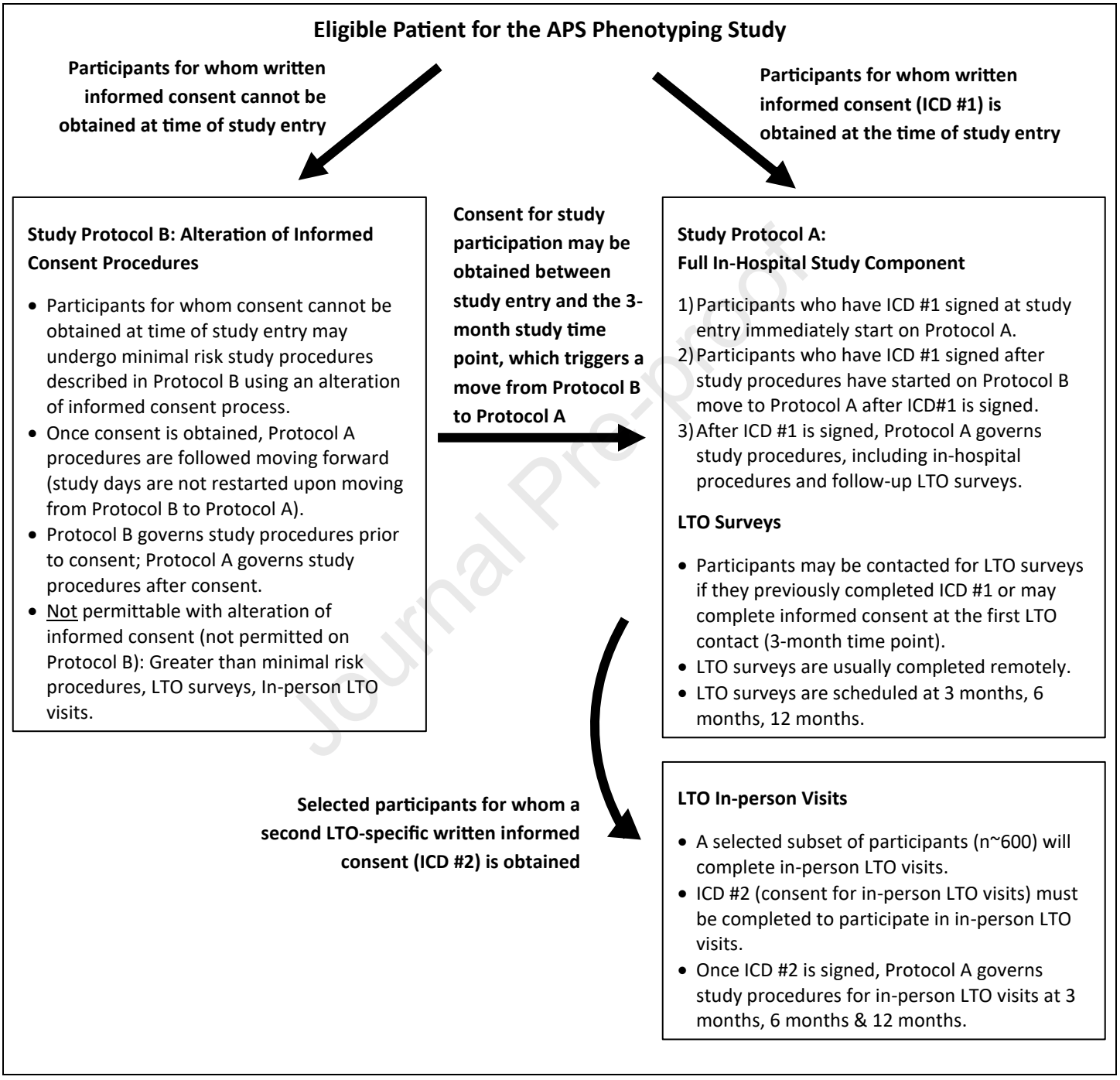
Figure 1. Flow diagram of informed consent procedures.



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11.2.3 Alteration of informed consent process

When a patient does not possess medical decision-making capacity for consent, and an LAR/surrogate is not available for informed consent discussions despite good faith efforts to reach an LAR/surrogate, written informed consent is considered impracticable and the study team may enroll the patient using an alteration of informed consent approach.

To enter a participant into the study without written informed consent for study participation using the alteration of informed consent process, the study team must complete each of the following before study procedures begin:

i. Confirm the patient lacks decision making capacity to consent for study participation. This assessment is completed by a study team member trained in good clinical practice for the conduct of clinical research who is in direct contact with the patient. If the patient cannot meaningfully engage with the study team member (such as, a state of intubation, chemical sedation, and/or not responsive to verbal stimuli), the study team member may consider the patient to lack decision making capacity for consent. If the patient can meaningfully engage with the study team member, the study team member will explain the APS study to the patient and review the informed consent document with the patient. After this explanation, if the patient cannot describe the basic elements of the study, including risks and benefits of study participation, the study team member may consider the patient as not possessing decision making capacity for consent. The study team member will document the process and outcome of capacity assessment in the APS Study Electronic Data Capture instrument.

ii. Confirm no LAR/ surrogate is available to discuss consent for study participation. The study team will make a good faith effort to identify an LAR/surrogate, including at a minimum:

    a. searching for family or other people accompanying the patient in the hospital;
    b. calling phone numbers for the patient, patient's family, and patient's decision makers listed in the medical record;
    c. asking the clinical care team for information on the whereabouts of the patient's family and/or other decision makers.

iii. Confirm with the clinical care team that they believe participation in the study is not in opposition of the patient's best interests.

iv. Confirm that study team will continue to seek consent for study participation from the patient or an LAR/surrogate iteratively while the patient is in the hospital through Study Day 14, and if needed, at 3-months (typically via telephone).

v. Place a participant notification document at the patient's bedside. The participant notification document states that the patient was entered into a research study, provides a description of the study in lay language, states that the research team would like to talk about the study with family and/or medical decision makers for the patient, and provide contact information for the study team. The goals of the participant notification document are to immediately make information about the study available to people who visit the patient in the hospital and facilitate communication between the patient's family/decision maker and the study team as soon as possible. The informed consent document for Protocol A (ICD #1) will be appended to the participant notification document.

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Once the steps above have been completed, the study team should document completion of each step in the APS Study Electronic Data Capture instrument. Thereafter, the study team may begin study procedures described in APS Study Protocol B.

11.2.4 Informed consent after study initiation with alteration of informed consent

The study team will assess the patient for capacity to provide consent for study participation and, if the patient does not possess capacity, search for an LAR/surrogate on at least 4 calendar days per week while the patient is in the hospital through Study Day 14. The study team will consult with the clinical team to identify any individuals who are making medical decisions for the patient during this time period and will attempt to contact these individuals. If consent is not obtained prior to the 3-month study time point for telephone contact (Study Day 85 through Study Day 135), the study team will attempt to obtain consent via telephone contact at that time. The study team will document these efforts to obtain informed consent in the study electronic data capture instrument.

The possible outcomes of the process for seeking informed consent after study entry and the implications of each outcome are detailed below and in Figure 2.

i. Category 1: Participant is enrolled with alteration of informed consent and later has written informed consent for study participation completed (either by the participant or an LAR/surrogate).

   Action for Category 1: Participant may complete minimal risk procedures described in Protocol B before consent is obtained. At the time of written informed consent, the participation moves from Protocol B to Protocol A. After informed consent is obtained, the participant may complete the study procedures described in Protocol A, including both minimal risk study procedures and greater than minimal risk study procedures. The study days remain continuous during the transfer from Protocol B to Protocol A, meaning that study days do not start over at Day 0 upon conversion from Protocol B to Protocol A, but rather Day 0 remains the same after conversion and Protocol A picks up on the Study Day on which consent was obtained. For example, if consent is obtained on Study Day 3, only minimal risk procedures may be done for Study Days 0, 1, and 2; then after consent is completed on Day 3, greater than minimal risk procedures may start on Day 3 for study procedures scheduled for Day 3 and later.

ii. Category 2: Participant is enrolled with alteration of informed consent and later the patient or LAR/surrogate is available for a decision about consent and declines full study participation moving forward but agrees to use of data and biospecimens already collected.

   Action for Category 2: Study procedures that involve direct contact between the study team and participant will cease. Medical record data collection will continue. Collected data and biospecimens will be retained in the study and available for use in the future.

iii. Category 3: Participant is enrolled with alteration of informed consent and later the patient or LAR/surrogate is available for a decision about consent and declines study participation moving forward and also declines use of data and biospecimens already collected.

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Action for Category 3: All study procedures will cease. Data with information and biospecimens stored at the enrolling site and coordinating center will be destroyed. This will be considered a study withdrawal. A record of a patient enrolling and then withdrawing will be maintained. The study team will attempt to collect the reason for withdrawal and record it in the APS Electronic Data Capture instrument. The study team will not attempt to contact the participant after the 3-month study window. If a participant contacts the study team after the 3-month study window to withdraw from the study, the study team will honor that request for withdrawal. If the participant withdraws from the study long after enrollment, some data and biospecimens may have already been used. In that case, deidentified data and biospecimens that have been transferred to NIH repositories or to laboratories for analysis may not be able to be destroyed. Completed works, such as manuscripts, that used the participant's data and/or biospecimens will not be revised or retracted and data to support those works will be retained.

iv. Category 4: Participant is enrolled with alteration of informed consent and informed consent is never obtained nor declined (the study team never discussed consent for study participation with the patient at a time when the patient had capacity or with an LAR/surrogate).

a. Category 4A: Consent was never obtained and the patient is known to be dead at or before the 3-month study time point for telephone contact (Study Day 85 through Study Day 135). The patient can be confirmed to be dead through several mechanisms, including but not limited to: death in the hospital, family member reports patient death at the 3-month telephone call, obituary for the patient identified.

Action for Category 4A: Participant completed Protocol B. At the time of death, use of data and biospecimens previously collected moves to a non-human subjects paradigm. Previously collected data and biospecimens may be used for future research under a non-human subjects paradigm.

b. Category 4B: Consent was never obtained and the patient is not known to be dead through the 3-month study time point for telephone contact (through Study Day 135). This scenario applies to participants who participated in the study under alteration of informed consent, never had informed consent obtained, and are either known to be alive at Study Day 135 or vital status is unknown at Study Day 135.

Action for Category 4B: Participant completed Protocol B. At Study Day 135, after consent has been shown to be impracticable by consent not being obtained despite a good faith effort to obtain consent while the patient was in the hospital and via telephone calls during the 3-month study window, the patient's participation converts to a waiver of informed consent paradigm. Only minimal risk study procedures will have been completed. Previously collected data and biospecimens may be used for future research under a waiver of informed consent paradigm. Genetic research that produces data that could result in identification of the patient will not be pursued with biospecimens collected from participants in this category.

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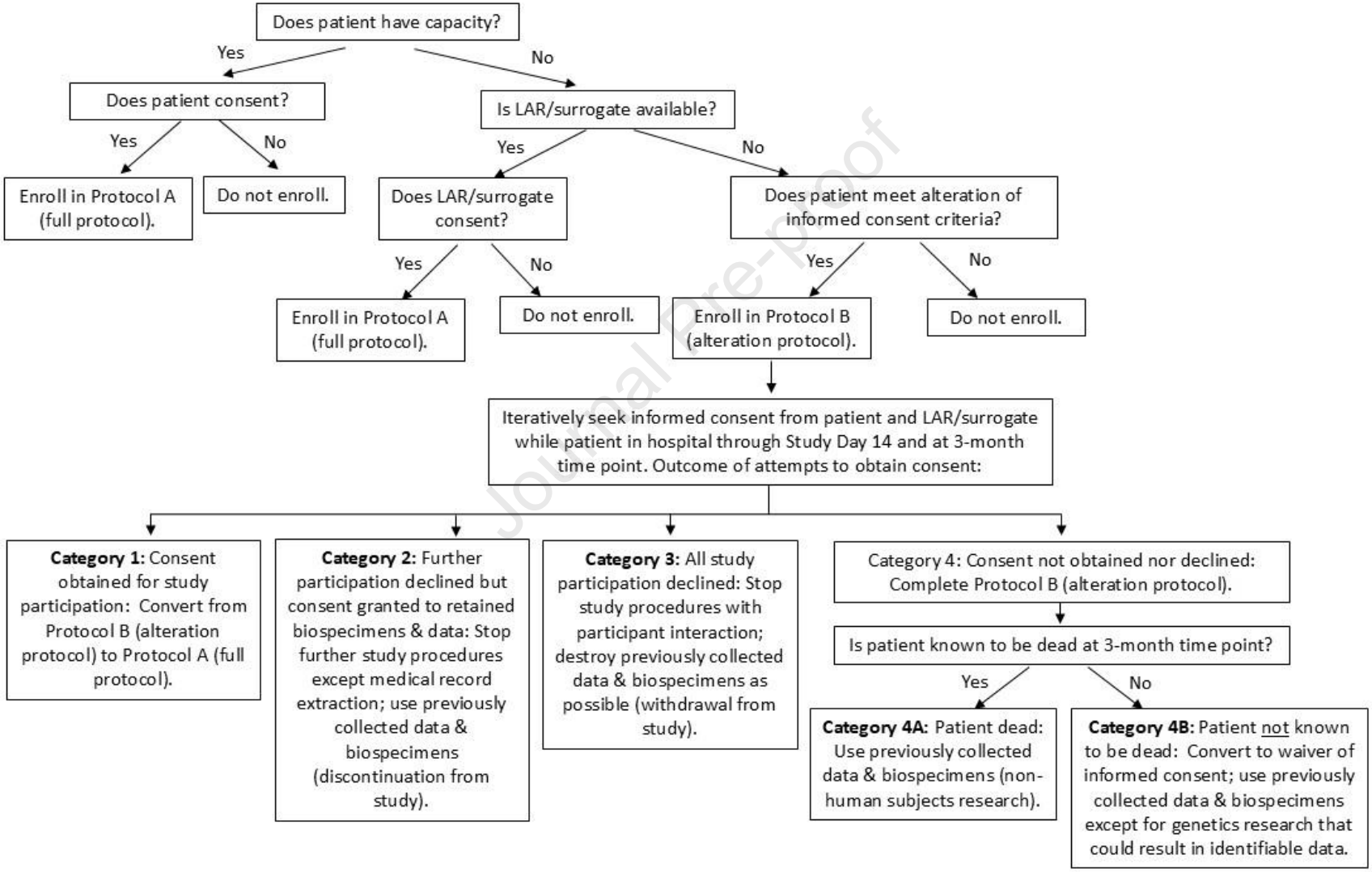
Figure 2. Approach to informed consent and alteration of informed consent after a patient has been confirmed as eligible for the study.



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11.3 Human subjects considerations for data and biospecimen banking and sharing

11.3.1 Storage of data and biospecimens

Data collected as part of the Consortium-wide study, including those captured from the EHR and other hospital databases, will be transferred into the study database via standardized electronic case report forms (eCRFs), which will reside in a centralized database located on secure servers. Study data will be entered and accessed via a secure, password-protected REDCap database website wherein all web-based information is encrypted. REDCap was developed specifically around HIPAA Security guidelines and is recommended by both the Vanderbilt University Privacy Office and Institutional Review Board. REDCap is available to all sites participating in the APS Consortium.

Data transferred to the APS Coordinating Center will include dates (for example, hospital admission date, and date of birth) and contact information to facilitate post-hospital follow-up visits. Biospecimens will be labeled with a study identification number without patient name, medical record number, or date of birth Access to personal health information in study databases will be limited to only those individuals requiring that level of access.

Study data and biospecimens will be stored for an indefinite period of time for future use. Deidentified data and biospecimens will be shared with researchers outside the APS Consortium. For long-term storage of data, personal identifiers will be removed once quality assurance has been confirmed and prior to data lock. All research records will be accessible for inspection by authorized representatives of the IRB, federal regulatory agency representatives, and NIH representatives.

11.3.2 Sharing data and biospecimens

The APS Consortium will develop data management and sharing plans consistent with NIH policies (https://sharing.nih.gov/).

APS Consortium investigators will be permitted to access and use data and biospecimens for the purpose of achieving the Consortium-wide and center-specific project aims directly from the Consortium Coordinating Center. Consortium-wide biospecimens and data will be sent to the central biorepository and study database housed at the Consortium Coordinating Center. Investigators seeking to perform approved ancillary studies with data and/or biospecimens collected by the APS Consortium may request data and biospecimens from the Consortium Coordinating Center before data and biospecimens are deposited in BioData Catalyst and BioLINCC.

Ultimately, the Consortium Coordinating Center will deposit de-identified data and biospecimens in BioData Catalyst and BioLINCC. Once data and biospecimens reach BioData Catalyst and BioLINCC, they will be available to investigators through the governance of BioData Catalyst and BioLINCC without involvement of the Consortium Coordinating Center.

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11.3.4 Co-enrollment with other studies

The APS Consortium steering committee, OSMB, and NHLBI will agree on co-enrollment procedures before participants in the APS study are co-enrolled with other studies. Principles for co-enrollment will include the following:

- Co-enrollment should not affect the scientific goals of the APS Consortium. Co-enrollment will not be permitted if it compromises the scientific integrity and/or statistical power of APS Consortium studies.
- Co-enrollment will be compliant with NIH and NHLBI guidelines and policies.
- Co-enrollment will only be allowed when study procedures for the APS Consortium studies can be achieved. Co-enrollment in the APS Consortium will not be permitted when study procedures in a co-enrolled study would prevent completion of data or biospecimen collection for the APS Consortium study.
- Safe blood collection procedures for critically ill patients will be followed as detailed by the PETAL Network Investigators.[13]

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12. Adverse Events and Safety Monitoring

The procedures for conducting safety monitoring and reporting adverse events are identical between APS Study Protocol A (full protocol) and APS Study Protocol B (alteration protocol).

12.1 Overview of Safety Monitoring

In this observational study that uses routine techniques for data and biospecimen collection, substantial numbers of serious, study-related adverse events are not anticipated. The study will not be overseen by the US Food and Drug Administration (FDA). Safety monitoring will be performed by the investigators, the study's single IRB at Vanderbilt University Medical Center, and an NHLBI-appointed Observational Safety Monitoring Board (OSMB).

12.2 Adverse Events

12.2.1 Paradigm for collecting Adverse Events

In this study, investigators will collect and report adverse events (AEs) that are classified as serious and related to study procedures, those prespecified as adverse events of special interest (AESIs), and those that potentially change the risk: benefit balance for patient participation (Unanticipated Problem (UP)). AEs that meet at least one of the following three criteria will be collected in this study:

1) both serious and study related;
2) AESI;
3) UP.

AEs that do not meet any of these criteria will not be collected.

Events that meet criteria for an AE in the population enrolled in this study will be numerous due to the severe medical conditions these patients have. The proportion of AEs experienced by the study population that are related to study procedures is anticipated to be extremely small. Thus, the paradigm of AE collection and reporting in this study was designed to capture all the events that could potentially represent a safety concern for the study while avoiding overly burdensome AE monitoring for participants, study teams, the IRB, and OSMB.

12.2.2 Definitions for Adverse Events

(i) Adverse Event

An AE is defined as any untoward medical occurrence.

(ii) Serious Adverse Event (SAE)

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A serious AE (SAE) is an untoward medical occurrence that directly causes at least one of the following in the judgement of the study team:

- Death
- Life-threatening condition that places the participant at immediate risk of death
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or a congenital anomaly/birth defect.
- An important medical event not meeting the criteria for one of the outcomes above but, based on medical judgment, jeopardized participant safety or required medical or surgical intervention to prevent one of the outcomes listed in this definition.

(iii) Relatedness of SAEs

SAEs will be evaluated for relatedness to study procedures using the definitions below:

- Definitely Related: The adverse event meets all three of the following criteria: (a) a temporal sequence from study procedure to the adverse event suggests relatedness, (b) the event cannot be explained by the known characteristics of the participant's clinical state or therapies, and (c) evaluation of the participant's clinical state indicates to the study team the experience is definitely related to study procedures.

- Possibly Related: In the study team's opinion, the adverse event has a reasonable possibility of being related to study procedures but one or more of the above criteria for "Definitely Related" are not met.

- Probably Not Related: The adverse event occurred at a time when it could have been caused by study procedures but, in the opinion of the study team, can reasonably be explained by the known characteristics of the participant's clinical state or therapies.

- Definitely Not Related: The adverse event was definitely produced by the participant's clinical state or therapies and not by the study procedures.

- Uncertain Relationship: The adverse event does not meet any of the criteria outlined above and the study team cannot ascertain enough information to classify relatedness of the event.

For the purposes of this study, an adverse event is considered related to study procedures if there is a "reasonable possibility" of a causal relationship between a study procedure and the adverse event or the relationship cannot be determined; this includes events that are classified as definitely related, possibly related, or of uncertain relationship.

(iv) Adverse events of special interest (AESIs)

An adverse event of special interest (AESI) is defined as a pre-specified event of scientific or medical concern that has the potential of being related to study procedures and is important to understand regardless of investigator classifications. There are no AESI for this protocol (Protocol B).

(v) Unanticipated Problem (UP)

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An unanticipated problem is a finding discovered during the conduct of the study that suggests participation in the study may have more risk than was anticipated at the time of study initiation. UPs have the potential to change the risk: benefit balance of the study compared to what was known at the time of study launch.

12.2.3 Reporting Adverse Events

AEs that meet criteria for reporting should be entered into the study's electronic data collection system within 3 calendar days of the study team becoming aware of the AE. Reporting an AE will include a clinical narrative explaining the context of the AE and rationale for the investigator's classification of the event as serious and related, an AESI, or an UP. The study team should also alert the Consortium Coordinating Center for reported events. The coordinating center will report the AE to the sIRB and OSMB within 4 calendar days of receiving the report of an AE (thus, within 7 calendar days of site awareness of the event). Reported events will be followed until resolution.

Figure 3 is a summary flow diagram to assist study teams with deciding which adverse events to report in this study.

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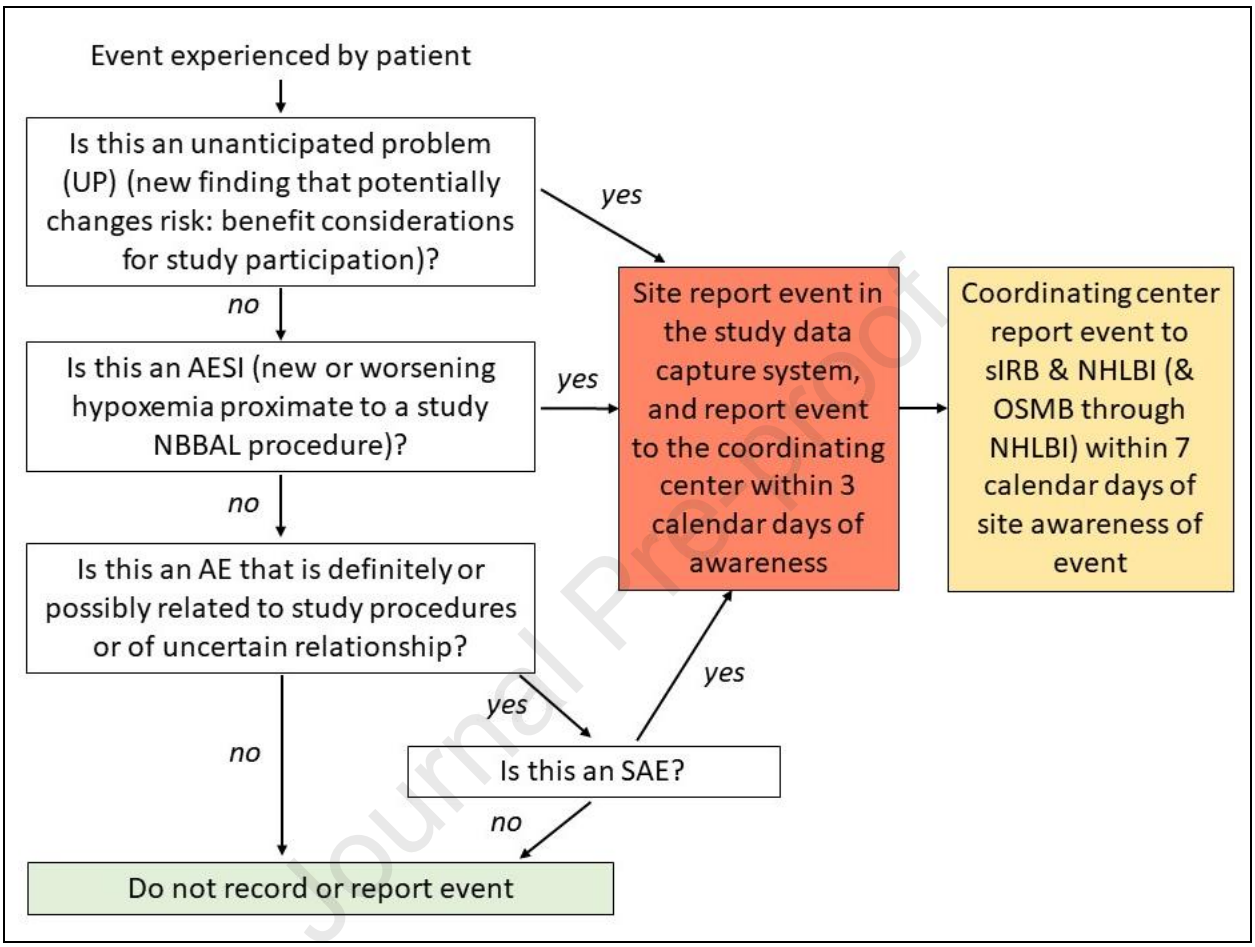
Figure 3. Flow diagram to assist with decisions about AE recording and reporting.



12.3 Observational Study Monitoring Board (OSMB)

The OSMB will be comprised of experts in APS syndromes and fields relevant for this study. The OSMB is appointed by NHLBI. The principal role of the OSMB is to evaluate the safety and integrity of the study. Full details of the OSMB structure and function will be provided in an OSMB charter, which will be reviewed with the OSBM at its first meeting with the study team.

Prior to initiation of study enrollment, the OSMB will review the study protocol and informed consent documents. OSMB meetings will be scheduled regularly in accordance with the OSMB charter. Additionally, the NIH, OSMB, and investigators may call ad hoc OSMB meetings.

The OSMB will regularly monitor several aspects of the study, including the safety, enrollment rates, protocol compliance, cohort demographics and geographic distribution, and data quality and completeness.

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Recommendations to end, modify, or continue aspects of the study will be communicated by the OSMB, through the OSMB executive secretary, to the Consortium Coordinating Center.

12.4 Institutional Review Boards (IRBs)

This is a multi-center cohort study for which a single IRB will be used for the ethical review of the proposed research per NIH policy (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-094.html). Vanderbilt University Medical Center will serve as the single IRB of record. Local context will be reviewed by local IRBs for each participating site.

This protocol (Protocol B) does not have an accompanying informed consent document; all patients participating in this protocol will be participating under alteration of informed consent.

Processes of obtaining informed consent and use of alteration of informed consent should be reviewed during local context review to ensure study procedures are consistent with local laws and standards.

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13. References

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APS Biospecimen Management for Sites

SOP Version:	4.0
Network:	The ARDS, Pneumonia, and Sepsis (APS) Consortium
Funders:	(1) National Heart, Lung, and Blood Institute (NHLBI) (2) National Institute for General Medical Sciences (NIGMS)
Clinical Center Hub Institutions:	(1) University of Pennsylvania (2) University of Michigan (3) Vanderbilt University Medical Center (4) University of Colorado (5) Intermountain Medical Center (6) University of California, San Francisco
Coordinating Center:	Vanderbilt University Medical Center
Coordinating Center Contacts:	Jillian Rhoads, PhD jillian.p.rhoads.1@vumc.org Adrienne Baughman adrienne.baughman@vumc.org Alyssa Merkel, MS alyssa.r.merkel@vumc.org Wanderson Rezende, PhD w.rezende@vumc.org
Purpose:	This document describes the standard operating procedures for managing and requesting APS biospecimen supplies; collecting and processing blood, respiratory, and microbiome biospecimens; and procedures for shipping biospecimens to the Consortium Coordinating Center. All procedures described herein were reviewed and approved by the members of the APS Steering Committee.
Scope:	This SOP applies to all trained personnel collecting, processing, shipping, and managing biospecimens. This document is comprehensive; specific protocols may not apply to all personnel.
Roles and Responsibilities:	All personnel conducting or assisting in the specimen collection and processing protocols must assiduously follow and execute the procedures in this SOP and maintain associated documentation of work performed. The APS CCC is responsible for ensuring that the most current version of this SOP is available to all sites within the consortium.
Training/Requirements:	Individuals participating in biospecimen collection, processing, and shipping are expected to adhere to all institutional policies including the completion and maintenance of required training(s). Biospecimens should be handled according to institutional guidelines/best practices at all times.

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1. Abbreviations

Definitions/Abbreviations	Definition
µL	Microliters
mL	Milliliters
APS	Acute Respiratory Distress Syndrome, Pneumonia, and Sepsis
BAL	Bronchoalveolar Lavage
BD	Becton Dickson
BSC	Biosafety Cabinet
BSL	Biosafety Level
CCC	Consortium Coordinating Center
COVID	Coronavirus disease caused by the SARS-CoV-2 virus.
CPT	Cell Preparation Tube with Sodium Citrate
CRC	Clinical Research Coordinator
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's Phosphate-Buffered Saline
EDTA	Ethylenediaminetetraacetic acid
FiO2	Fraction of inspired oxygen.
HME	Heat & Moisture Exchange
INR	International Normalized Ratio
LN2	Liquid Nitrogen
NBBAL	Non-bronchoscopic bronchoalveolar lavage
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-buffered Saline
PEEP	Positive End-Expiratory Pressure
PPE	Personal Protective Equipment
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RCP	Respiratory Care Practitioner
RNA	Ribonucleic acid
RPM	Revolutions per minute
RT	Respiratory Therapist
SARS	Severe Acute Respiratory Syndrome
SOP	Standard Operating Procedure
SpO2	Oxygen Saturation
TA	Tracheal Aspirate
TB	Tuberculosis
VUMC	Vanderbilt University Medical Center

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3. Biospecimen Collection and Processing Materials

3.1. Materials Provided By The APS Consortium Coordinating Center

Item	Manufacturer/Supplier	Catalog Number
6 mL EDTA vacutainer	BD 367863	02-683-99D
10 mL EDTA vacutainer	BD 366643	02-657-32
8 mL CPT vacutainer	BD 362761	02-685-125
2.5 mL RNA PAXgene tube	BD 762165	23-021-01
2.7 mL Sodium Citrate vacutainer	BD 363083	02-683-172
Urine/Stool specimen cup	Fisher Sci	Generic (Any)
RNA/DNA Shield swab kit (1 mL fill)	Zymo Research	R1107
RNA/DNA Shield (2 mL bead tube)	Zymo Research	R1103
RNA/DNA Shield (2x Concentrate)	Zymo Research	R1200-25
Airlife HME filter	McKesson	003003
25mL conical	Eppendorf™	0030122437
OMNImet/OMNIgene-GUT LTO Stool kit	DNA GENOTEK	MP-013
OMNImet-GUT stool collection tube	DNA GENOTEK	ME-200
Cryovials (multi-color)	SARSTEDT	Various
Cryovial tube caps (green and white)	VWR	Various
Mini BAL catheter 16Fr 142	McKesson	984479
BAL Supply kit	McKesson	1137591
Cell strainer, 100µm (sterile, nylon)	Falcon	352360
Lab Tape	Fisher Sci	Generic (Any)
6x9 bio-Specimen collection bag[^]	Fisher Sci	23-700-212
Commode collector	Fisher Sci	02-544-208
12x12 bio-specimen bag with handle[^]	RD Plastics/Fisher Sci	21-465-300
Return Shipping Label	FedEx	Custom
Primary Specimen Labels	Custom	NA
10x12 non-bio Ziplock bag[$]	Fisher Sci	Generic (Any)
6x9 non-bio Ziplock bag[$]	Fisher Sci	Generic (Any)
TA Trap supply kit	Fisher Sci	8884724500

[^]*Can be used for post-collection specimen transport/secondary containment. Sites can use alternative preferred and institutionally approved containers.*

[$]*Can be used for pre-collection supply transport. Sites can use alternative preferred and institutionally approved containers.*

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3.2. Materials to be supplied by APS sites

Item	Suggested Vendor/Manufacturer, Model (catalogue number, if applicable)
Phlebotomizing supplies	i.e., needles, gauze, bandages etc.
Lab processing supplies	i.e., pipettes, 1.5 mL microcentrifuge tubes, 15 mL and 50 mL conical tubes, cleaning supplies etc.
PPE	i.e., gloves, disposable lab coats, goggles, masks etc.
Box and dividers for PAXGene storage and shipping	NUNC Box: Thermo Sci., cat#369647, *or* *Equivalent* 49 Cell divider: Fisher Sci, cat# 50-190-2293, *or* Fisher Sci, cat#50-727-640, *or* VWR, cat#82021-122, or *Equivalent*
Box with divider for cryovial storage and shipping	Standard 5x5x2" (81cell divider): VWR #89214-766 *or* Fisher #03-395-455, *or* *Equivalent*
Dulbecco PBS (DPBS) without $CaCl_2$ and $MgCl_2$	Gibco #14190250 *or equivalent*
Barcode scanner (must be able to scan QR codes)	AirTrack, CodeCorp, or similar

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3.1. Collection Supplies

Blood Specimens	**6 mL EDTA** (Purple Cap)	**10 mL EDTA** (Purple Cap)	**RNA PAXGene**	**Sodium Citrate** (Blue Cap)	**8 mL CPT™** (Blue/Black Tiger Cap)
Respiratory Specimens	**HME Filter**	**Tracheal Aspirate (TA) Trap**	**NBBAL Kit**		
Microbiome Specimens	**Microbiome Zymo Research / RNA/DNA Shield Swab Kits** (Oral, Nasal, Rectal)	**Sterile Specimen Cup**	**Commode Collection Device and Omnimet Gut Stool Kit**	**Omnigene & Omnimet Gut Home Stool Kit**	

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3.1.1. NBBAL Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (2x) 8 mL CPT™
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) NBBAL Kit

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3.1.2. Day 0 Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (3x) 6 mL EDTA (1x) RNA PAXGene (1x) Sodium Citrate
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (2x) 6x9 Biohazard Specimen Bag (1x) HME Filter (1x) Tracheal Aspirate Trap
Microbiome	(2x) 10x12 Non-Bio Ziplock Bag (1x) 12x12 Bio-Specimen Bag (w/ handle) (2x) 6x9 Non-Bio Ziplock Bag (6x) 6x9 Bio-Specimen Bags (3x) Microbiome Zymo Research / RNA/DNA Shield Swab Kits Oral (1) Nasal (1), Rectal(1) (2x) Sterile Specimen Cup (1x) Commode Collection Device (1x) OMNImet-GUT stool collection kit

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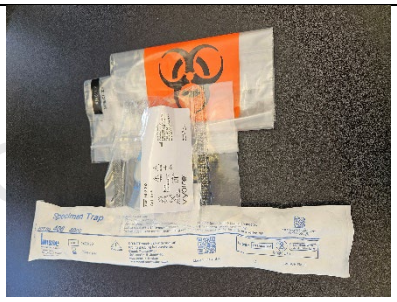
3.1.3. Day 2 Collection Kit assembly

Specimen Collection	Supplies Needed	
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) 10 mL EDTA (1x) RNA PAXGene (1x) Sodium Citrate	
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) HME Filter (1x) Tracheal Aspirate Trap	
Microbiome	(1x) 10x12 Non-Bio Ziplock Bag (1x) 12x12 Bio-Specimen Bag (w/ handle) (1x) 6x9 Non-Bio Ziplock Bag (4x) 6x9 Bio-Specimen Bags (3x) Microbiome Zymo Research / RNA/DNA Shield Swab Kits Oral (1) Nasal (1), Rectal (1) (1x) Sterile Specimen Cup (1x) Commode Collection Device (1x) OMNImet-GUT stool collection kit	

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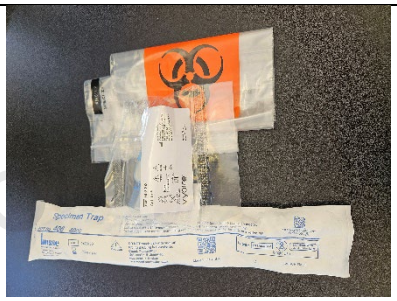
3.1.4. Day 4 Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen bag (1x) 6 mL EDTA.
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) HME Filter (1x) Tracheal Aspirate Trap. 

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3.1.5. Day 6 Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (2x) 6 mL EDTA (1x) RNA PAXGene (1x) Sodium Citrate
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) HME Filter (1x) Tracheal Aspirate Trap
Microbiome	(1x) 10x12 Non-Bio Ziplock Bag (1x) 12x12 Bio-Specimen Bag (w/ handle) (1x) 6x9 Non-Bio Ziplock Bag (4x) 6x9 Bio-Specimen Bags (3x) Microbiome Zymo Research / RNA/DNA Shield Swab Kits Oral (1)Nasal (1), Rectal (1) (1x) Sterile Specimen Cup (1x) Commode Collection Device (1x) OMNImet-GUT stool collection kit

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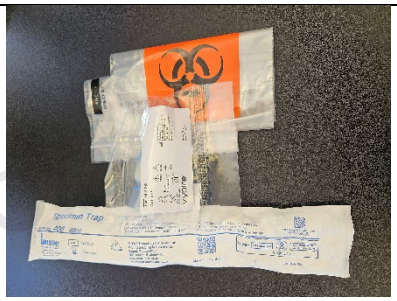
3.1.6. Day 14 Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) 6 mL EDTA (1x) RNA PAXGene
Respiratory	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) HME Filter (1x) Tracheal Aspirate Trap 

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3.1.7. LTO Timepoint 1 (3 to 6 months) Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) 6 mL EDTA (1x) 10 mL EDTA (1x) RNA PAXGene
Microbiome	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Non-Bio Ziplock Bag (2x) 6x9 Bio-Specimen Bags (2x) Microbiome Zymo Reasearch / RNA/DNA Shield Swab Kits Oral (1) Nasal (1) (1x) OMNImet/OMNIgene-GUT LTO Stool kit

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3.1.8. LTO Timepoint 2 (12-Months) Collection Kit Assembly

Specimen Collection	Supplies Needed
Blood	(1x) 6x9 Non-Bio Ziplock Bag (1x) 6x9 Biohazard Specimen Bag (1x) 10 mL EDTA (1x) RNA PAXGene
Microbiome	(1x) 10x12 Non-Bio Ziplock Bag (1x) 6x9 Non-Bio Ziplock Bag (2x) 6x9 Bio-Specimen Bags (2x) Microbiome Zymo Research / RNA/DNA Shield Swab Kits Oral (1), Nasal (1) (1x) OMNImet/OMNIgene-GUT LTO Stool kit

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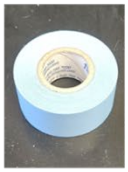
3.2. Processing and Aliquoting Supplies

Blood Specimens	**Purple Cap 2.0 mL Cryovials**	**Black Cap 2.0 mL Cryovials**	**Orange Cap 2.0 mL Cryovials**	**Blue Cap 2.0 mL Cryovials**	**Clear Cap 2.0 mL Cryovials**

Respiratory Specimens	**Yellow Cap 2.0 mL Cryovials**	**Dark Green Cap 2.0 mL Cryovials with beads**	**Clear 2.0 mL Cryovials with Green & White Cap Inserts**	**Pink Cap 2.0 mL Cryovials**	**Clear Cap 2.0 mL Cryovials**	**Lab Tape**	**25 mL Conical Tube**
							

Microbiome Specimens	**Red Cap 2.0 mL Cryovials**	**Green Cap 2.0 mL Cryovials**	**Brown Cap 2.0 mL Cryovials**	**Clear Cap, low-binding , 2.0 mL Cryovials**	**Amber 2.0 mL Cryovials**

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3.2.1. NBBAL Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(4x*) Blue Cap 2.0 mL Cryovials (4x) Clear Cap 2.0 mL Cryovials
Respiratory	(15x*) Pink Cap 2.0 mL Cryovials • (5x) Metagenomics • (10x*) Fluid (10x) Clear Cap 2.0 mL Cryovials • (5x) Cryostor • (10x*) DNA/RNA Shield (1x) Cell strainer, 100µm (sterile, nylon)

This is the **maximum number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.*

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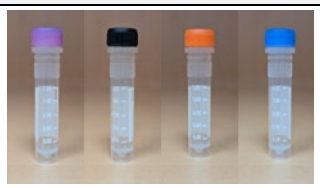
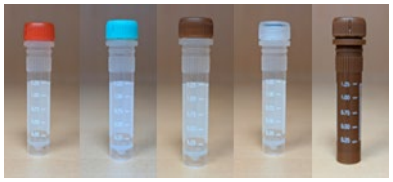
3.2.2. Day 0 Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(30x*) Purple Cap 2.0 mL Cryovials (1x) Black Cap 2.0 mL Cryovial (1x) Orange Cap 2.0 mL Cryovial (5x*) Blue Cap 2.0 mL Cryovials 
Respiratory	(10x*) Yellow Cap 2.0 mL Cryovials (2x) Dark Green Cap 2.0 mL Cryovials with beads (2x) 2.0 mL Clear Cryovials with Green cap insert (2x) 2.0 mL Clear Cryovials with White cap insert (1x) Lab Tape (1x) 25 mL Conical Tube
Microbiome	(2x) Red Cap 2.0 mL Cryovials (2x) Green Cap 2.0 mL Cryovials (2x) Brown Cap 2.0 mL Cryovials (5x) Clear Cap, low-binding, 2.0 mL Cryovials (4x) Amber 2.0 mL Cryovials 

This is the maximum number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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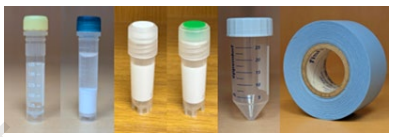
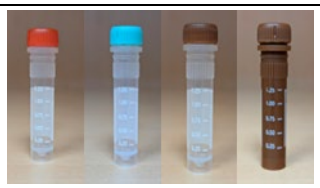
3.2.3. Day 2 Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(10x*) Purple Cap 2.0 mL Cryovials (3x*) Blue Cap 2.0 mL Cryovials
Respiratory	(10x*) Yellow Cap 2.0 mL Cryovials (2x) Dark Green Cap 2.0 mL Cryovials with beads (2x) 2.0 mL Clear Cryovials with Green cap insert (2x) 2.0 mL Clear Cryovials with White cap insert (1x) Lab Tape (1x) 25 mL Conical Tube 
Microbiome	(2x) Red Cap 2.0 mL Cryovials (2x) Green Cap 2.0 mL Cryovials (2x) Brown Cap 2.0 mL Cryovials (4x) Amber 2.0 mL Cryovials 

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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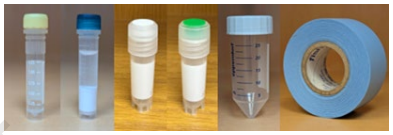
3.2.4. Day 4 Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(6x*) Purple Cap 2.0 mL Cryovials.
Respiratory	(10x*) Yellow Cap 2.0 mL Cryovials, (2x) Dark Green Cap 2.0 mL Cryovials with beads, (2x) 2.0 mL Clear Cryovials with Green cap insert (2x) 2.0 mL Clear Cryovials with White cap insert (1x) Lab Tape, (1x) 25 mL Conical Tube. 

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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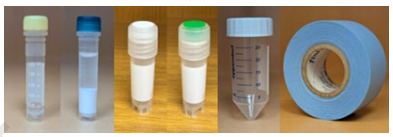
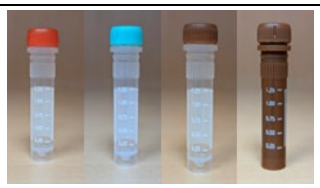
3.2.5. Day 6 Processing and Aliquoting Kit Assembly

Specimen Alituots	Supplies Needed
Blood	(12x*) Purple Cap 2.0 mL Cryovials, (3x*) Blue Cap 2.0 mL Cryovials.
Respiratory	(10x*) Yellow Cap 2.0 mL Cryovials, (2x) Dark Green Cap 2.0 mL Cryovials with beads, (2x) 2.0 mL Clear Cryovials with Green cap insert (2x) 2.0 mL Clear Cryovials with White cap insert (1x) Lab Tape, (1x) 25 mL Conical Tube. 
Microbiome	(2x) Red Cap 2.0 mL Cryovials, (2x) Green Cap 2.0 mL Cryovials, (2x) Brown Cap 2.0 mL Cryovials, (4x) Amber 2.0 mL Cryovials. 

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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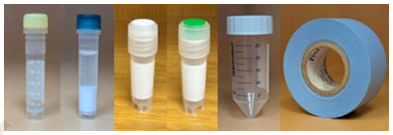
3.2.6. Day 14 Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(6x*) Purple Cap 2.0 mL Cryovials
Respiratory	(10x*) Yellow Cap 2.0 mL Cryovials (2x) Dark Green Cap 2.0 mL Cryovials with beads (2x) 2.0 mL Clear Cryovials with Green cap insert (2x) 2.0 mL Clear Cryovials with White cap insert (1x) Lab Tape (1x) 25 mL Conical Tube 

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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3.2.7. LTO Timepoint 1 (3 to 6 months) Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(16x*) Purple Cap 2.0 mL Cryovials (1x) Orange Cap 2.0 mL Cryovials
Microbiome	(2x) Red Cap 2.0 mL Cryovials (2x) Green Cap 2.0 mL Cryovials (8x) Amber 2.0 mL Cryovials

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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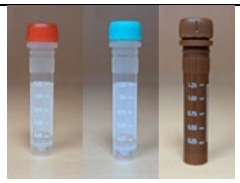
3.2.8. LTO Timepoint 2 (12-Months) Processing and Aliquoting Kit Assembly

Specimen Aliquots	Supplies Needed
Blood	(10x*) Purple Cap 2.0 mL Cryovials (1x) Orange Cap 2.0 mL Cryovials
Microbiome	(2x) Red Cap 2.0 mL Cryovials (2x) Green Cap 2.0 mL Cryovials (8x) Amber 2.0 mL Cryovials 

This is the <u>maximum</u> number of aliquots that can be produced for this specimen type. The number of aliquots will vary based on the actual volume of processed specimen. Return all unused cryovials to bulk supplies.

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3.3. Biospecimen Labels

Each site will receive two sets of specimen labels for each participant: collection labels and processing labels.

- Blue Packet: Collection Labels
  - Labels for primary collection tubes are separated into envelopes with color-coded timepoint stickers. Each envelope is labeled “Primary” with the patient ID and timepoint (see Figure 1).
  - Ensure the first 2 digits of each label match the Site Number for your site provided in Section 3.3.2.1of this SOP.
- White Packet: Lab Processing Labels
  - Cryovial labels for lab processing are separated into envelopes with color-coded timepoint stickers. Each envelope is labeled “Lab” with the patient ID and timepoint (see Figure 2).
  - Ensure the first 2 digits of each label match the Site Number for your site provided in Section 3.3.2.1of this SOP.

Figure 1: Collection Labels (blue envelope)

Figure 2: Lab Processing Labels (white envelope)

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3.3.1. Label Structure

Understanding the components of both the collection and processing labels is vital for the correct labeling of specimens. Both collection and processing specimen labels contain two specific QR Codes: one Participant ID Barcode, and one Unique Barcode.

- *The* Participant ***ID Barcode*** *contains the full participant ID and timepoint along with the specimen type, collection tube number and volume specific to timepoint.*
- The ***Unique Barcode*** *is different for every single label. This ID number should never repeat itself.*
- Use *only the labels provided by the APS Consortium Coordinating Center. Specimens with handwritten labels will not be accepted by the Coordinating Center lab.*

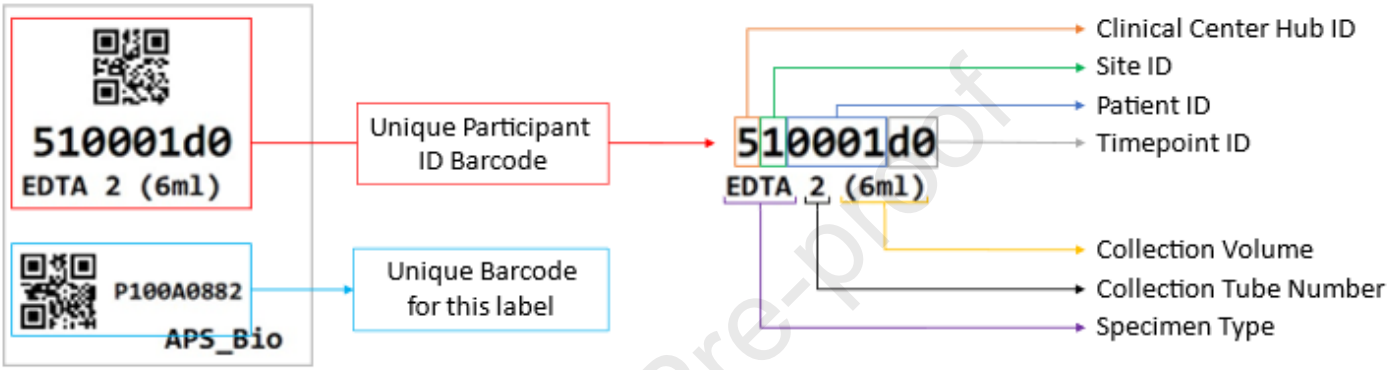

Figure 3: Components of Primary Collection Labels

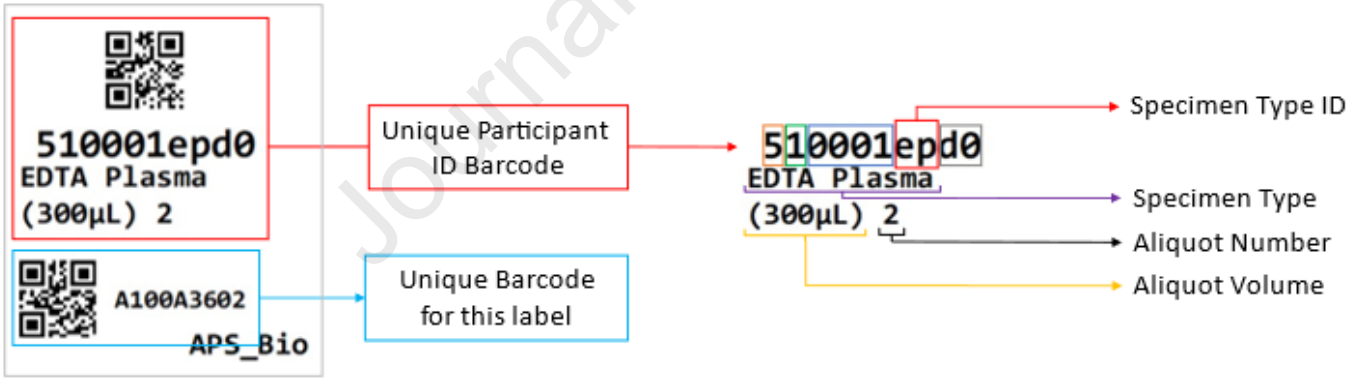

Figure 4: Components of Lab Processing Labels

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3.3.2. Label Nomenclature

3.3.2.1. APS Site Numbers

Clinical Center	Site	Site Number
Pennsylvania/ University of Pennsylvania Hospital	Penn HUP	11
	Penn Presbyterian (PPMC)	12
	Penn Cedar	13
	Penn LGH	14
Great Lakes/University of Michigan	University of Michigan	21
	University of Cincinnati	22
	Henry Ford Health	23
	University of Chicago	24
	Northwestern University	25
MUSIC/ Vanderbilt University Medical Center	Vanderbilt University Medical Center	31
	Meharry Medical College	32
	Washington University, St. Louis	33
Colorado/University of Colorado	University of Colorado Hospital	41
	Denver Health	42
	Medical Center of the Rockies	43
	Poudre Valley Hospital	44
	St. Joseph Hospital	45
InterACT/ Intermountain Medical Center	Intermountain Medical Center	51
	Johns Hopkins Hospital	52
	Duke University	53
	University of Utah	54
	Bayview Medical Center	55
California/ University of California San Francisco	UCSF San Francisco	61
	UCSF Fresno	62
	San Francisco General Hospital	63
	Stanford University	64

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3.3.2.2. Clinical Specimens and Laboratory Aliquots Label Nomenclature

Table 1: A breakdown of primary specimen collection and processing aliquot label nomenclature, and corresponding color-coded cryovials.

Label Nomenclature and Corresponding Cryovials			
Clinical Specimens Collection	**Processing Aliquots**		**Processing Cryovials**
EDTA 10 mL/ 6 mL	EDTA plasma	ep	Purple cap 2.0 mL cryovial
	Buffy coat	bc	Black cap 2.0 mL cryovial
	RBCs	rc	Orange cap 2.0 mL cryovial
Sodium Citrate	Sodium citrate plasma	sp	Blue cap 2.0 mL cryovial[#]
PAXgene	RNA PAXgene	rp	N/A
CPT	CPT plasma	cp	Blue cap 2.0 mL cryovial[#]
	CPT(PBMC) cells	cc	Clear cap 2.0 mL cryovial[#]
Zymo Nasal Swab	Nasal swab	ns	Green cap 2.0 mL cryovial
Zymo Oral Swab	Oral swab	os	Red cap 2.0 mL cryovial
Zymo Rectal Swab	Rectal swab	rs	Brown cap 2.0 mL cryovial
Whole Stool	Whole stool	ws	Solid amber cryovial 2.0 mL cryovial
Urine	Urine	ur	Clear, low binding 2.0 mL cryovial
Omnigene-Gut Stool (Red)	Omnigene-Gut (red)	og	Solid amber cryovial 2.0 mL cryovial
Omnimet-Gut Stool (Purple)	Omnimet-Gut (purple)	om	Solid amber cryovial 2.0 mL cryovial
HME Filter	HME filter Fluid	hf	Yellow cap 2.0 mL cryovial
Tracheal Aspirate	Tracheal aspirate (transcriptomics)	ta	Dark green cap, with beads 2.0 mL cryovial
	Tracheal aspirate (neat)	tan	2.0 mL clear cryovial with green cap insert
	Tracheal aspirate (proteomics)	tap	2.0 mL clear cryovial with white cap insert
NBBAL	NBBAL Fluid	nf	Pink cap 2.0 mL cryovial
	NBBAL Metagenomics	mg	Pink cap 2.0 mL cryovial
	NBBAL paired CPT PBMC cells	nc	Clear cap 2.0 mL cryovial[#]
	NBBAL Cells (CryoStor)	ncc	Clear cap 2.0 mL cryovial[#]
	NBBAL paired CPT Plasma	ncp	Blue top 2.0 mL cryovial[#]
Paperwork *(Optional)*	Paperwork *(Optional)*	pw	Optional for paper lab sheets

[#]*Some aliquot cryovial colors are used for multiple clinical specimen types*

3.3.3. Label Orientation

3.3.3.1. Label orientation is critical to ensure the barcode scanner reads specimen labels accurately. Collection Tubes

- Ensure *that the label is pressed down firmly against the collection tube to avoid wrinkles.*
- Place the APS specimen label over the paper tube label to ensure specimen volume can be seen clearly during processing (see Figure 5)

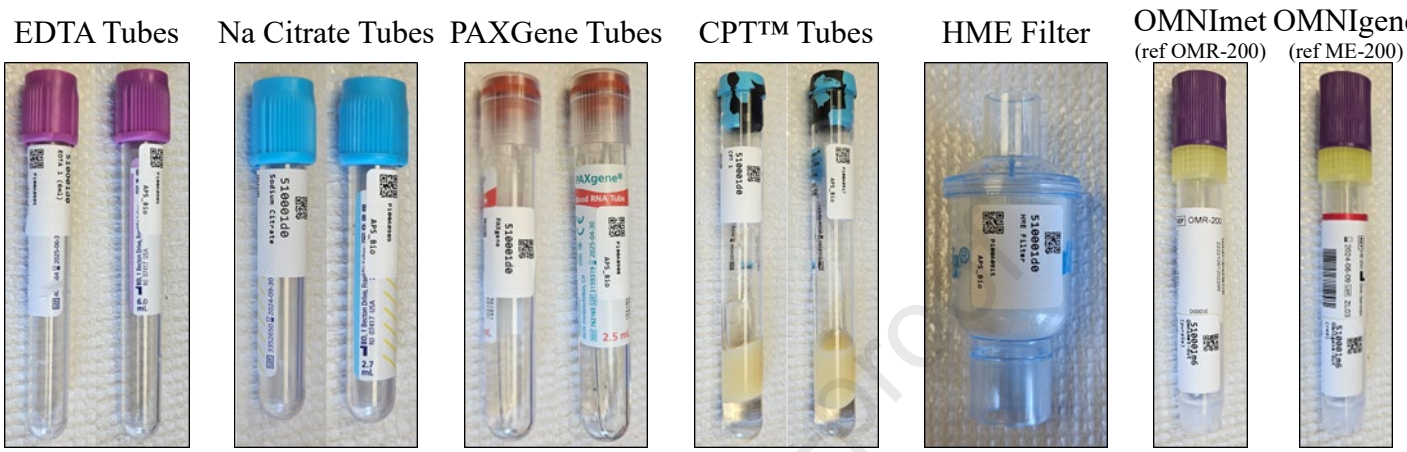

Figure 5: Correctly labeled specimen collection tubes

3.3.3.2. Aliquot Cryovials

- Labels should be placed on the cryovial vertically along the length of the tube. Labels should never be placed horizontally across the middle of the tube (see Figure 6).

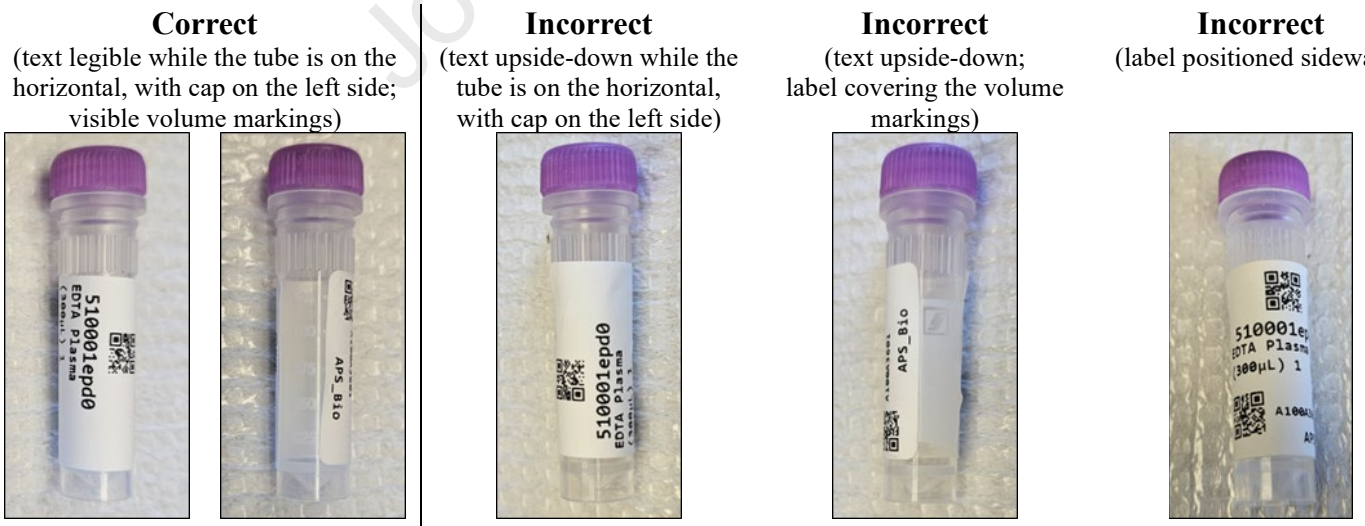

Figure 6: Correct and incorrect positioning of labels in cryovial aliquoting tubes

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3.4. Ordering supplies from the Consortium Coordinating Center

To order materials from the Consortium Coordinating Center, please click the REDCap survey link below. This survey will provide the necessary instructions for completing your order.

APS Supply Order Survey

Full link: https://redcap.link/aps_supply_request

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4. Blood Specimens Collection and Processing

4.1. Materials

Materials required for blood collection, processing, and aliquoting are summarized here. More details on supplies are available in Section 3 of this SOP.

4.1.1. Collection Supplies

- 10 mL EDTA tube
- 2.5 mL RNA PAXgene tube
- 2.7 mL Sodium Citrate Tube
- 6 mL EDTA tube
- CPT™ tubes
- 6x9 Specimen Bags (generic)
- Primary Specimen Labels

4.1.2. Processing and Aliquoting Supplies

- CryoStor® CS10 freezing medium
- Black Cryovial
- Blue Cryovial
- Purple Cryovial
- Clear Cryovial
- Orange Cryovial
- Lab Processing Labels

4.1.3. Additional Supplies not provided by the CCC

Item	*Manufacturer/Vendor*	*Catalog Number*
DPBS without Calcium or Magnesium	Gibco, or equivalent	14190250, or equivalent
50 mL conical tube	Various	Various
Box with 49-cell divider for PAXGene storage and shipping	NUNC Box: Thermo Sci.	369647, or equivalent
	49 Cell divider: Fisher Sci	50-190-2293, or equivalent
	Fisher Sci	50-727-640, or equivalent
	VWR	82021-122, or equivalent
Standard 5x5x2” box with 81-cell divider for	VWR	89214-766

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Item	*Manufacturer/Vendor*	*Catalog Number*
cryovial storage and shipping	Fisher	03-395-455

4.1.4. Equipment

Equipment Name	*Required features/capabilities*
Micropipettes	P1000, P200 (calibrated) paired with sterile filtered tips
Serological Pipettes	Various volumes, sterile
Slow freeze apparatus	Mr. Frosty, CoolCell, or equivalent
Tabletop Centrifuge	Swing bucket with a 16 x 125 mm tube adapter

4.2. Biosafety

All personnel must follow applicable Institutional Biosafety requirements and guidelines and/or other Institutional policies. Wear all appropriate personal protective equipment while working in the laboratory and follow all appropriate protocols for working safely in a Biosafety Cabinet (BSC) when applicable.

4.3. Schedule of Events

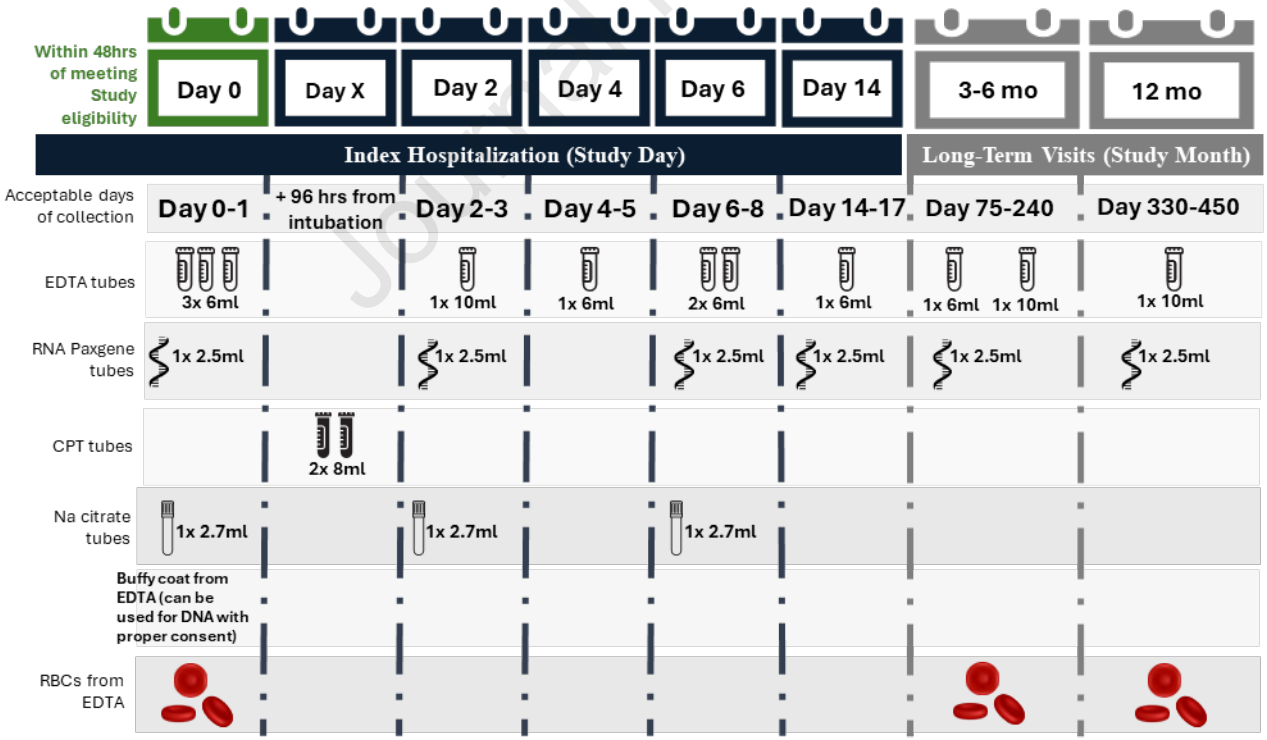

Figure 7: Schedule of events: Blood Collection

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4.4. Blood Specimen Collection

4.4.1. Blood Collection Best Practices

- Follow the approved institutional phlebotomy procedure, including all local institutional safety protocols.
- When placing aliquot vials into a cryobox, prioritize filling each cryobox with as many vials of the **same biospecimen type** as possible. If there is remaining space after one blood specimen type has been placed into a cryobox, fill the rest of the cryobox with other types of blood biospecimens, leaving an empty row between the different blood biospecimen types. However, do not mix blood biospecimens with respiratory or microbiome biospecimens in the same cryobox. Please refer to Section 8 of this SOP for shipping details.
- Please note that, unlike other blood specimen types, RNA PAXgene tubes should be placed in their own cryobox. Please refer to Section 8 of this SOP for shipping details.
- All collection times should be entered in the APS REDCap database at the time of specimen collection.
- If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 "Specimen Chain of Custody" of this SOP.
- When collecting multiple blood tubes during a single draw, follow the order of draw outlined below. Even if not all blood tubes are collected on a given collection time point, the order of draw should be followed for the applicable tubes.

Order	Collection Tube
1	Sodium citrate
2	EDTA
3	CPT
4	RNA PAXgene

4.4.2. EDTA Blood Collection

- NOTE: The number of EDTA tubes collected may vary between sites:
  - CPT Sites (sites processing PBMCs): TWO (2) purple top EDTA tubes.
  - Non-CPT Sites (sites NOT processing PBMCs): THREE (3) purple top EDTA tubes.
- **PURPLE TOP**

Time point	Collection Tube	Number of tubes
Day 0	6 mL EDTA	3
Day 2	10 mL EDTA	1
Day 4	6 mL EDTA	1
Day 6	6 mL EDTA	2
Day 14	6 mL EDTA	1

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Time point	Collection Tube	Number of tubes
LTO Timepoint 1 (3 to 6 months)	6 mL EDTA 10 mL EDTA	1 1
LTO Timepoint 2 (12 months)	10 mL EDTA	1

4.4.3. Sodium Citrate Blood Collection

- **BLUE TOP**

Time point	Collection Tube	Number of tubes
Day 0	2.7 mL Sodium Citrate	1
Day 2	2.7 mL Sodium Citrate	1
Day 6	2.7 mL Sodium Citrate	1

4.4.4. CPT™ Collection

- CPT™ tubes must be at room temperature (18-25 °C) before collection.
- After collection, invert tube 8 to 10 times to mix anticoagulant additive with blood. **DO NOT SHAKE.** Vigorous mixing can cause hemolysis.
- Store the tubes in an upright position at room temperature until centrifugation.
- **IMPORTANT:** Centrifuge tubes within 2 hours of collection (See Section 4.5.5 for centrifugation conditions)
- **TIGER TOP** glass tube with a gel barrier

Time point	Collection Tube	Number of tubes
Day X (NBBAL)	8 mL CPT™	2

4.4.5. PAXGene Collection

- **RED TOP**

Time point	Collection Tube	Number of tubes
Day 0	2.5 mL RNA PAXGene	1
Day 2	2.5 mL RNA PAXGene	1
Day 6	2.5 mL RNA PAXGene	1
Day 14	2.5 mL RNA PAXGene	1
LTO Timepoint 1 (3 to 6 months)	2.5 mL RNA PAXGene	1
LTO Timepoint 2 (12 months)	2.5 mL RNA PAXGene	1

4.5. Processing Blood Specimens

4.5.1. EDTA Blood Tubes (Figure 9A – All Timepoints)

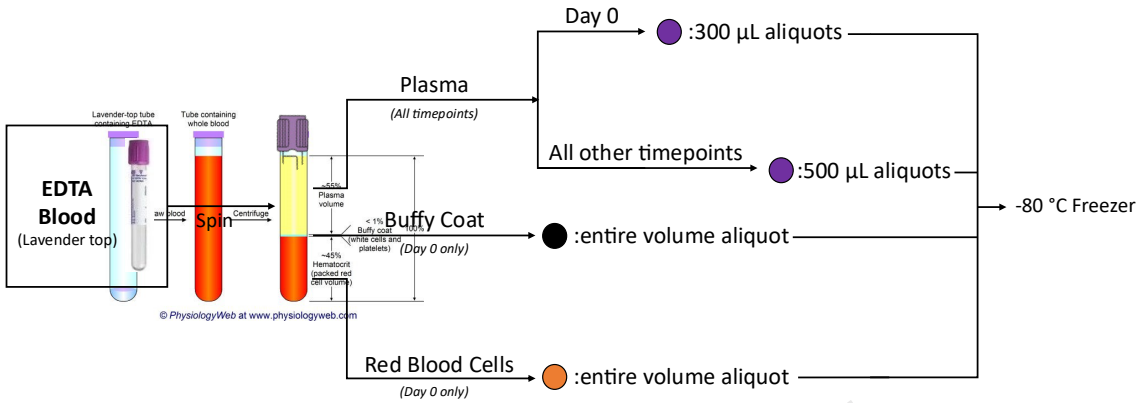

Figure 8: Processing Visual Summary - EDTA Blood Tubes

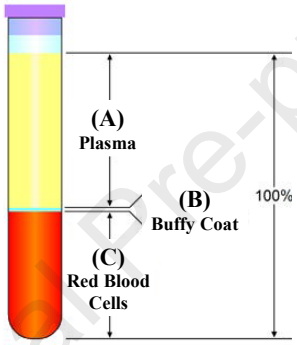

Figure 9: Blood separation composition in EDTA collection tube after centrifugation.

4.5.1.1. Important notes

- Blood should ideally be processed within **4 hours of collection**.
- The tubes must be kept at room temperature before centrifugation.

4.5.1.2. Procedure

1. Verify that collection tubes are correctly labeled.
2. Centrifuge the EDTA tubes for **10 minutes at 1,200 RCF (xg force) at room temperature** (18-25 ºC) leaving acceleration and deceleration ON. Ensure that the centrifuge rotor is appropriately balanced.
3. While samples are spinning, label the 2.0 mL screw cap aliquoting cryovials according to the

table below.

a. **PURPLE** cap for EDTA Plasma.

b. **BLACK** cap for EDTA Buffy Coat.

c. **ORANGE** cap for Red Blood Cells Pellet.

Time point	Specimen (tube cap color)	Number of cryovials	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	**EDTA Plasma (purple)**	30*	300 µL	epd0
	EDTA Buffy Coat (black)	1	Entire fraction	bcd0
	***Red Blood Cells* (orange)**	1	Entire fraction	rcd0
Day 2	**EDTA Plasma (purple)**	10*	500 µL	epd2
Day 4	**EDTA Plasma (purple)**	6*	500 µL	epd4
Day 6	**EDTA Plasma (purple)**	12*	500 µL	epd6
Day 14	**EDTA Plasma (purple)**	6*	500 µL	epd14
LTO Timepoint 1 (3 to 6 months)	**EDTA Plasma (purple)**	16*	500 µL	epm3
	***Red Blood Cells* (orange)**	1	Entire fraction	rcm3
LTO Timepoint 2 (12 months)	**EDTA Plasma (purple)**	10*	500 µL	epm12
	***Red Blood Cells* (orange)**	1	Entire fraction	rcm12

* *The number of these aliquots are dependent on the volume of processed specimen. Sites may generate up to this number of cryovials.*

4. After samples have finished spinning, remove tubes from the centrifuge.

5. Using a P1000 micropipette and filtered tips, set the volume according to the respective timepoint (see table on Step 3 above), and aliquot the EDTA plasma into labeled PURPLE screw cap cryovial tubes.

   a. Do not disrupt the buffy coat layer.

- *NOTE*: **Do not underfill tubes.** If there is insufficient plasma for a 300 µL aliquot, discard the remaining plasma.

- *NOTE*: **Do not discard unused cryovials.** If cryovials were labeled and were not used (i.e., because of low blood volume in collection tube), retain for future use. Destroy the original label (e.g., by

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removal or covering the information using a permanent marker). Original labels can be overlaid with new labels.

6. Once all aliquots are filled for the specific time point, transfer all cryovials to an 81-cell cryobox dedicated to EDTA specimens and store in a -80 °C freezer.
7. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.
8. For **Day 0** timepoint specimens only:
    a. Save two EDTA tubes for the next steps and continue to sections 4.5.2 and 4.5.3
9. For **Day 0**, and **LTO** timepoint specimens only:
    a. Save two EDTA tubes for the next steps and continue to section 4.5.3

4.5.2. EDTA Buffy coat (Figure 9B) – Day 0 Timepoint.

1. After aliquoting the plasma, retrieve the first EDTA tube.
2. Use a P1000 micropipette set to 1000 µL to retrieve as much buffy coat layer (Figure 9B) as possible in a single draw.
    a. *NOTE:* Volume is typically <500 µL.
3. Transfer to the labeled **BLACK** screw cap cryovial tube.
4. Retrieve the second EDTA tube and repeat Step 2**,** pooling the buffy coat layer into the same labeled **BLACK** screw cap cryovial.
5. Transfer the cryovial to an 81-cell cryobox dedicated to Buffy Coat specimens and store in a -80 °C freezer.
6. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.
7. Save the two EDTA tubes for the next section.

4.5.3. Red Blood Cells pellet (Figure 9C) – Day 0, LTO Timepoints 1 and 2.

1. Retrieve the two EDTA tubes containing the red blood cells pellet.
2. Using a P1000 micropipette set to 1000 µL, gently stir red blood cells and any leftover buffy coat/plasma by pipetting up and down until homogenous.
3. Transfer the entire RBC fraction to the labeled **ORANGE** screw cap cryovial tube.
4. Repeat Step 2, pooling the RBC layer of the second EDTA tube into the same labeled

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ORANGE screw cap cryovial tube.

5. Discard all waste into a red biohazard bin.
6. Transfer the aliquot cryovial to an 81-cell cryobox dedicated to RBC specimens and store in a -80 °C freezer.
7. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.

    If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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4.5.4. Sodium Citrate Blood Tubes

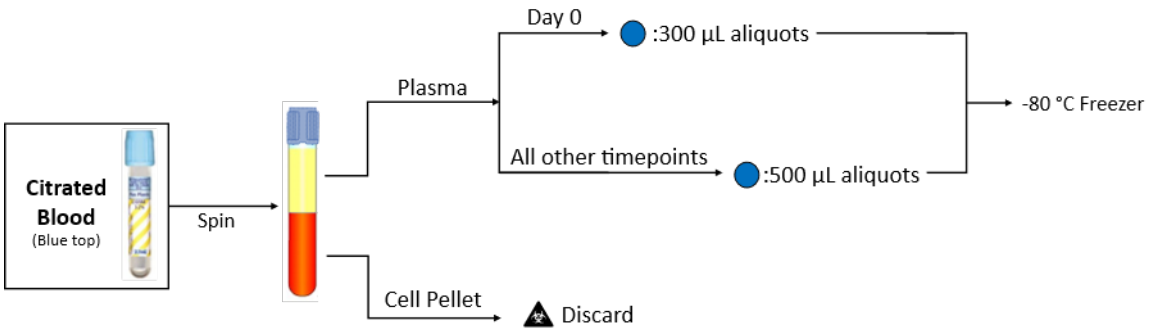

Figure 10: Processing Visual Summary - Sodium Citrate Blood Tubes

4.5.4.1. Important notes

- Process the collected blood **within 2 hours of collection**.
- Keep the tube(s) in an upright position at room temperature until centrifugation.

4.5.4.2. Procedure

1. Centrifuge the Sodium Citrate tube(s) for **10 minutes at 1,200 RCF (xg force) at room temperature** (18-25 ºC) leaving acceleration and deceleration ON. Ensure that the centrifuge rotor is appropriately balanced.
2. While samples are spinning, label the necessary number of 2.0 mL screw cap aliquoting cryovials as described in the table below.
    a. **BLUE** for Citrated Plasma.

Time point	Specimen (tube cap color)	Number of tubes	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***Citrated Plasma* (blue)**	5*	300 µL	spd0
Day 2	***Citrated Plasma* (blue)**	3*	500 µL	spd2
Day 6	***Citrated Plasma* (blue)**	3*	500 µL	spd6

* *The number of these aliquots are dependent on the volume of processed specimen. Sites may generate up to this number of cryovials.*

3. After samples have finished spinning, remove tubes from the centrifuge.
4. Using a P1000 micropipette and filtered tips, set the volume according to the respective timepoint (see table on Step 2)2, and aliquot the Citrated plasma into labeled **BLUE** screw cap

cryovial tubes.

   a. *NOTE*: **Do not discard unused cryovials.** If cryovials were labeled and were not used (i.e., because of low blood volume in collection tube), retain for future use. Destroy the original label (e.g., by removal or covering the information using a permanent marker). Original labels can be overlaid with new labels.

5. Discard the tube containing the remaining plasma and cell pellet.
6. Once all aliquots are fulfilled for the specific time point, transfer all cryovials to an 81-cell cryobox dedicated to Sodium Citrate specimens and store in a -80 °C freezer.
7. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
   a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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4.5.5. CPT™ Tubes

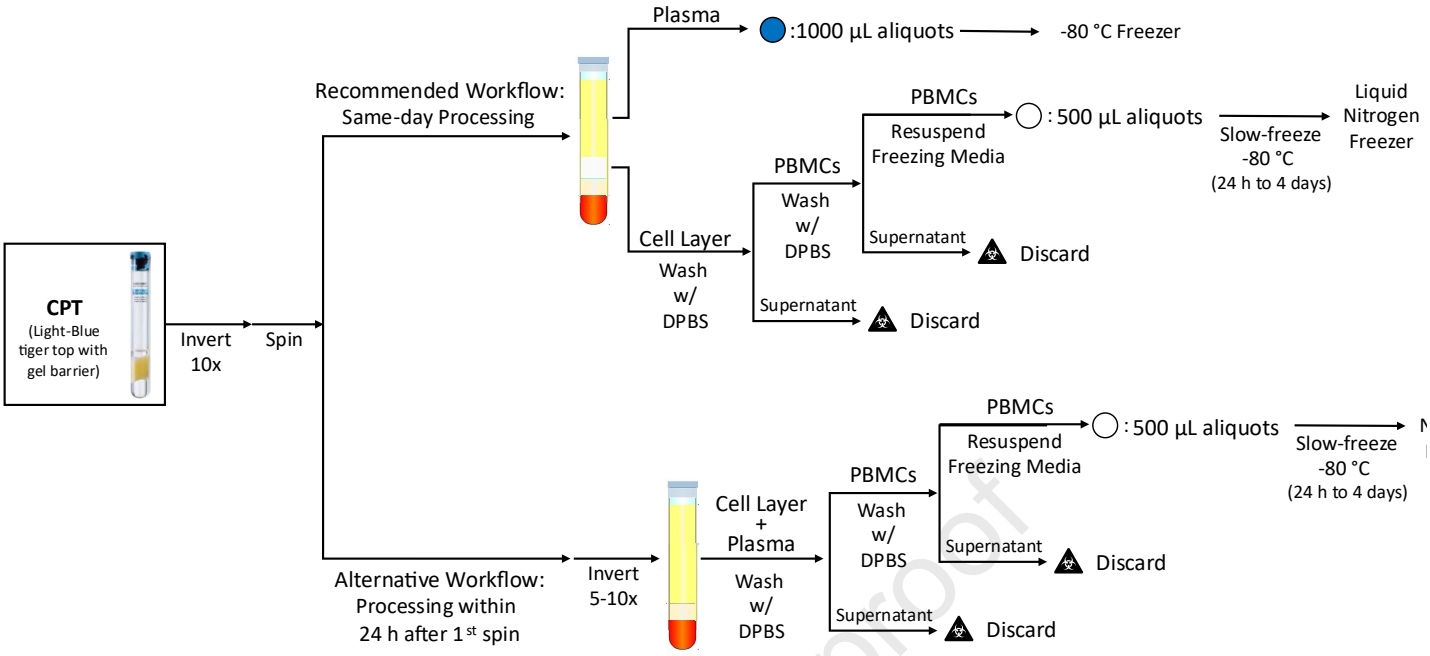

Figure 11: Processing Visual Summary - CPT Blood Tubes

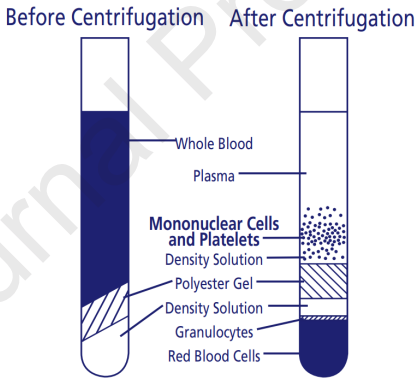

Figure 12: BD Vacutainer® CPT™ tube layering of formed elements before and after centrifugation.

4.5.5.1. Important notes

- To ensure proper barrier formation, blood samples must be **centrifuged within 2 hours of blood collection**.
- Keep the tubes in an upright position at room temperature until centrifugation.
- Process the CPT™ tube collecting the cells **within 24 hours after initial centrifugation**.
  - In the exceptional case the CPT™ tubes cannot be processed immediately after centrifugation, resuspend the cells into the plasma by gently inverting the unopened CPT™ tubes 5 to 10 times. After that, the tubes can be stored in the upright position at room temperature for up to 24 hours.

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4.5.5.2. PBMC Layer Isolation

1. Immediately before placing the CPT™ tube in the centrifuge, gently invert the tubes 10 times.

- *NOTE*: This step is critical. Failing to mix blood and reagent thoroughly prevents the red blood cells from migrating below the gel layer.

2. Ensure the tubes are placed in the proper centrifuge carrier/adapter (16 x 125 mm tube adapter). Ensure that the centrifuge rotor is appropriately balanced.
3. Centrifuge the CPT™ tubes for **25 minutes at 1,500 RCF (xg force) at room temperature** (18-25 ºC) leaving acceleration and deceleration at the middle-point setting (e.g., Level 5).
4. After samples have finished spinning, remove tubes from the centrifuge.
5. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see Figure 12).
6. Ensure all red blood cells have moved below the gel barrier. If not, re-centrifuge the tubes at 1,500 RCF (xg force) for 3 minutes leaving acceleration and deceleration OFF. Ensure that the centrifuge rotor is appropriately balanced.
7. If processing the CPT™ tubes immediately after the first centrifugation, remove tubes from the centrifuge and continue to the next Section 4.5.5.3.
8. If CPT™ tubes cannot be processed immediately after the first centrifugation (i.e., shipping the tubes to another processing lab), ensure to send the correct set of labels and the **CLEAR** cryovials together with the CPT™ tubes. The processing lab shall continue the protocol from Section 4.5.5.6.

4.5.5.3. Washing PBMCs

9. Label the necessary number of aliquoting cryovials according to the table below.
    a. **CLEAR cryovials** for PBMC from CPT™ tubes.
    b. **BLUE** cryovials for Citrated Plasma from CPT™ tubes.

Time point	Specimen (cryovial cap color)	Number of cryovial	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day X (NBBAL)	***PBMC* (clear)**	4	500 µL	ncdx
	***Citrated Plasma*(blue)**	4*	1000 µL	ncpdx

* *The number of these aliquots are dependent on the volume of processed specimen. Sites may generate up to this number of cryovials.*

10. Label two new 50 mL conical tubes as "Plasma" and "Cells".
11. Pipette approximately half the plasma volume (~2 mL) from both CPT™ tubes into the

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"Plasma" 50 mL conical tube. Set the tube aside.

a. **DO NOT** disturb the bottom half of the plasma layer where cells are located.

12. Pipette the bottom half of the plasma+cells layer (everything above the polyester gel layer, see Figure 12) from the CPT™ tubes into the "Cells" 50 mL conical tube.
13. Add sterile DPBS to the "Cells" conical tube using a serological pipette, bringing the volume to 15 mL.
14. Securely cap the tube and gently mix cells by inverting the tube 5 times.
15. Centrifuge for **15 minutes at 300 RCF (xg force) at room temperature** (18-25 ºC) leaving acceleration and deceleration ON. Ensure that the centrifuge rotor is appropriately balanced.
16. Remove the tube from the centrifuge without disturbing the cell pellet.
17. Carefully decant or pipette the supernatant into a waste container. Without disturbing the cell pellet, carefully pipette any remaining supernatant into the waste container.
18. Add sterile DPBS to the "Cells" conical tube using a serological pipette, bringing the volume to 10 mL.
19. Securely cap the tube and gently mix cells by inverting the tube at least 5 times.
    a. Gently tap the tube to break up the cell pellet if necessary.
20. Centrifuge the "Cells" conical tube for **10 minutes at 300 RCF (xg force) at room temperature** (18-25 ºC) leaving acceleration and deceleration ON. Ensure that the centrifuge rotor is appropriately balanced.

4.5.5.4. Freezing PBMCs

- **CRITICAL STEP:** Cells must not sit at room temperature longer than 5 minutes after adding the freezing medium.Prepare slow freeze device (e.g. Nalgene's Mr. Frosty or CoolCell) according to manufacturer instructions.

21. Retrieve the 50 mL "Cells" conical tube containing PBMCs after centrifugation.
22. Without disturbing the cell pellet, carefully pipette any remaining supernatant into the waste container.
23. Using a P1000 pipette set to 1000 µL, resuspend the cells in 2 mL of CryoStor® freezing medium.
24. Using a P1000 pipette set to 500 µL, aliquot resuspended cells into four labeled **CLEAR** screw cap cryovial tubes.
25. Immediately transfer the cryovials into the slow-freeze device (e.g. Nalgene's Mr. Frosty or CoolCell prepared in Step 21).
26. Place the slow-freeze device into a -80 °C freezer; leave it for at least **24 hours and no longer than 4 days**.
27. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection

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and Processing form.

a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

28. Without allowing the cells to thaw, transfer all cryovials from the slow-freeze device into an 81-cell cryobox dedicated to PBMCs specimens and store in the LN2 freezer.

- **IMPORTANT:** If onsite liquid nitrogen is unavailable, PBMCs stored at -80 °C should be shipped to VUMC within one month of collection.

4.5.5.5. Aliquoting CPT Citrated Plasma

29. Retrieve the 50 mL conical tube labeled "Plasma" from Step 11.
30. Using a P1000 micropipette and filtered tips, set the volume according to the respective timepoint (see table on Step 9), and aliquot the Citrated plasma into labeled **BLUE** screw cap cryovial tubes.
31. Once all aliquots are fulfilled for the specific time point, transfer all cryovials to an 81-cell cryobox dedicated to CPT Plasma specimens and store in a -80 °C freezer.
32. Enter on REDCap specimen log the date/time when specimen was processed.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

4.5.5.6. Isolating PBMCs from CPT™ tubes after storage or transport within 24 h post-collection

33. Retrieve the CPT™ tubes maintaining them in an upright position.
34. Label a new 50 mL conical tube as "Cells".
35. Label the necessary number of aliquoting cryovials.
    a. **CLEAR cryovials** for PBMC from CPT™ tubes.

Time point	Specimen (cryovial cap color)	Number of cryovial	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day X (NBBAL)	***PBMC*** **(clear)**	4	500 µL	ncdx

36. Carefully open the CPT™ tube and pipette the entire contents of the tube above the gel into the “Cells” conical tube.
37. Continue the PBMC isolation protocol starting from Step 13 in the “Washing PBMCs” section.
    a. **IMPORTANT:** If isolating PBMCs from stored or transported CPT™ tubes, the plasma fraction will not be collected.

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4.5.6. PAXgene Tubes

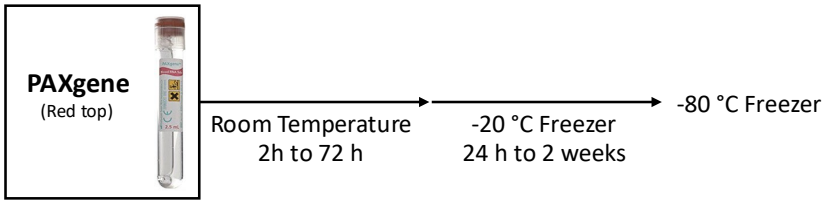

Figure 13: Processing Visual Summary - PAXGene Blood Tubes

1. After receiving the PAXgene tube from the collection site, label it according to the table below.

Time point	Specimen (tube cap color)	Number of tubes	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***PAXGene*** **(red tiger top)**	1	N/A	rpd0
Day 2	***PAXGene*** **(red tiger top)**	1	N/A	rpd2
Day 6	***PAXGene*** **(red tiger top)**	1	N/A	rpd6
Day 14	***PAXGene*** **(red tiger top)**	1	N/A	rpd14
LTO Timepoint 1 (3 to 6 months)	***PAXGene*** **(red tiger top)**	1	N/A	rpm3
LTO Timepoint 2 (12 months)	***PAXGene*** **(red tiger top)**	1	N/A	rpm12

2. Leave the PAXgene tube upright at room temperature (18−25˚C) for a **minimum of 2 hours and a maximum of 72 hours**.
3. Place the PAXgene tube upright in a -20 °C freezer for a **minimum of 24 hours and a maximum of 2 weeks**.

- *NOTE*: Do not freeze PAXgene tubes in a Styrofoam™ tray. The CCC recommends the box for PAXgene storage and shipping itemized in Section 4.1.

4. Move the PAXgene tube into a -80 °C freezer.
5. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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4.6. References

- CPT™ tubes Instruction for Use (BD Website, accessed in July 2024):
  - https://www.bdbiosciences.com/content/dam/bdb/products/global/blood-collection/blood-collection-tubes/362xxx/3627xx/362760_base/pdf/VDP40394.pdf
- PAXgene tubes Instruction for Use (BD Website, accessed in July 2024):
  - https://www.bdbiosciences.com/content/dam/bdb/products/global/blood-collection/blood-collection-tubes/762xxx/7621xx/762165_base/pdf/VDP40400.pdf

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5. Respiratory Specimens Collection and Processing

5.1. Materials

Materials required for respiratory collection, processing, and aliquoting are summarized here. More details on supplies are available in Section 3 of this SOP.

5.1.1. Collection Supplies

- 6x9 Specimen Bags (generic)
- BAL Supply kit
- Ballard Mini BAL sampling catheter
- Covidien Argyle Specimen Trap 40mL
- HME Filter
- Primary Specimen labels

5.1.2. Processing and Aliquoting Supplies

- 25 mL Eppendorf tube
- 100 μm Cell Strainer
- CryoStor® CS10 freezing medium
- BD Pharm 10x Lysis Buffer
- DNA/RNA Shield (2x)
- Lab Tape
- Yellow cryovials
- Clear cryovials
- Clear cryovials with green cap inserts
- Clear cryovials with white cap inserts
- Pink cryovials
- Dark green cryovials with DNA/RNA shield
- Lab Processing Labels

5.1.3. Additional supplies not provided by the CCC

Item	*Manufacturer/Vendor*	*Catalog Number*
1.5 mL microcentrifuge tube	Various	Various
DPBS	Gibco, or equivalent	14190250, or equivalent

5.1.4. Equipment

Equipment Name	*Required features/capabilities*
Micropipettes	P1000, P200 (calibrated) paired with sterile filtered tips
Mini Vortex	N/A.
Serological Pipettes	Various volumes, sterile
Slow freeze apparatus	Mr. Frosty, CoolCell, or equivalent
Tabletop Centrifuge	Swing bucket, temperature control (cooling)

5.2. Biosafety

Concentrations of respiratory infectious agents (e.g., SARS-CoV-2 or TB) can be very high in respiratory specimens compared to blood specimens. **DO NOT collect respiratory specimens on patients with suspected or confirmed TB.** All personnel must follow applicable Institutional Biosafety requirements and guidelines and/or other Institutional policies regarding laboratory best practices to safely process respiratory samples that may contain various infectious agents (e.g., if COVID+ respiratory samples must be processed in a BSL2+ laboratory).

Wear all appropriate personal protective equipment while working in the laboratory and follow all protocols for working safely in a BSC when applicable.

5.3. Schedule of Events

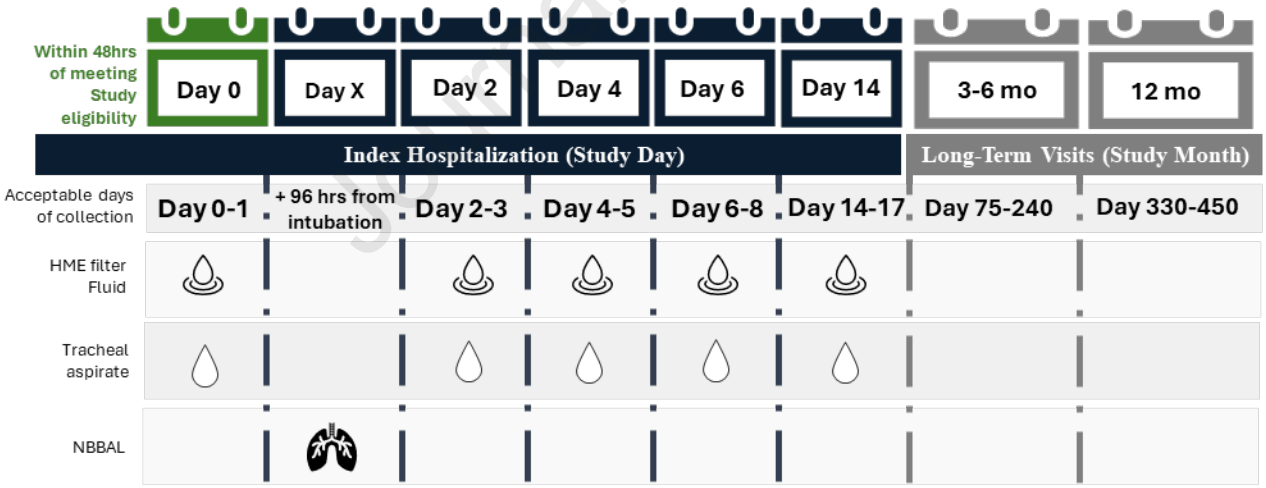

Figure 14: Schedule of Events: Respiratory Specimens

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5.4. Respiratory Specimen Collection

5.4.1. Respiratory Specimen Collection Best Practices

- All collection times should be entered in the APS REDCap database at the time of specimen collection.
- If access to REDCap is not readily available, use the paper specimen log sheet available in the "Specimen Chain of Custody" section of this SOP.
- When placing aliquot vials into a cryobox, prioritize filling each cryobox with as many vials of the same biospecimen type as possible. If there is remaining space after one respiratory specimen type has been placed into a cryobox, you may fill the rest of the cryobox with other types of respiratory biospecimens, leaving an empty row between the different microbiome biospecimen types. However, do not mix respiratory biospecimens with blood or microbiome biospecimens in the same cryobox. Please refer to the shipping section of this SOP for more details.

5.4.2. Heat Moisture Exchange Filter (HME Filter)

Time point	Collection Device	Quantity
Day 0	HME Filter	1
Day 2	HME Filter	1
Day 4	HME Filter	1
Day 6	HME Filter	1
Day 14	HME Filter	1

5.4.2.1. Important notes

- HME filters are placed by Respiratory Care Practitioners (RCPs) or by study personnel with properly documented training.
- HME filters sit in line with the endotracheal tube for four hours.
- As described in Section 5.2. Biosafety, confirm Institutional policies regarding collection and processing of HME filter fluid for COVID+.

5.4.2.2. Safety screen

- HME filters should not be changed if the patient fails the safety screen. **A Patient fails if <u>any</u> of the following criteria are met at the time of daily assessment** for HME placement:
  - High FiO2 requirements (>80%).
  - High PEEP (>15 cm H2O).
  - Low SpO2 (<88%).
  - Actively receiving continuous inhaled epoprostenol (Flolan) or nitric oxide
  - Actively receiving nebulizer therapy.

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- Need nebulizer treatment in the next 4 hours.
- Need humidified air in the next 4 hours.
- And/or per staff discretion.
  - If secretions are too profuse or thick, RCP may decline to turn off the heated humidifier.
    - *Sometimes, patients are placed on 100% FiO2 just after intubation and then are rapidly titrated down. If this is the case, re-consider in a few hours.*

- While not a safety screen-out, intubated patients are often on q4 or q6 RCP breathing treatments. If possible, try timing the placement of HME filter around these treatments so that the four-hour saturation is not interrupted by breathing treatments.

5.4.2.3. Placing the HME Filter

1. Label the HME Filter with the provided patient collection label.
2. Temporarily set the ventilator's oxygen setting to 100% for 1-2 minutes.
3. Place the HME filter adjacent to the endotracheal mouthpiece.
   a. If a suction catheter is present, leave it in place and position the HME filter between the catheter and the vent circuit.

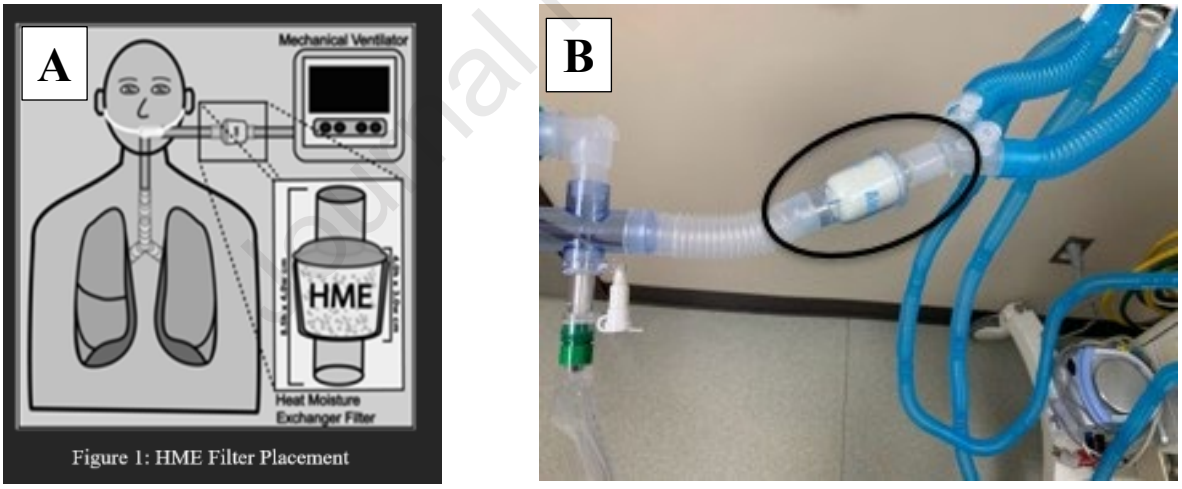

Figure 15: HME Filter Placement. A) Adjacent to mouthpiece; B) between suction catheter and vent circuit.

4. If the patient is on a heated circuit, the heated circuit must be turned off to avoid excessive condensation in the circuit. The heated circuit can be turned off in two different ways:
   a. RCP can press the "off" button on the humidifier to turn off the heater, or
   b. RCP can bypass the humidifier by rearranging the ventilator and humidifier tubing.

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[See linked video in Section 5.6 for demonstration]

5. Let the HME filter sit in line and saturate for **four (4) hours**.
    a. Minimum of two (2) hours, maximum of six (6) hours.
6. Enter the time of the HME Filter retrieval in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.
7. Take the filter for processing **within 1 hour after collection**.

5.4.3. Tracheal Aspirate (TA)

Time point	Collection Device	Quantity
Day 0	Specimen Trap 40mL	1
Day 2	Specimen Trap 40mL	1
Day 4	Specimen Trap 40mL	1
Day 6	Specimen Trap 40mL	1
Day 14	Specimen Trap 40mL	1

5.4.3.1. Important notes

- A Respiratory Care Practitioner (RCP) will collect research TA samples as part of their routine clinical care for intubated patients.
- As described in Section 5.2, Biosafety, confirm Institutional policies regarding collection and processing of TA fluid for COVID+ patients.
- If tracheal aspirate and NBBAL are going to be collected on the same day for a particular participant, collect the tracheal aspirate first.
- If saline is used in the Tracheal Aspirate collection, this must be documented in the REDCap Biospecimen Collection and Processing Form

5.4.3.2. Guidelines for RCPs

- If the patient was intubated <12 hours, allow typical workflow for RTs without mandating replacement of in-line suction.
- If intubated >12 hours, remind the RCP to please change out the ballard/in-line suction device before the research specimen is collected (as they would for a clinical TA collection)
- Exclude patients with high suspicion or confirmed TB.
- When to involve a Study Investigator:
    - If the bedside staff express discomfort with the request to replace in-line suction for samples obtained <24 hours after intubation.

5.4.3.3. Procedure

1. Collect the Tracheal Aspirate specimen following the locally approved procedures and training protocols.
2. Enter the time of the HME Filter retrieval in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

5.4.4. Non-bronchoscopic Bronchoalveolar Lavage (NBBAL)

Time point	Collection Device	Quantity
Day of Intubation	Mini BAL catheter 16Fr. 142	1
	BAL Supply kit	1

5.4.4.1. Important notes

- Collect the NBBAL specimen following the locally approved procedures and training protocols.
- Do not conduct NBBAL on patients with bronchial or tracheal stents.
- If a full bronchoscopic bronchioalveolar lavage (BAL) is performed clinically within 96 hours following intubation and sample can be obtained for the APS study, an NBBAL does not need to be performed. Clinical BAL samples can be used in place of NBBAL. This should be documented on the NBBAL sample form in REDCap.
- Sites have a set instillation volume of either 40 mL or 80 mL. Follow the steps below for the volume to which your site has been assigned.
- Ensure that all safety criteria have been met as specified in the APS Master Protocol.
- NBBAL should be performed as soon as possible following initiation of mechanical ventilation. If safety criteria are not met, NBBAL can be delayed for up to 96 hours after intubation.
- Sites should make a local determination as to whether to hold tube feeds prior to the NBBAL procedure.

5.4.4.2. Prior to the procedure

1. Perform closed suctioning of respiratory secretions using in-line suction device.
2. Place the patient on 100% FiO2 for a minimum of 10 minutes to “pre-oxygenate”. This can be done while setting up for the procedure.

5.4.4.3. Procedure setup

3. **40 mL instillation sites:** Fill one syringe with 40 mL sterile saline. Leave 5 mL of air behind the saline to ensure that all the saline gets flushed through the catheter channel during the

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procedure.

4. **80 mL instillation sites:** Fill two syringes, each with 40 mL sterile saline. Leave 5 mL of air behind the saline to ensure that all the saline gets flushed through the catheter channel during the procedure.
5. Open non-bronchoscopic catheter kit, maintaining sterile conditions (Ballard Mini BAL sampling catheter 16Fr. 142).
6. Attach the suction adapter to the distal end of the three-way stopcock.
7. Attach the specimen trap to the suction adapter. Attach the other hub of the specimen trap to wall suction.
8. Attach the saline and air-filled syringe to the three-way stopcock.
9. Remove the protective cover from the NBBAL catheter tip and insert the catheter tip through the access port elbow. Extend the catheter tip approximately 1.5 inches past the elbow adapter.
10. Disconnect the ventilator circuit from the endotracheal tube and quickly attach the elbow adapter.
11. Reattach the ventilator circuit.
12. Rotate the oxygen port to direct the catheter tip to go down either the right or left side.
13. Advance the NBBAL outer catheter until the markings on the outside of the catheter match with the markings on the endotracheal tube, and then advance an additional 5 cm.
14. Clear the tip of the catheter by instilling 2 mL of saline through the catheter.
15. Hold the outer catheter in place and gently advance the inner catheter until resistance is met. Pull back slightly (~ 1 cm).
16. Holding the syringe upright (so that air is on top of the saline), instill 40 mL of saline into the lungs.
17. **80 mL instillation sites only:** Close the stopcock to suction. Place the second syringe on the stopcock and instill the second aliquot of saline.
18. Hold the collection trap upright and then turn the three-way stopcock to enable suctioning of fluid from the lungs into the collection trap.
19. Turn off suction. Withdraw the NBBAL catheter until the tip can be seen in the connector elbow.
20. Remove the NBBAL catheter and elbow together and reconnect the endotracheal tube to the ventilator circuit.
21. Disconnect the specimen trap from the NBBAL catheter circuit and seal the specimen trap. *The specimen is ready for processing*.
22. Return the patient to their prior ventilator settings. Restart enteral nutrition if this was stopped.
23. Enter the volume of recovered fluid and time of collection in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available

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in Section 7.2 of this SOP.

5.5. Processing Respiratory Specimens

5.5.1. Heat Moisture Exchange Filter (HME Filter)

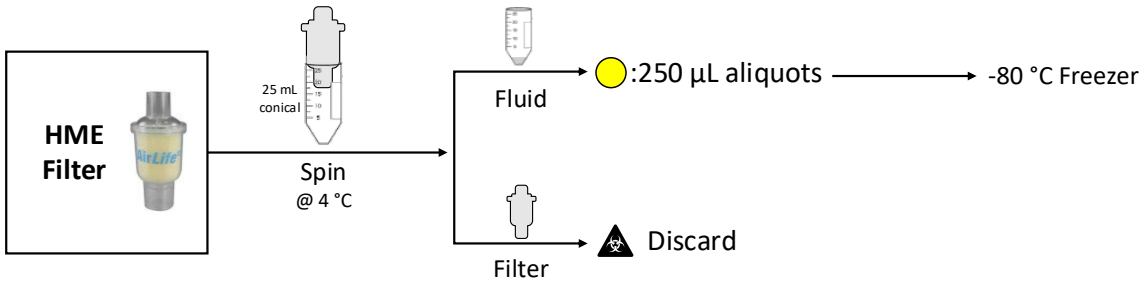

Figure 16: Processing Visual Summary - HME Filter

Figure 17: Securing the HME filter on a 25 mL conical tube adaptor.

5.5.1.1. Important Notes

- As described in Section 5.2. Biosafety, confirm Institutional policies regarding collection and processing of HME filter fluid for COVID+.
- Processing of HME filter fluid should be completed following BSL2 guidelines, and the staff members should wear appropriate PPE, such as a surgical mask.

5.5.1.2. Procedure

1. Equilibrate the centrifuge temperature to 4 °C.
    a. *NOTE*: if access to a temperature-controlled centrifuge is not available, perform the centrifugation at room temperature (18-25 °C).
2. Using a permanent marker, label a 25 mL conical tube pairing it with a respective HME filter.
3. Tape the HME filter to the correspondent 25 mL centrifuge tube. (See Figure 17)
4. Verify that the centrifuge temperature is 4 °C.
5. Ensure the centrifuge has enough clearance for properly attaching shields to the top of the tube

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holders to contain aerosols.

6. Ensure that centrifuge buckets are correctly balanced.
    a. To achieve this balance for a single HME sample, tape an unused HME filter to a 25 mL conical tube. This empty tube can be re-used for balancing the centrifuge.
7. Place the adapted HME filter in the centrifuge and spin for **10 minutes at 1,500 RCF (xg force) at 4 °C**.
8. Label the 2.0 mL screw cap aliquoting cryovials as described in the table below:
    a. **YELLOW** for HME Filter Fluid aliquots.

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters of label)
Day 0	***HME Filter Fluid*** **(yellow)**	10	250 µL	hfd0
Day 2	***HME Filter Fluid*** **(yellow)**	10	250 µL	hfd2
Day 4	***HME Filter Fluid*** **(yellow)**	10	250 µL	hfd4
Day 6	***HME Filter Fluid*** **(yellow)**	10	250 µL	hfd6
Day 14	***HME Filter Fluid*** **(yellow)**	10	250 µL	hfd14

9. Once the spin is complete, allow the filters to rest in the centrifuge buckets for 5 minutes before removing them from the centrifuge.
10. Carefully remove the tape separating the HME filter from the 25 mL conical tube.
11. Secure the 25 mL conical tube in a tube rack.
    a. The 25 mL conical tube should contain the HME filter fluid.
12. Dispose of the HME filter in a biohazard waste.
13. Using a P1000 micropipette set to 250 µL, aliquot the HME filter fluid into up to ten (10) labeled cryovials.
14. Place cryovials in an 81-cell cryobox dedicated to HME specimens and store in a -80 °C freezer.
15. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP .

5.5.2. Tracheal Aspirate (TA)

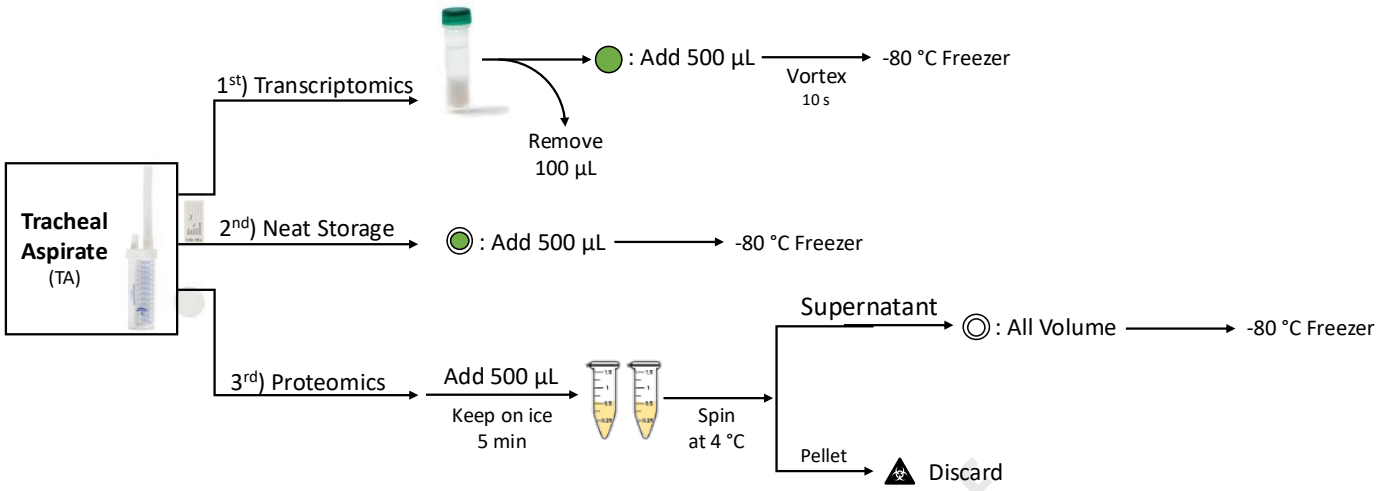

Figure 18: Processing Visual Summary - Tracheal Aspirate (TA) Fluid

5.5.2.1. Important notes

- It is important to keep the TA fluid on ice or at ≤4 °C. Samples should be processed within 30 minutes or no more than 2 hours from the time of collection.
- If insufficient TA volume is available, prioritize aliquots in the order listed below. Document in REDCap which aliquots were successfully collected.
  - First Priority: 2 × 500 µL transcriptomic aliquots (dark green caps with RNA Shield + beads)
  - Second Priority: 2 × 500 µL neat storage aliquots (clear/green cap inserts, no processing)
  - Third Priority: Up to 2 × 500 µL proteomics aliquots (clear/white cap inserts requires immediate processing)

5.5.2.2. Procedure for Transcriptomics Aliquots (Figure 18A)

1. Label the Zymo RNA Shield + bashing beads tubes as described in the table below.
   a. **DARK GREEN cap with beads** for TA Fluid aliquots.

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***TA Fluid*** **(dark green)**	2	500 µL	tad0
Day 2	***TA Fluid*** **(dark green)**	2	500 µL	tad2
Day 4	***TA Fluid*** **(dark green)**	2	500 µL	tad4
Day 6	***TA Fluid*** **(dark green)**	2	500 µL	tad6

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Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 14	***TA Fluid*** **(dark green)**	2	500 µL	tad14

2. Retrieve the two labeled bead lysis cryovials and let them stand until all beads settle on the bottom of the tube.
3. Using a P200 micropipette with filter tips set to 100 µL, discard 100 µL of the buffer from each tube into a waste container, without disturbing the beads.
4. Bring the tracheal aspirate to the BSC. Using a P1000 micropipette with filter tips set to 500 µL, aliquot the tracheal aspirate from the specimen trap into two labeled bead lysis tubes containing 1.0 mL Zymo RNA Shield + bashing beads.

- *NOTE*: aliquot carefully. The pre-filled tube can accommodate 500 µL of TA fluid.

5. Vortex tubes for 10 seconds.
6. Place cryovials in an 81-cell cryobox dedicated to Respiratory specimens and store in a -80 °C freezer.
7. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

5.5.2.3. Neat Storage Aliquots (Figure 18B)

1. Label the 2.0 mL screw cap aliquoting cryovials for neat TA storage as described in the table below.
    a. **CLEAR** cryovial with **GREEN** cap insert for neat TA aliquots (no reagent added).

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***TA Fluid*** **(green cap insert)**	2	500 µL	tand0
Day 2	***TA Fluid*** **(green cap insert)**	2	500 µL	tand2
Day 4	***TA Fluid*** **(green cap insert)**	2	500 µL	tand4
Day 6	***TA Fluid*** **(green cap insert)**	2	500 µL	tand6
Day 14	***TA Fluid*** **(green cap insert)**	2	500 µL	tand14

2. Using a P1000 micropipette with filter tips set to 500 µL, aliquot 500 µL of tracheal aspirate from the specimen trap into each of the two labeled 2.0 mL cryovials.
    a. *NOTE*: These aliquots contain NO reagent and are stored neat (unprocessed).
    b. *NOTE:* For thick TA samples that cannot be pipetted with standard tips, see Biospecimen FAQs for alternative processing methods.
3. Place neat TA cryovials in an 81-cell cryobox dedicated to Respiratory specimens and store in a -80 °C freezer immediately.
4. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

5.5.2.4. Proteomics Aliquots (Figure 18C)

1. Label the 2.0 mL screw cap aliquoting cryovials for proteomics aliquots as described in the table below.
    a. **CLEAR** cryovial with **WHITE** cap insert for proteomics TA aliquots

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***TA Fluid*** **(white cap insert)**	2	500 µL	tapd0
Day 2	***TA Fluid*** **(white cap insert)**	2	500 µL	tapd2
Day 4	***TA Fluid*** **(white cap insert)**	2	500 µL	tapd4
Day 6	***TA Fluid*** **(white cap insert)**	2	500 µL	tapd6
Day 14	***TA Fluid*** **(white cap insert)**	2	500 µL	tapd14

2. Obtain two 1.5 mL microcentrifuge tubes
3. Using a permanent marker, label the 1.5 mL microcentrifuge tubes, pairing it to is respective Tracheal Aspirate specimen ID
4. Using a P1000 micropipette with filter tips set to 500 µL, aliquot 500 µL of tracheal aspirate from the specimen trap into each labeled 1.5 mL microcentrifuge tube

- *NOTE*: These samples must be processed immediately as they are temperature sensitive.
- *NOTE:* For thick TA samples that cannot be pipetted with standard tips, see Section 5.5.2.1 for

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alternative processing methods.

5. Keep samples on ice for 5 minutes if using a room temperature centrifuge or proceed directly if using a refrigerated centrifuge.
6. Using a refrigerated centrifuge (set to 4 °C), spin the TA sample at **15,000 RCF (xg force) for 5 minutes**.
    a. **Sites without refrigerated centrifuge**: Keep samples on ice for 5 minutes prior to spinning at room temperature.
7. Pipette the supernatant into the labeled 2.0 mL **CLEAR** cryovial with **WHITE** cap insert. This supernatant is the TA-proteomics sample.
    a. The supernatant should be clear to cloudy with a distinct line of separation from the solid material that settles at the bottom after centrifugation.

- **CRITICAL**: Do not contaminate the supernatant with pellet material. If the supernatant is disturbed during pipetting, re-spin the sample at 15,000 RCF (xg force) for 5 minutes.
    b. Leave the pellet at the bottom of the original microcentrifuge tube and discard.
8. Place processed proteomics supernatant samples in an 81-cell cryobox dedicated to Respiratory specimens and store in a -80 °C freezer immediately.
9. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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5.5.3. Non-bronchoscopic Bronchoalveolar Lavage (NBBAL)

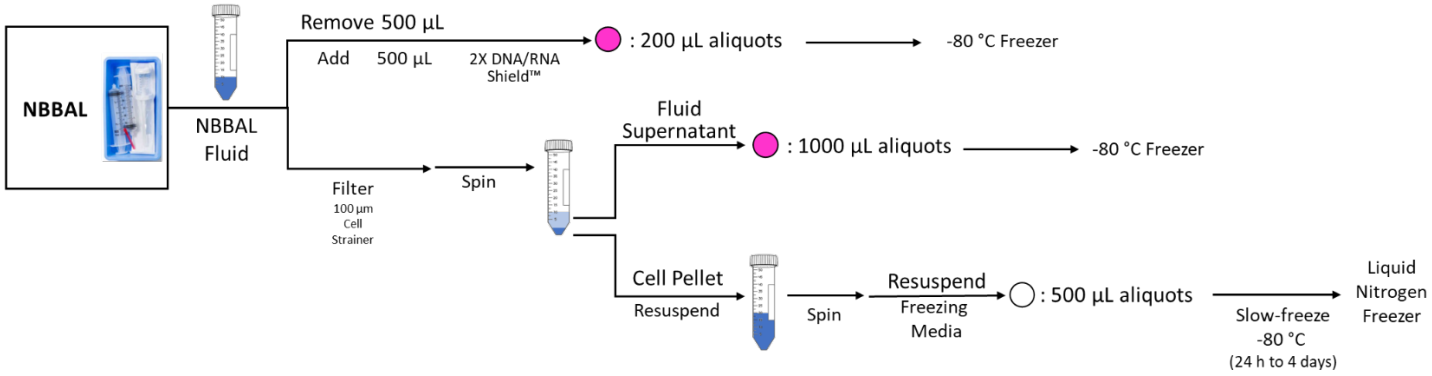

Figure 19: Processing Visual Summary - NBBAL (Fluid and Cells)

5.5.3.1. NBBAL Fluid – Metagenomics

1. Using a permanent marker, label a 1.5 mL microcentrifuge tube, pairing it to the respective NBBAL specimen ID.
2. In a BSC, use a P1000 pipette to transfer 500 µL of unprocessed fluid for Metagenomics into the 1.5 mL microcentrifuge tube.
3. Add 500 µL of 2x DNA/RNA Shield™ to the tube and gently homogenize by pipetting up-and-down.
4. Label the 2.0 mL screw cap aliquoting cryovials as described in the table below:
    a. **PINK** for NBBAL Fluid aliquots.

Time point	Specimen (tube color)	Number of tubes	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day of intubation	***NBBAL Fluid* (pink)**	5	200 µL	mgdx

5. Using a P1000 micropipette set to 200 µL and filtered tips, aliquot the NBBAL fluid as 200 µL aliquots into five (5) labeled **PINK** cryovials.
6. Reserve the remaining NBBAL fluid for the next step (see section 5.5.3.2)
7. Place cryovials in an 81-cell cryobox dedicated to NBBAL specimens and store in a -80 °C freezer.
8. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available

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in Section 7.2 of this SOP.

5.5.3.2. NBBAL Fluid

1. Using a permanent marker, label a sterile 50 mL conical tube, pairing it to the respective NBBAL specimen ID.
2. Place a sterile 100 µm cell strainer (yellow) onto the labeled 50 mL conical tube.
3. Pour the remaining NBBAL fluid through the strainer to eliminate mucus and debris.
4. Centrifuge the 50 mL conical tube **for 10 minutes at 300 RCF (xg force) at room temperature (18-25 °C)**.
5. Label the 2.0 mL screw cap aliquoting cryovials according to the table below.
    a. **PINK** for NBBAL Fluid aliquots.

Time point	Specimen (tube color)	Number of tubes	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day of intubation	***NBBAL Fluid*** **(pink)**	10*	1000 µL	nfdx

* *The number of these aliquots are dependent on the volume of processed specimen. Sites may generate up to this number of cryovials.*

6. Remove the 50 mL tube from the centrifuge and wipe it with a paper towel moistened with 70% ethanol before placing it back into the BSC.
7. Using a P1000 micropipette set to 1 mL and filtered tips, aliquot the NBBAL fluid supernatant as 1 mL aliquots into labeled **PINK** cryovials without disturbing the cell pellet.

- *NOTE*: **Do not discard unused tubes.** If tubes were labeled and were not used (i.e., because of low blood volume in collection tube), retain for future use. Destroy the original label (e.g., by removal or covering the information using a permanent marker). Original labels can be overlaid with new labels.

8. Place cryovials in an 81-cell cryobox dedicated to NBBAL specimens and store in a -80 °C freezer.
9. Discard any remaining NBBAL fluid supernatant without disturbing the cell pellet. Reserve the tube with the cell pellet for the next step (see section 5.5.3.3).
10. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

5.5.3.3. NBBAL Cells – preparation

- ***NOTE:*** If NBBAL is grossly bloody (i.e., sample appears red, not "pinkish"), follow Steps 1 and

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2. If NBBAL is not bloody (i.e., sample appears pink or lighter) proceed to Step 5.

1. Prepare 1x lysis buffer:
    a. In a clean 50 mL conical tube, mix 1.0 mL of 10x BD Pharm Lyse™ solution into 9.0 mL of sterile distilled water.
2. Retrieve the 50 mL conical tube with the cell pellet (from Section 5.5.3.2, Step 9). Resuspend the pellet in 5 mL of 1x lysis buffer, vortex, and incubate at room temperature for 5 minutes.
3. Add DPBS to bring the volume in the tube to 50 mL and centrifuge **for 5 minutes at 300 RCF (xg force) at room temperature (18-25 °C)** to pellet cells.
4. Open the tubes and carefully pour the supernatant into a waste container without disturbing the cell pellet.
5. Resuspend the pellet in 10 mL of DPBS.
6. Vortex and mix well.
7. Prepare slow freeze device (e.g. Nalgene's Mr. Frosty or CoolCell) according to manufacturer instructions.

5.5.3.4. NBBAL Cells – resuspension in CryoStor®

8. Label screw cap 2.0 mL cryovial tubes as described in the table below:
    a. **CLEAR** cap for NBBAL Cells in CryoStor® aliquots.

Time point	Specimen (tube color)	Number of tubes	Aliquot Volume	Label specimen and timepoint convention (last 4-5 characters)
Day of intubation	***NBBAL Cells*** **(clear)**	5	500 µL	nccdx

9. Collect the 10 mL cell suspension from Step 5 and centrifuge **for 5 minutes at 300 RCF (xg force) at room temperature (18-25 °C)**.
10. Open the tube and carefully pour the supernatant into a waste container. Without disturbing the cell pellet, carefully pipette any remaining supernatant into the waste container.
11. Resuspend the cell pellet in 2.5 mL of CryoStor® freezing medium. Thoroughly resuspend the cell pellet by swirling or gentle pipetting.

- ***CRITICAL STEP*****:** Aliquot the cells quickly. After adding a freezing medium, cells must not sit at room temperature for longer than 5 minutes.

12. Using a P1000 pipette set to 500 µL, aliquot resuspended cells into five (5) labeled **CLEAR** screw cap cryovial tubes.
13. Immediately transfer the cryovial(s) into a slow-freeze device (e.g., Nalgene's Mr. Frosty or CoolCell).
14. Place the slow-freeze device into a -80 °C freezer; Leave it for at least **4 hours and no longer**

than 4 days.

- ***NOTE*:** The minimum manufacturer recommended freeze time for Mr. Frosty and CoolCell slow freeze devices **is 4 hours**. If using a different brand of slow-freeze device, refer to manufacturer instructions for minimum freeze time.

15. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.
16. Without allowing the cells to thaw, transfer cryovials to the LN2 freezer.
    a. If onsite liquid nitrogen is unavailable, NBBAL cells stored at -80 °C should be shipped to VUMC within one month of collection.

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5.6. References

HME filter fluid collection video

HME filter fluid processing video

NBBAL Instructional Video.url

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6. Microbiome Specimens Collection and Processing

6.1. Materials

Materials required for microbiome specimens collection, processing, and aliquoting are summarized here. More details on supplies are available in section 3 of this SOP.

6.1.1. Collection Supplies

- 12x12 specimen bag (for stool collection)
- 6x9 specimen bags (generic)
- Commode collectors (stool)
- OMNImet-GUT collection tube for in-hospital stool collection
- OMNImet/OMNIgene-GUT collection kit for LTO stool collection
- Specimen cup
- Zymo Research DNA/RNA Shield swab kit
- Primary Specimen Labels

6.1.2. Processing and Aliquoting

- 25 mL Eppendorf tube
- CryoStor® CS10 freezing medium
- Lab tape
- Lab processing labels
- Green cryovials
- Red cryovials
- Brown cryovials
- Clear, low binding cryovials
- Amber cryovials

6.1.3. Additional Supplies not provided by the CCC

Item	*Manufacturer/Vendor*	*Catalog Number*
DPBS without Calcium or Magnesium	Gibco, or equivalent	14190250, or equivalent

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6.1.4. Equipment

Equipment Name	*Required features/capabilities*
Micropipettes	P1000, P200 (calibrated) paired with sterile filtered tips
Tabletop Centrifuge	Swing bucket, temperature control (cooling)

6.2. Biosafety

All personnel must follow applicable Institutional Biosafety requirements and guidelines and/or other institutional policies. Wear all appropriate personal protective equipment while working in the laboratory and follow all protocols for working safely in a BSC when applicable.

6.3. Schedule of Events

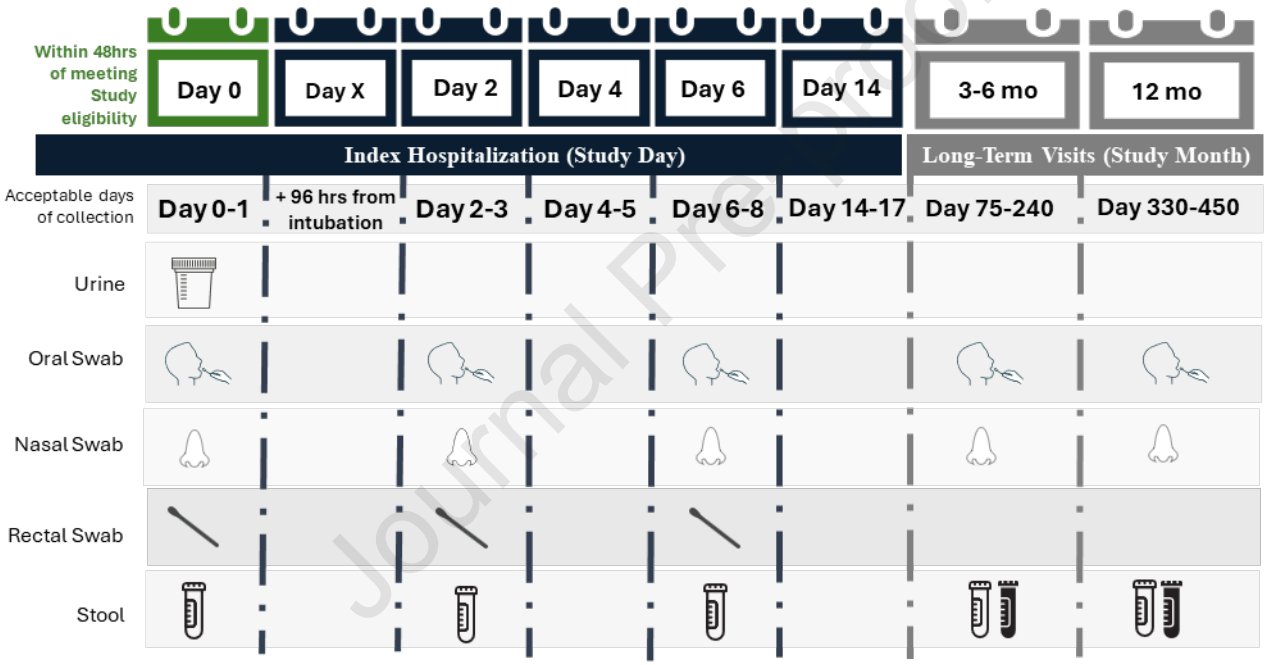

Figure 20: Schedule of Events: Microbiome Specimens

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6.4. Microbiome Specimen Collection

6.4.1. Microbiome Specimen Collection Best Practices

- All collection times should be entered into the APS REDCap database at the time of specimen collection.
  - If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP .
- When placing aliquot vials into a cryobox, prioritize filling each cryobox with as many vials of the same biospecimen type as possible. If there is remaining space after one microbiome specimen type has been placed into a cryobox, you may fill the rest of the cryobox with other types of microbiome biospecimens, leaving an empty row between the different respiratory biospecimen types. However, do not mix microbiome biospecimens with blood or respiratory biospecimens in the same cryobox. Please refer to Section 8, "Biospecimen Shipping" of this SOP for more details.
- If an ostomy is present, do not collect a stool sample. Rectal swabs can be collected from patients with an ostomy. When entering data on rectal swab collection into the EDC, document that an ostomy is present.

6.4.2. Oral Swabs

Time point	Collection Device	Quantity
Day 0	Zymo Swab Kit	1
Day 2	Zymo Swab Kit	1
Day 6	Zymo Swab Kit	1
LTO Timepoint 1 (3 to 6 months)	Zymo Swab Kit	1
LTO Timepoint 2 (12 months)	Zymo Swab Kit	1

6.4.2.1. Procedure

1. Peel open the sterile pouch and extract the sterile swab without contacting any surfaces
2. Swab the patient's cheeks, tongue, and upper palate for 15-20 seconds, applying moderate pressure.
3. Open the collection tube and insert swab tip into the tube/reagent.
4. Break the swab tip (at the 80 mm break point) into the tube.
5. Cap and invert the vial several times.
6. Enter the collection time in the APS REDCap database.
   a. If access to REDCap is not readily available, use the paper specimen log sheet available

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in Section 7.2 of this SOP.

6.4.3. Nasal Swabs

Time point	Collection Device	Quantity
Day 0	Zymo Swab Kit	1
Day 2	Zymo Swab Kit	1
Day 6	Zymo Swab Kit	1
LTO Timepoint 1 (3 to 6 months)	Zymo Swab Kit	1
LTO Timepoint 2 (12 months)	Zymo Swab Kit	1

6.4.3.1. Important notes

- Patients should NOT perform self-collection.
- Every swab should be administered to both nares.

6.4.3.2. Procedure

1. Peel open the sterile pouch and extract the sterile swab without contacting any surfaces.
2. Insert the swab in the patient's nostril to a depth of approximately one inch (2.5 cm).
3. Rotate the swab against all surfaces for approximately 3 seconds.
4. Using the same swab, repeat the same procedure for the contralateral nostril.
5. Open the collection tube and insert the swab tip into the tube/reagent.
6. Break the swab tip (at the 80 mm break point) into the tube.
7. Cap and invert the vial several times.
8. Enter the time of collection in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

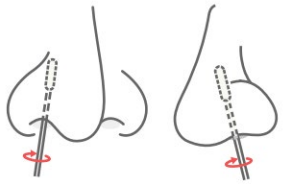

Figure 21: Nasal Swab Collection

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6.4.4. Rectal Swabs

Time point	Collection Device	Quantity
Day 0	Zymo Swab Kit	1
Day 2	Zymo Swab Kit	1
Day 6	Zymo Swab Kit	1

6.4.4.1. Important notes

- Swabs are intended to sample fecal matter and can thus be performed on fresh stool (evacuated within the preceding two hours), contents of fecal management systems (rectal tubes), or directly on patient rectums.
- Rectal swabs can be collected from a patient with an ostomy (ileostomy/colostomy). When entering rectal swab collection data into the EDC, document that an ostomy is present.
- **IMPORTANT:** do not swab the patient's rectum if the patient's serum platelet count is below 5,000/µL or if INR is greater than 5.

6.4.4.2. Procedure

1. For fresh stool and fecal management systems, directly swab the fecal matter to attain visible soilage of the swab tip.
2. For patient rectums, insert the swab into the rectum roughly 1-2 inches (2.5-5 cm) past the anal margin (the outside of the anus). Gently rotate the swab for approximately 5 seconds.
3. Open the collection tube and insert the swab tip into the tube/reagent.
4. Break the swab tip (at the 80 mm break point) into the tube.
5. Cap and invert the vial several times.
6. Enter the time of collection in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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6.4.5. Urine Specimens

Time point	Collection Device	Quantity
Day 0	Specimen Cup (sterile)	1

6.4.5.1. Procedure

1. Urine can be collected from Foley or condom catheter or if patient is spontaneously voiding (without catheter).
    a. For freshest sample, coordinate with the clinical team to collect urine next time the patient voids, if possible.
    b. If waiting for sample is necessary, leave specimen cup with the clinical staff along with the research team contact information
2. Collect ~5 mL of urine.
    a. Volumes lower than 5 mL are acceptable.
    b. Collected urine specimen is stable at room temperature for ~6 hours and for a maximum of 24 hours when refrigerated at 4 °C.
3. Enter the time of collection in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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6.4.6. Stool Specimens

Time point	Collection Device	Quantity
Day 0	OMNImet-GUT stool collection kit	1
	Commode collector	1
	12x12 specimen bag	1
Day 2	OMNImet-GUT stool collection kit	1
	Commode collector	1
	12x12 specimen bag	1
Day 6	OMNImet-GUT stool collection kit	1
	Commode collector	1
	12x12 specimen bag	1
LTO Timepoint 1 (3 to 6 months)	OMNImet/OMNIgene-GUT LTO collection kit	1
LTO Timepoint 2 (12 months)	OMNImet/OMNIgene-GUT LTO collection kit	1

6.4.6.1. Important notes

- Do not collect stool from a patient with an ostomy (ileostomy/colostomy).
- Specimens can be collected from freshly evacuated stool (within the preceding two hours) or directly from fecal management systems port.
  - Collection route priority 1: Freshly evacuated stool
    - If collecting fresh evacuated stool, use a sterile specimen container and sterile scoop or spatula to collect stool.
    - Collection from a diaper is allowable if sampling occurs within a maximum of 2 hours from evacuation of bowels
  - Collection route priority 2:
    - If collecting stool from fecal management systems, follow the institution's established procedures to collect from system's port most proximal to the patient.

6.4.6.2. Procedure

1. Collect approximately 5 g (roughly 5 mL, the size of a marble) of freshly evacuated stool either from a diaper, by using the commode collector, or through the fecal management systems port.
2. While holding the yellow tube top of the OMNImet-GUT collection tube, unscrew ONLY the purple cap and set aside for later use.

- NOTE: Handle the stabilization liquid with extra care

3. Use the spatula included in the collection kit to transfer a small amount of fresh stool into the

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collection tube.

4. Transfer the stool into the top portion of the yellow tube top.
5. Scrape horizontally across the tube top to level the sample and remove any excess. Wipe the exterior of the tube and top as needed.
6. Replace the purple cap onto the yellow tube top and screw until tightly closed.
7. Shake the sealed tube **for a minimum of 30 seconds** as fast as possible in a back and forth motion. The stool sample will be mixed with the stabilizing liquid in the tube. Not all particles will dissolve.
    a. Continue shaking if large particles remain.
8. Send specimen for processing. Samples will remain stable at room temperature for up to 7 days.
9. Enter the time of collection in the APS REDCap database.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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6.5. Processing Microbiome Specimens

6.5.1. Oral, Nasal, and Rectal Swabs

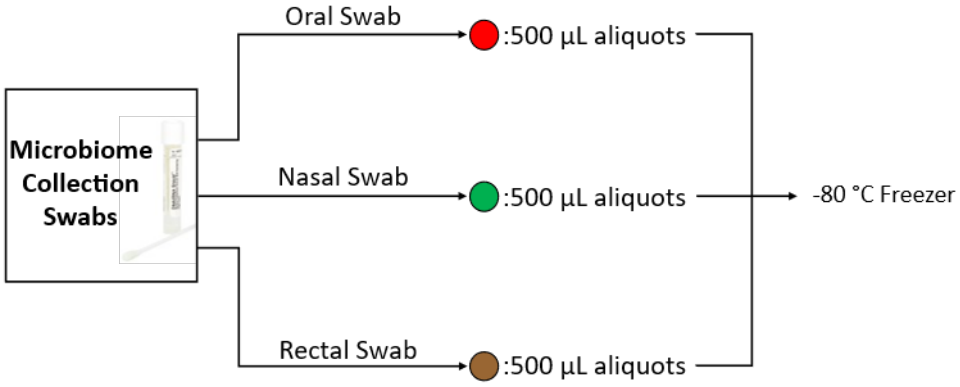

Figure 22: Processing Visual Summary - Oral, Nasal, and Rectal Swabs

6.5.1.1. Procedure

1. Retrieve the swab collection tubes.
2. Label the 2.0 mL screw cap aliquoting cryovials for each swab specimen as described in the tables below.
    a. **RED** cap cryovials for Oral swab aliquots.

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***Oral Swab*** **(red)**	2	500 µL	osd0
Day 2	***Oral Swab*** **(red)**	2	500 µL	osd2
Day 6	***Oral Swab*** **(red)**	2	500 µL	osd6
LTO Timepoint 1 (3 to 6 months)	***Oral Swab*** **(red)**	2	500 µL	osm3
LTO Timepoint 2 (12 months)	***Oral Swab*** **(red)**	2	500 µL	osm12

    b. **GREEN** cap cryovials for Nasal swab aliquots.

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***Nasal Swab*** **(green)**	2	500 µL	nsd0

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Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 2	***Nasal Swab*** **(green)**	2	500 µL	nsd2
Day 6	***Nasal Swab*** **(green)**	2	500 µL	nsd6
LTO Timepoint 1 (3 to 6 months)	***Nasal Swab*** **(green)**	2	500 µL	nsm3
LTO Timepoint 2 (12 months)	***Nasal Swab*** **(green)**	2	500 µL	nsm12

c. **BROWN** cap cryovials for Rectal swab aliquots.

Time point	Specimen (tube cap color)	Number of tubes/aliquots	Volume of aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***Rectal Swab*** **(brown)**	2	500 µL	rsd0
Day 2	***Rectal Swab*** **(brown)**	2	500 µL	rsd2
Day 6	***Rectal Swab*** **(brown)**	2	500 µL	rsd6

3. Vortex the collection tube containing the swab for approximately 10 seconds.
4. Using a P1000 micropipette set to 500 µL, transfer 500 µL of the specimen into each respective labeled color-coded cryovial according to the respective specimen.
    a. **RED** cap cryovials for Oral swab aliquots.
    b. **GREEN** cap cryovials for Nasal swab aliquots.
    c. **BROWN** cap cryovials for Rectal swab aliquots.
5. Place all cryovials in an 81-cell cryobox dedicated to Microbiome specimens and store in a -80 °C freezer.
6. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

6.5.2. Urine Specimens

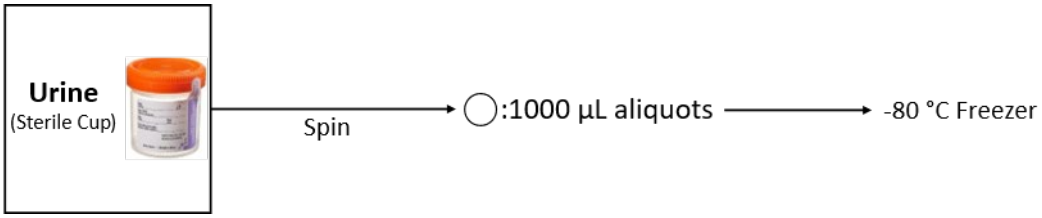

Figure 23: Processing Visual Summary - Urine Specimens

6.5.2.1. Procedure

1. Transfer urine into two 15 mL conical tubes. Fill each with equal amounts to counterbalance each other.
2. Securely screw the tube caps and transfer them to the centrifuge.
3. Centrifuge the tubes for **10 minutes at 1,200 RCF (xg force) at room temperature (18-25 °C)**, leaving acceleration and deceleration ON.
4. While tubes are spinning, label the 2.0 mL screw cap aliquoting cryovials as described in the table below.
    a. **CLEAR**, **low binding tubes** for Urine Specimen aliquots.

Time point	Specimen (tube cap color)	Number of tubes	Amount per aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***Urine*** **(clear)**	5*	1000 µL	urd0

* *The number of these aliquots are dependent on the volume of processed specimen. Sites may generate up to this number of cryovials.*

5. Gently retrieve the tubes from the centrifuge.
6. Using a P1000 pipette set to 1000 µL, aliquot the urine supernatant (careful to not collect the sediment) into the labeled **CLEAR** screw cap cryovial tubes.
7. Discard the 15 mL conical tubes containing any residual urine and sediments into a biohazard trash container.
8. Store all cryovials in 81-cell cryobox dedicated to urine specimens and store in a -80 °C freezer.
9. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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6.5.3. In-Hospital Stool Specimens

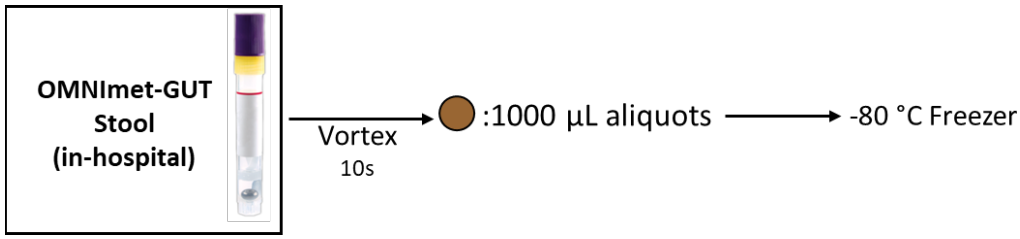

Figure 24: Processing Visual Summary - In-Hospital Stool Specimens

6.5.3.1. Procedure

1. Sterilize and decontaminate BSC according to local laboratory procedures.
    a. For example, wipe BSC surfaces with bleach and 70% ethanol for decontamination.
2. Label the 2.0 mL screw cap aliquoting cryovials as described in the table below:
    a. **AMBER BROWN** for Stool Specimen aliquots.

Time point	Specimen (tube cap color)	Number of tubes	Amount per aliquot	Label specimen and timepoint convention (last 4-5 characters)
Day 0	***In-hospital Stool*** **(amber brown)**	4	1000 µL	omd0
Day 2	***In-hospital Stool*** **(amber brown)**	4	1000 µL	omd2
Day 6	***In-hospital Stool*** **(amber brown)**	4	1000 µL	omd6

3. Retrieve the OMNImet-GUT collection tube.
4. Vortex collection tube (with stool and stabilizing reagents) for approximately 10 seconds.
5. Retrieve the four (4) 2.0 mL cryovial tubes labeled for in-hospital stool.
6. Using a P1000 pipette set to 1000 µL, make four 1.0 mL aliquots of OMNImet-GUT.
7. Once aliquoting is complete, wipe the work area with bleach wipes, followed by 70% ethanol.
8. Place all cryovials in 81-cell cryobox dedicated to stool specimens and store in a -80 °C freezer.
9. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

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6.5.4. Home Stool Collecting Kit

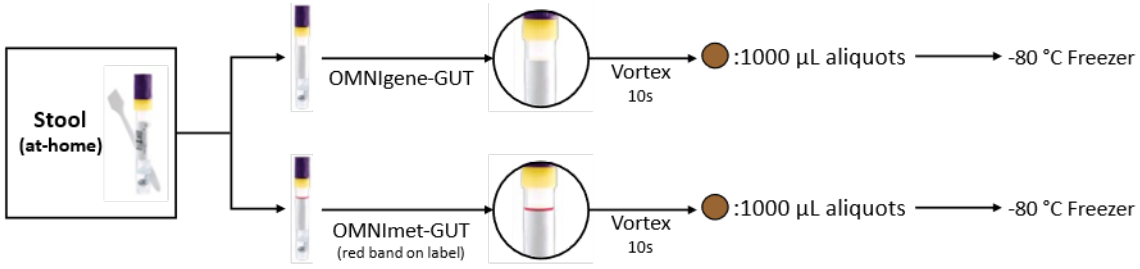

Figure 25: Processing Visual Summary - At-Home Stool Specimens

6.5.4.1. Procedure

1. Retrieve the at-home collection kit, which contains two tubes:
    a. OMNIgene-GUT
    b. OMNImet-GUT
2. Label the 2.0 mL screw cap aliquoting cryovials as described in the table below:
    i. **AMBER BROWN** for Stool Specimen aliquots. Four labeled for OMNIgene-GUT, and four labeled for OMNImet-GUT specimens.

Time point	Specimen (tube cap color)	Number of tubes	Amount per aliquot	Label specimen and timepoint convention (last 4-5 characters)
LTO Timepoint 1 (3 to 6 months)	***At-home Stool*** **(amber brown)**	4	0.5 – 1.0 g	ogm3
		4	0.5 – 1.0 g	omm3
LTO Timepoint 2 (12 months)	***At-home Stool*** **(amber brown)**	4	0.5 – 1.0 g	ogm12
		4	0.5 – 1.0 g	omm12

3. Vortex both collection tubes (with stool and stabilizing reagents) for approximately 10 seconds.
4. Retrieve the four (4) 2.0 mL cryovial tubes labeled for the OMNIgene-GUT specimens.
5. Using a P1000 pipette set to 1000 µL, make four 1.0 mL aliquots of OMNIgene-GUT.
6. Retrieve the four (4) 2.0 mL cryovial tubes labeled for the OMNImet-GUT specimens.
7. Using a P1000 pipette set to 1000 µL, make four 1.0 mL aliquots of OMNImet-GUT.
8. Place all cryovials in an 81-cell cryobox dedicated to stool specimens and store in a -80 °C freezer.
9. Enter the date/time when the specimen was processed in the REDCap Biospecimen Collection and Processing form.
    a. If access to REDCap is not readily available, use the paper specimen log sheet available in Section 7.2 of this SOP.

7. Biospecimen Chain of Custody

7.1. Specimen Chain of Custody

Collection

1. Access the *Biospecimen Collection and Processing* form from the respective day of collection on REDCap.
2. When collecting specimens for Day X, use the *NBBAL & Paired CPT* on REDCap.
    - If no access to REDCap, print and use the forms available in Section 11 of the Chain of Custody document.
3. Keep the specimens in secondary container and in the proper transport temperature.
4. Deliver specimens (and proper tracking form, if applicable) to the processing laboratory.

Processing

1. Retrieve specimens from the clinical team and the respective specimen tracking form, if applicable.
2. Verify the label on the form and labels on the collection tube.
3. Process specimens according to the APS Biorepository SOPs.
4. Annotate in the REDCap specimen tracking from:
    - Information must be recorded concordantly with processing, in real time. If access to REDCap is not readily available, print and use the forms available in Section 11 of the Chain of Custody document.

Shipping

1. Schedule to be determined on a site basis.
2. Fill up the shipping manifesto information on REDCap.
3. Send a copy of the manifesto to the APS email address.
    - **aps_biocclab@vumc.org**
4. From VUMC
    1. APS personnel will compare the shipping manifesto contents to the information on REDCap.
    2. APS personnel will inform the site about any discrepancies or missing information. (collecting and processing)
    3. If there are no discrepancies, APS personnel will send shipping labels and instructions.
5. Once confirmation is received, prepare shipment boxes per APS Biorepository SOP.
6. After placing shipment, send tracking info to:
    - **aps_biocclab@vumc.org**

At VUMC

1. Once the shipment arrives, APS personnel will match the information in the shipping manifesto against the contents.
2. Quality indications of successful shipment:
    - visual inspection
    - vials integrity
    - adequately labeled vials
    - apparent volume
    - documentation
    - tubes in the manifesto match shipped tubes
    - all corresponding specimen tracking forms have been sent (scanned PDFs are acceptable)
3. APS personnel will mark "received" next to each tube on REDCap.
4. APS personnel will store the received batch into two separate locations.
5. Specimens will be placed sequentially in labeled 81-vials cardboard boxes.
6. Boxes will be placed in -80 °C staging freezers for short-term, in-house storage.
7. Once transferred to long term storage, location will be entered in Freezerworks:
    - -80 °C and LN2 freezers located on the MCN 6th floor.
    - -80 °C freezer located in the MCN 3rd floor freezer farm.

7.2. Specimen Tracking Forms

For use if access to REDCap is unavailable or otherwise preferred by laboratory personnel. Data should be entered into the REDCap APS EDC at the earliest opportunity.

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APS Specimen Tracking Form / Lab Form

Day 0

Patient Label

Patient Label

Subject ID/Initials: _____

Collector's name (print): _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C or in LN2 Freezer?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes (-80° C) __ No			
	__ Buffy Coat				__ Yes (-80° C) __ No			
	__ RBC				__ Yes (-80° C) __ No			
	__ Na Citrate				__ Yes (-80° C) __ No			
	__ PAXgene				__ Yes (-80° C) __ No			
Respiratory	__ HME Filter				__ Yes (-80° C) __ No			
	__ Tracheal Aspirate				__ Yes (-80° C) __ No			
Microbiome	__ Nasal Swab				__ Yes (-80° C) __ No			
	__ Oral Swab				__ Yes (-80° C) __ No			
	__ Rectal Swab				__ Yes (-80° C) __ No			
	__ Stool				__ Yes (-80° C) __ No			
	__ Urine				__ Yes __ No			

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Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

Day X (NBBAL)

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C or in LN2 Freezer?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	___ CPT plasma				__ Yes (-80° C) __ No			
	___ CPT cells				__ Yes (LN2) __ No			
Respiratory	___ NBBAL Fluid Metagenomics				__ Yes (-80° C) __ No			
	___ NBBAL Fluid				__ Yes (-80° C) __ No			
	___ NBBAL Cells in CryoStor®				__ Yes (LN2) __ No			
	___ NBBAL Cells in DNA/RNA Shield™				__ Yes (-80° C) __ No			

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Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

Day 2

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				→ PROCESSING LAB ONLY				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C or LN2 Freezer?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes (-80° C) __ No			
	__ Na Citrate				__ Yes (-80° C) __ No			
	__ PAXgene				__ Yes (-80° C) __ No			
Respiratory	__ HME Filter				__ Yes __ No			
	__ Tracheal Aspirate				__ Yes __ No			
Microbiome	__ Nasal Swab				__ Yes __ No			
	__ Oral Swab				__ Yes __ No			
	__ Rectal Swab				__ Yes __ No			
	__ Stool				__ Yes __ No			

Journal Pre-proof

Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

Day 4

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes __ No			
Respiratory	__ HME Filter				__ Yes __ No			
	__ Tracheal Aspirate				__ Yes __ No			

Journal Pre-proof

Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

Day 6

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				→ PROCESSING LAB ONLY				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C or LN2 Freezer?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes (-80° C) __ No			
	__ Na Citrate				__ Yes (-80° C) __ No			
	__ PAXgene				__ Yes (-80° C) __ No			
Respiratory	__ HME Filter				__ Yes (-80° C) __ No			
	__ Tracheal Aspirate				__ Yes (-80° C) __ No			
Microbiome	__ Nasal Swab				__ Yes (-80° C) __ No			
	__ Oral Swab				__ Yes (-80° C) __ No			
	__ Rectal Swab				__ Yes (-80° C) __ No			
	__ Stool				__ Yes (-80° C) __ No			

Journal Pre-proof

Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

Day 14

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes __ No			
	__ PAXgene				__ Yes __ No			
Respiratory	__ HME Filter				__ Yes __ No			
	__ Tracheal Aspirate				__ Yes __ No			

Journal Pre-proof

Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

LTO Timepoint 1

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C or LN2 Freezer?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes (-80° C) __ No			
	__ RBC				__ Yes (-80° C) __ No			
	__ Na Citrate				__ Yes (-80° C) __ No			
	__ PAXgene				__ Yes (-80° C) __ No			
Microbiome	__ Nasal Swab				__ Yes (-80° C) __ No			
	__ Oral Swab				__ Yes (-80° C) __ No			
	__ Stool				__ Yes (-80° C) __ No			

Journal Pre-proof

Journal Pre-proof

APS Specimen Tracking Form / Lab Form

Patient Label

Patient Label

LTO Timepoint 2

Subject ID/Initials: _____

Collector's name (print): _____Date: _____

Specimen Collected (Check all that apply)

				PROCESSING LAB ONLY →				
		Volume Collected (mL)	Date and time of Collection	Date and time of Processing	Processed and stored at -80°C?	Number of Aliquots	Comments and Deviations	Operator Initials
Blood	__ EDTA				__ Yes __ No			
	__ RBC				__ Yes __ No			
	__ PAXgene				__ Yes __ No			
Microbiome	__ Nasal Swab				__ Yes __ No			
	__ Oral Swab				__ Yes __ No			
	__ Stool				__ Yes __ No			

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Journal Pre-proof

8. Biospecimen Shipping

8.1. Materials

Sites are responsible for all storage and shipping supplies.

Item
Insulated shipper
SAFPAK bag (clear bag) and Tyvek SAFPAK envelope (White) *or equivalent 95kPa certified bag or secondary container*
Absorbent material (Therapak™ Absorbent Sheet, 3x6 in; catalog nubmer: 22-130-040) *or other IATA or DOT-approved equivalent*
Dry ICE UN1845 label (See appendix)
UN3373 Category B Specimen label (See appendix)

8.2. Biospecimen Shipping Best Practices

8.2.1. Biospecimen cryoboxes

- Ensure the cryobox is clearly identified with the collection type
- Specimens should be consolidated and organized by collection type (blood, respiratory, or microbiome).
  - Do not consolidate different collection types into a single cryobox. For example, blood specimens should never be consolidated into the same box with microbiome or respiratory specimens.
  - For specimens that do not fill an entire box, consolidate cryovials together by specimen type, leaving an empty row between specimen types.
- Once all specimens have been consolidated, start a new box for the next collection type.
- Cryoboxes of specimens should be shipped closed, fastened with rubber bands, and securely placed in sealed 95kPa sealable bags with absorbent material. No specimens should be sent loose inside specimen bags.
- There are 3 types of cryoboxes:
  - Blood cryobox
  - Microbiome cryobox
  - Respiratory cryobox

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8.2.1.1. Blood biospecimen cryoboxes

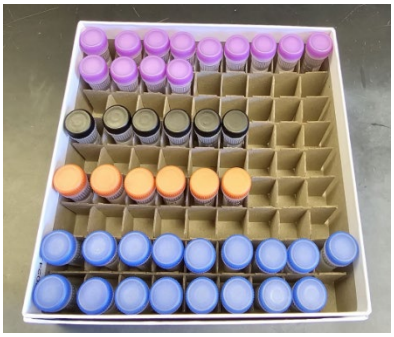

Figure 26: Consolidation of multiple blood specimen types

- The following biospecimen types may be placed into blood cryoboxes (Figure 26):
  - EDTA Plasma
  - CPT Plasma
  - CPT PBMCs
  - EDTA Buffy Coats
  - Na Citrate Plasma
  - Red Blood Cells
- For RNA PAXgene tubes, if the box is not full, stabilize lid by evenly distributing tubes along the box sides. Place a rubber band around the box to secure lid before shipping.

8.2.1.2. Microbiome specimen cryoboxes

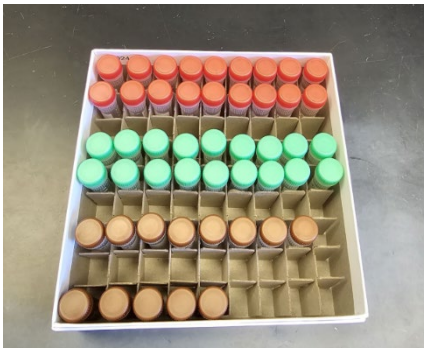

Figure 27: Consolidation of multiple microbiome specimen types

- The following biospecimen types may be placed into microbiome cryoboxes (Figure 27):
  - Oral swab
  - Nasal swab
  - Rectal swab
  - Stool

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- Urine

8.2.1.3. Respiratory biospecimen cryoboxes

Figure 28: Consolidation of multiple respiratory specimen types

- The following biospecimen types may be placed into respiratory cryoboxes (Figure 28)
  - HME Filter Fluid
  - TA
  - NBBAL Fluid (i.e., NBBAL fluid and NBBAL fluid for metagenomics)
  - NBBAL Cells

8.2.2. Preparing a shipment

- All specimen work taking place outside of the freezer should be done on dry ice, ensuring all specimens remain fully frozen at all times.
- Specimen manifests should be prepared as described in Section 8.3 and provided to the CCC at least one week in advance of the proposed shipment date.
  - The site and the CCC must acknowledge and agree to the manifest prior to shipment.
  - The site should confirm that all specimens listed in the shipping manifest are included before packing the shipment
- Shipping labels will be provided by the APS Biorepository Lab
- Shipments should be packaged as described in Section 8.4 and comply with all institutional guidelines and federal regulations.

8.2.3. Shipping schedule

- Specimens will be shipped every 4 weeks starting January 2025.
- Per the Coordinating Center, sites will be designated to ship either the second or the fourth week of the month.
- Shipment dates will be agreed upon by both parties prior to shipment.

- Site staff should take note of the date of the planned shipment to ensure that specimens will arrive on days when they can be received.
  - Shipments must occur between Monday to Wednesday. Deliveries cannot be accepted from Friday through Sunday.
  - Please be wary of holiday schedules and inclement weather alerts and how they may impact shipping.
- Any questions regarding this schedule can be sent to APS_biocclab@vumc.org.

8.3. Shipping Manifest

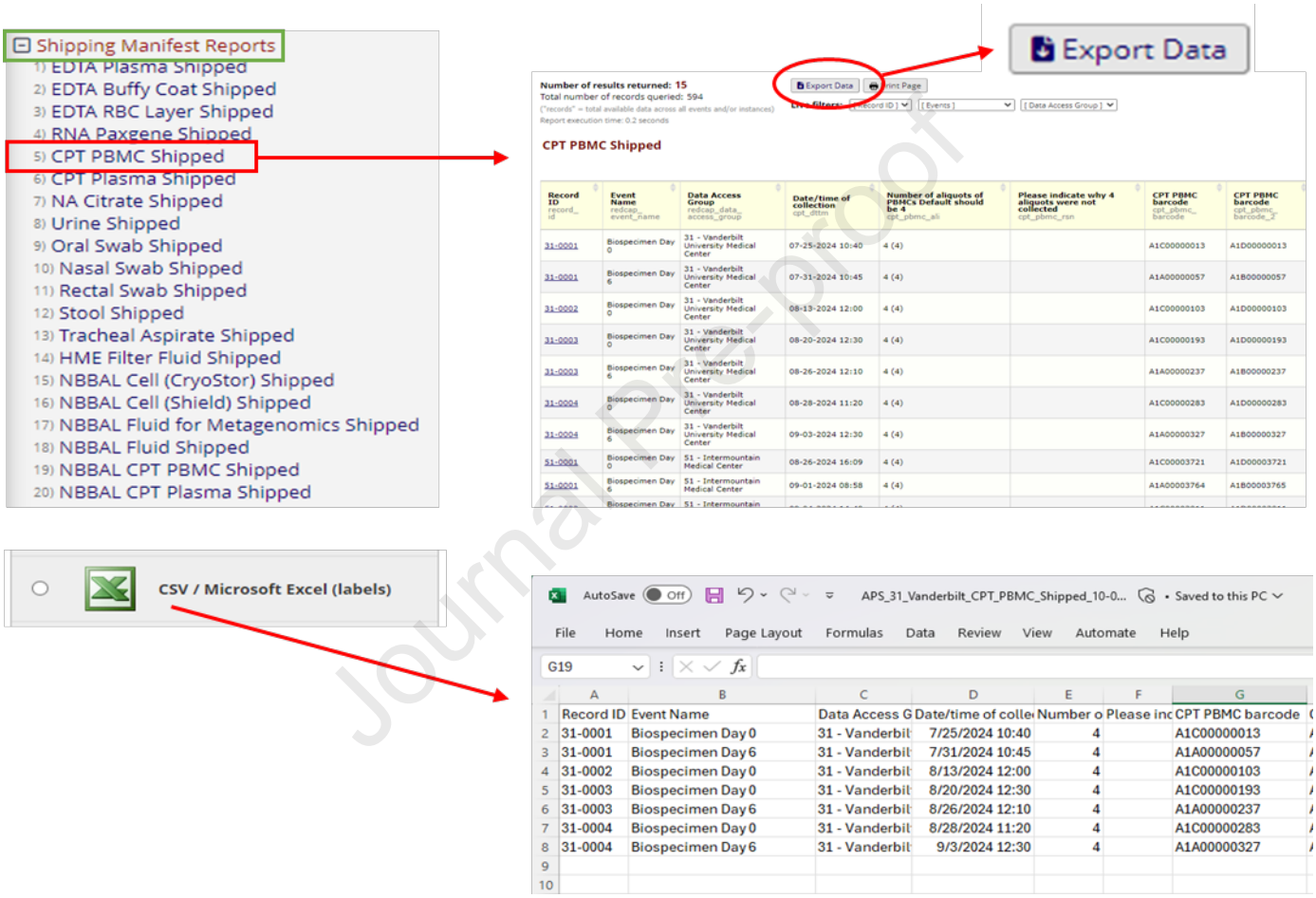

Figure 29: Generating a shipment manifest in REDCap

1. For the specimens being shipped, enter the planned shipment date in the “Date Shipped” field of the appropriate REDCap Biospecimen Collection and Processing form. This date can be

populated ahead of the shipment.

2. Run the manifest report for each specimen type being shipped.
3. Export the manifest report into a CSV/Microsoft Excel File.
4. Rename the data file for your site name and number
    a. Example:
    31-VUMC-APSEDC-CPTPBMCShipped_DATA_LABELS_2024-10-25_1015
5. Any biospecimens included in the report but not included in the shipment should be removed from the shipping manifest
6. Send a copy of the CSV Manifest to the APS Biorepository Lab at aps_biocclab@vumc.org.
7. A printed copy of the manifest must also accompany all shipments.

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8.4. Packing a shipment

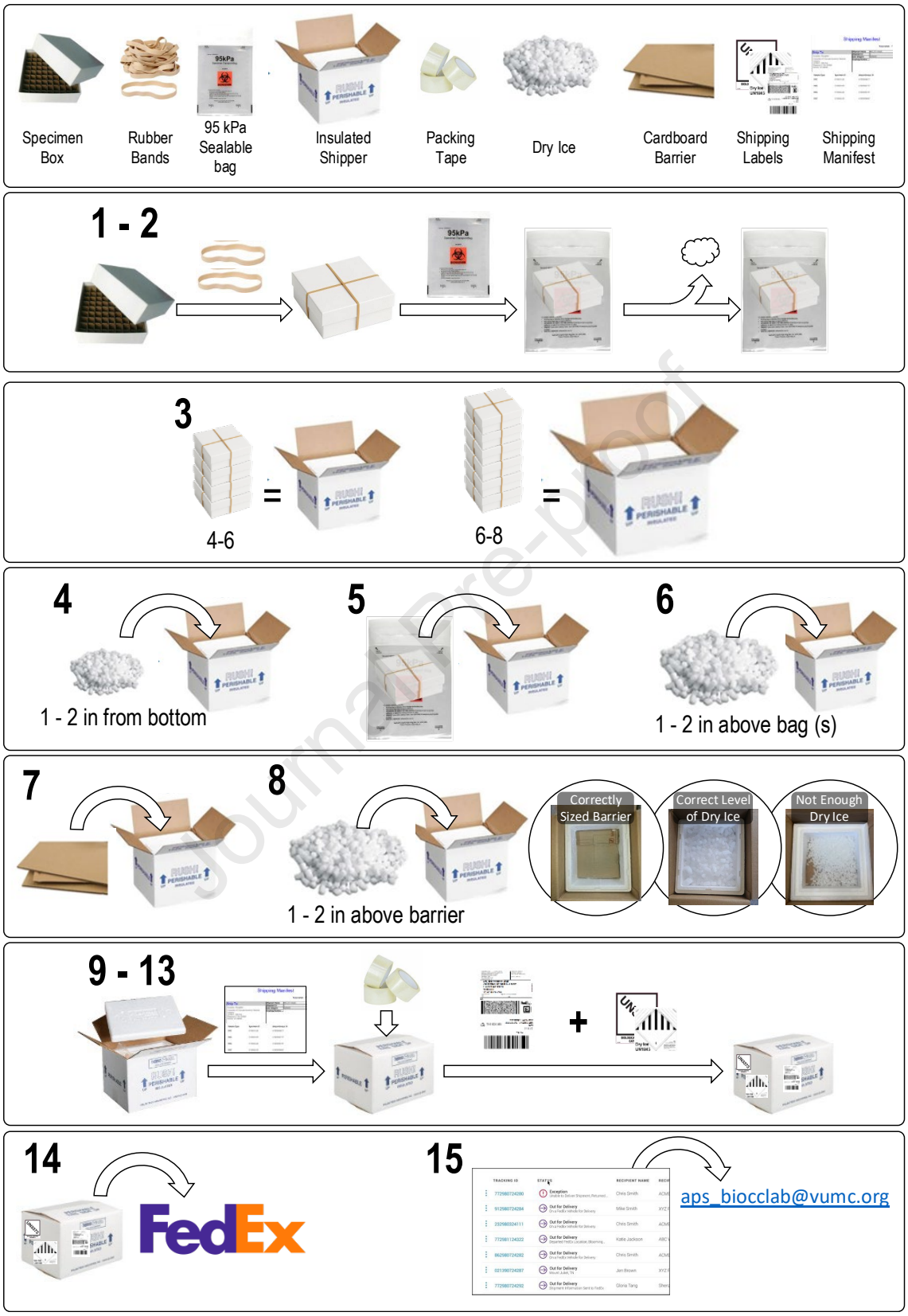

Figure 30: Summarized Shipping Instructions – Follow Steps from Section 8.4

1. Secure specimen cryoboxes with rubber bands and place in 95 kPa sealable bags with absorbent material.
2. Remove excess air from bags and seal.
3. Choose the appropriate size shipper:
    a. Medium: 4 – 6 cryoboxes.
    b. Large: 6 – 8 cryoboxes.
4. Fill the bottom of the shipper with a layer of dry ice (approximately one inch).
5. Place the bagged specimen boxes in the insulated shipper.
    a. Be sure to leave enough room to top the specimens with dry ice
6. **Cover the specimens completely with dry ice (1-2 inches above bags)** and fill empty spaces with packing material if needed.
    a. Ensure the boxes are not rattling or loose inside the shipper.
7. Cover the dry ice with carboard barrier (must fit tight in the shipper).
8. Fill the box with dry ice (1-2 inches above the barrier).
9. Close the insulated shipper with the insulated top.
    a. Do not tape the inside insulated foam box shut; it needs to vent.
10. Place a copy of the shipping manifest(s) outside the insulated shipper and inside the outer box.
11. Close and seal the outer box with packing tape.
12. Place the return shipping label(s) provided by the APS Biorepository Lab on the outer box.
13. Add the "UN1845 Dry Ice" and "UN3373 Biological Substance, Category B" labels (see Appendix) to the outside of the shipper
    a. Fill in the shipping information on the UN1845 Dry Ice label as required.
14. Drop off the shipment at a well-known FedEx location where you can ensure the package will be promptly retrieved by FedEx. If there is no pickup location available, arrange for FedEx pick up or take it to FedEx in person.
15. Please track your specimen shipment the day after shipping to ensure they are arriving on time. If it appears your specimens have been held up in shipping and will not arrive when expected, notify aps_biocclab@vumc.org immediately.

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9. SOP Revision History

SOP Version #	SOP Version Date	Summary of Revisions
1.0	July 24, 2024	New SOP adapted from procedures and protocols given by APS investigators.
2.0	October 21, 2024	Kit assembly, biospecimen collection and processing SOPs, chain of custody, and shipping SOPs have been combined into a single document. Kit assembly SOP revisions: Added 100µm cell strainer to list of materials that need to be supplied by APS sites and in list of supplies needed for Day X respiratory specimen processing. Added link to survey for ordering supplies from CCC (section 3.1). Blood specimen collection and processing SOP revisions: CPT processing figure was updated to reflect correct aliquot volumes. Removed repetitive language from steps for freezing PBMCs. Added clarification on proper practices for placing vials in cryoboxes under "general notes" section. Added reminders to enter specimen data into REDCap and to use specimen tracking forms if EDC is not available. Added order of draw to general notes section. Added the preferred order of collection for blood specimens. Removed requirement to process samples in BSC. Respiratory specimen collection and processing SOP revisions: Adjusted minimum freezing time (4 hours) before transferring samples from controlled-rate freezing device (CoolCell or Mr. Frosty) to long-term storage to reflect manufacturer recommendations. Removed step instructing staff to process HME filter fluid in a BSC. Added reminders to enter specimen data into REDCap and to use specimen tracking forms if EDC is not available. Added clarification on proper practices for placing vials in cryoboxes under "general notes" section. Added note on order of collection stating that if TA and NBBAL are collected on the same day, the TA collection should occur before NBBAL. Clarified that "grossly bloody" NBBAL samples refers to samples that appear red and not "pinkish." Updated centrifuge settings for HME filter fluid processing to 10 minutes at 1,500 RCF (xg force) at 4°C. Added language to clarify that patients with bronchial or tracheal stents are excluded from NBBAL specimen collection. Microbiome specimen collection and processing SOP revisions: Indicated that swab specimen collection tubes should be vortexed for approximately 10 seconds. Modified urine processing to remove language about aliquoting in a BSC. Added reminders to enter specimen data into REDCap and to use specimen tracking forms if EDC is not available. Added clarification on proper practices for placing vials in cryoboxes under "general notes" section. Modified urine collection procedure to encourage coordination with clinical team to facilitate collection of freshest sample. Corrected time points for rectal swabs, which are only collected on days 0, 2, and 6. Added language to clarify that stool specimens placed on ice should be processed within 24 hours of collection, and stool cannot be placed in biospecimen refrigerator in lieu of placing on ice. Added language on how to handle stool and rectal swab collections when ostomy is present. Chain of custody SOP revisions: Removed aliquot barcode columns from the specimen tracking forms. Removed consortium contacts table. Updated specimen tracking forms to match EDC forms. Added biospecimen shipping SOP.
3.0	September 15, 2025	Updated the Coordinating Center Contacts Added a List of Figures (section 2) Updated the Tracheal Aspirate processing protocol: Added new materials to the Materials Provided by the APS Consortium Coordinating Center table in section 3.1 and the Processing and Aliquoting Supplies figures in section 3.2; Added new label nomenclature to Table 1; Updated Figure 18 to reflect the new processing protocol; Added procedure for neat storage aliquots (section 5.5.2.3) and procedure for proteomics aliquots (section 5.5.2.4) Figure 1 and Figure 2 were updated to reflect the new envelope labeling scheme for participant label kits

		Added site 25 - Northwestern University to the APS Site Numbers table in section 3.3.2 Added "Best Practices" to Blood Specimen Collection (section 4.4), Respiratory Specimen Collection (section 5.4), Microbiome Specimen Collection (section 6.4), and Biospecimen Shipping (section 8.2) Added "Troubleshooting" section to the Blood Specimens Collection and Processing SOP (section 4.6) and Respiratory Specimens Collection and Processing SOP (section 5.6) Section 5.2 updated to clarify NOT to collect respiratory specimens on patients with suspected or confirmed TB Added Respiratory Biospecimen Cryobox figure (Figure 29) Added Summarized Shipping Instructions figure (Figure 31) General reformatting for a more cohesive document
4.0	February 3, 2026	Updated Blood Specimens Collection and Processing procedures: Removed CPT collection at Day 0, Day 6, and 3-month (section 4.4.4), added 1 extra 6-mL EDTA tube collection at Day 0, Day 6, and 3-month (section 4.4.2), removed "Blood (when isolating PBMCs)" from kit assembly tables in section 3.2 Updated NBBAL cell processing protocol: removed processing of cells in RNA Shield Buffer (section 5.5.3), updated NBBAL cell processing visual summary (figure 19) Updated In-Hospital stool collection and processing protocols: Added OMNImet-GUT stool collection for Day 0, Day 2, and Day 6 (section 6.4.6), added new supplies to kit assembly tables in section 3.2, updated biospecimen labels (table 1), updated stool processing visual summary (figure 25) Updated LTO Timepoints: Removed references to "Month 6" and clarified "LTO Timepoint 1 (3 to 6 months)" and "LTO Timepoint 2 (12 months)"; updated schedule of events (Figures 7, 14, 20) Removed troubleshooting sections 4.6 and 5.6 to create a separate Frequently Asked Questions (FAQs) document

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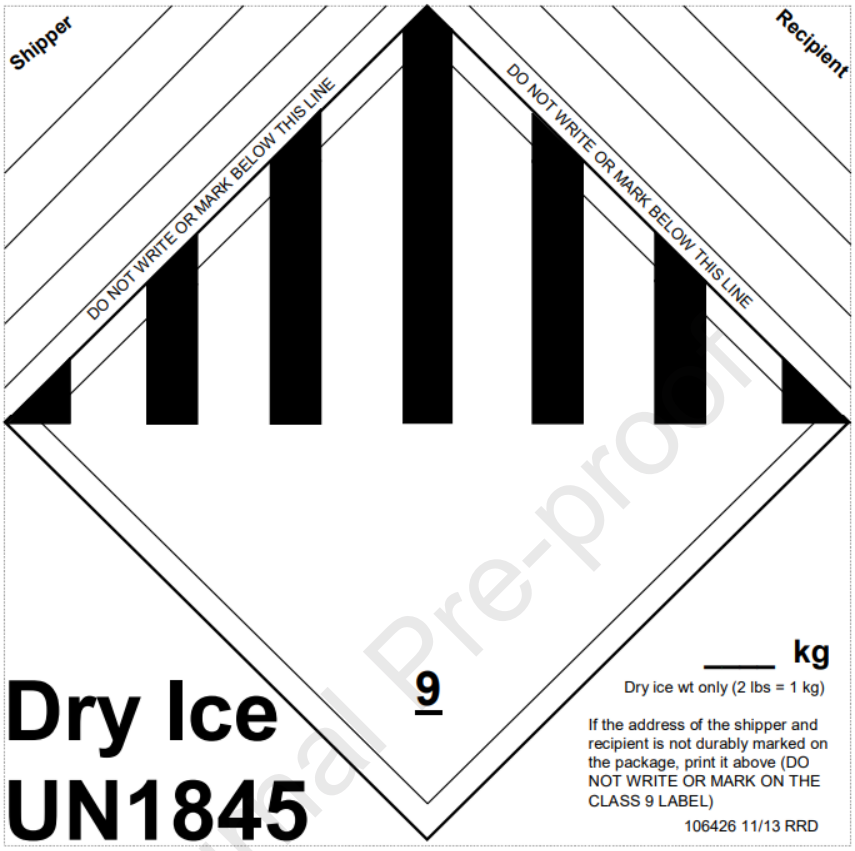
10. Appendix

10.1. Dry Ice UN1845 Shipping Label



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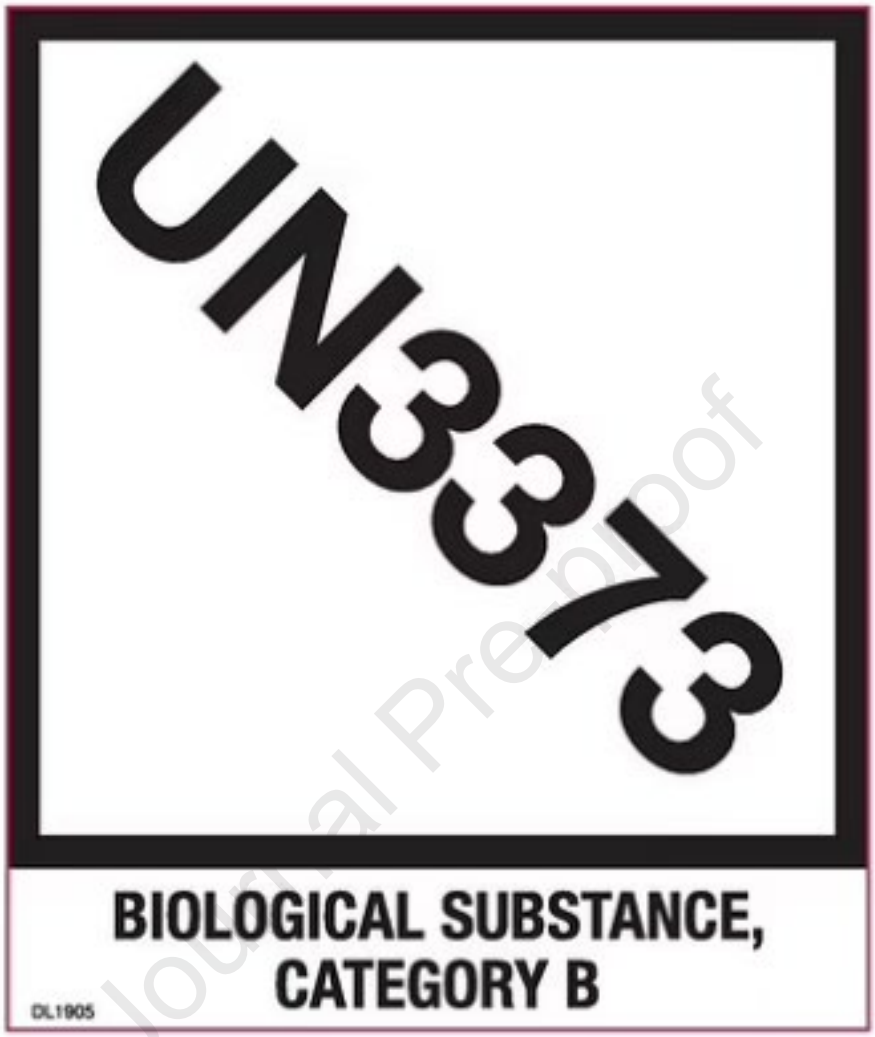
10.2. UN3373 Biological Substance Category B Shipping Labels



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