**Title:** NIH RECOVER: A Multi-site Observational Study of Post-Acute Sequelae of SARS-CoV-2 Infection in Adults

**Short Title:** Understanding the Long-term Impact of COVID-19 in Adults

**Study ID:** S21-01226

**Sponsor**: National Heart Lung and Blood Institute of the National Institutes of Health

**Protocol Version**: Version 14.0

**Revision history**

| Revision # | Date | Changes |
|---|---|---|
| 1.0 | 9/16/2021 | Original release |
| 1.1 | 10/08/2021 | Revision to inclusion/exclusion criteria; revisions to appendices 2 and 3 |
| 2.0 | 10/18/2021 | Revisions to appendices 2, 3 and 4 |
| 3.0 | 12/01/2021 | Footer: Added protocol version date.<br>List of abbreviations: Added additional abbreviations.<br>Section 4.1: Deleted risk for ventilation/perfusion scan since that procedure was previously removed.<br>Section 7.1: Clarified inclusion criteria to include patients who will have positive SARS-Cov-2 infection-specific antibody testing<br>Section 7.2: Added incarceration as an exclusion criterion.<br>Section 7.3: Added language regarding enrollment in the pediatric protocol<br>Section 9.7: Added text about the data management plans from the Data Resource Core, including two new figures<br>Section 13.3.2: Revised to indicate the study will be posted on clinicaltrials.gov<br>Section 13.6: Added an opt-in for future genetic testing<br>Section 15.3: Clarified that participant compensation is site-specific<br>Appendix 1:<br>• Schedule of Assessments revised to provide more detail;<br>• Clarified assessment schedule for subjects > 48 months post-infection.<br>Appendix 3:<br>• Removed procalcitonin from Tier 2 and moved EKG from Tier 1 to Tier 2<br>• Added the NIH Toolbox picture sequence age 7+ v2.1+ as a Tier 3 procedure (inadvertently removed in previous version);<br>• Deleted ENT as a Tier 3 procedure, since it's in Tier 2.<br>Appendix 4: Added one laboratory study – Anti-Mullerian hormone. |
| 4.0 | 01/27/2022 | Section 4.1:<br>* clarified that pregnant participants cannot undergo cardiopulmonary exercise testing<br>* added language regarding risks to bronchoscopy, right heart cardiac catheterization, upper endoscopy, colonoscopy<br>* removed text allowing return of research laboratory results<br>* clarified risk of genetic testing language<br>Section 6.3.1: Added that subjects may receive information about the study through videos, slides, pamphlets or website information. |

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| | | Section 8.4: Added new section permitting one-time off schedule visits during on-study infections<br>Section 9.6: Clarified which PHI elements are in the central database and which are retained locally by sites<br>Section 9.7.6: Noted that only data that have already been shared for research will not be destroyed if a subject withdraws from the study<br>Section 12.1: Updated hypotheses to be tested according to latest version of statistical analysis plan<br>Table 2: Added an additional row for sample size calculations; corrected data in one other row<br>Section 12.3.1: Corrected number of aliquots that can be collected per SST tube<br>Section 12.5: Removed analytic details and referred instead to more detailed SAP (included as attachment)<br>Section 13.3.1: Added that materials such as videos, slide presentation and scripts may be used to aide in the consent process.<br>Section 13.4.3.1: Removed option to return research lab results<br>Appendix 2: Corrected table to indicate Tier 1 lab tests are performed at 0, 3, 6 months<br>Appendix 3: Clarified which procedures pregnant participants cannot do; moved fecal viral load to Tier 2<br>Appendix 4: Clarified which procedures pregnant participants cannot do; indicated which procedures may be performed under sedation |
| 5.0 | 03/22/2022 | Section 4.1: Added risk of brain infection to Lumbar Puncture<br>Section 7.1: Updated the recruitment window from 24 to 36 months<br>Section 7.2: Amended exclusion criteria to exclude some types of pregnant subjects from adult cohort sites<br>Section 8.1: Added that uninfected pregnant individuals begin study schedule on delivery date<br>Section 9.4: Removed option for MITRA or other home blood collection device<br>Section 10.3.1: Changed IRB reporting deadline from 5 to 10 days<br>Section 10.4: Section added to describe process for ensuring participant safety when survey response indicates suicidal ideation<br>Section 14.3 Changed IRB reporting deadline from 5 to 10 days; clarified type of protocol deviations to be reported to OSMB<br>Appendix 1: Added figure legend. Indicated that Office visit, labs, biospecimens may be collected at 48 months after infection and thereafter if participant is still within study follow up period<br>Appendix 2: Replaced “infection” with “enrollment” throughout<br>Appendix 3: Added that participants <3 months post-partum cannot have any tests that pregnant participants cannot have |

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| | | Appendix 4: Specified methylmalonic acid to be drawn with serum B12; added that participants <3 months post-partum or breastfeeding cannot have any tests that pregnant participants cannot have |
| 5.1 | 03/28/2022 | Appendix 2: Corrected clinical assessment and laboratory timeline to start from infection not enrollment |
| 6.0 | 8/11/2022 | Protocol Summary: Revised the earliest date of possible infection from March to January 2020<br>Section 4.1: Removed reference to lung plethysmography<br>Section 6.1: Revised Figure 1 to include acute reinfected participants<br>Section 6.3.1: Removed 15% cap on self-referral participants<br>Section 6.3.2: Added additional retention strategies<br>Section 7.1: Added note that participants should be willing to participate in the overall protocol and should expect to be available for the duration of the study to meet inclusion criteria; added several clarifications to inclusion criteria to match MOP.<br>Section 7.3.2: Added section on Prisoners to indicate long-term incarcerated subjects are not eligible.<br>Section 7.3.3: Added section on “Inclusion of Students and Employees in Research” to clarify the measures taken to include these vulnerable populations if passively recruited.<br>Section 7.4: Added section “Loss to Follow-Up” to document how sites should proceed if participants cannot be reached or choose not to continue with participation<br>Section 7.5: Clarified “Subject Withdrawal” to differentiate withdrawal of consent from cessation of participation or loss to follow-up.<br>Section 8.1: Added instructions for starting study schedule at time of acute reinfection (enrollment) for previously infected participants enrolling during an acute infection.<br>Section 9.4: Revised the biospecimen tube protocol to include the reduction in CPT tubes and addition of sodium citrate and EDTA tubes. Added instructions for biospecimen collection for acute on-study reinfection.<br>Section 9.7: New section on reading centers<br>Section 10.1: Revised definition of adverse event for clarity to clearly exclude data collected as potential PASC outcomes; additional textual clarifications<br>Section 10.2: Clarified recording of adverse events in REDCap<br>Section 10.3: Clarified reporting of new information to the IRB<br>Section 12.3.1: Removed details of biospecimen processing in favor of referral to biospecimen MOP<br>Section 14.3: Noted that protocol deviations captured by data reports do not need to be individually filed in REDCap |

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| | | Appendix 1: Changed the color of the comorbidity row to indicate participation completion only. Clarified that the post 48-month study schedule repeats starting at 39-month schedule<br>Appendix 2: Moved ANA, anti-CCP, RF, EBV to Tier 2; removed reference to lung volumes<br>Throughout: Revised minor formatting and grammatical errors |
| 6.1 | 9/29/2022 | Section 6.3.1: Added waiver of authorization language |
| 7.0 | 12/15/2022 | Section 4.1: Added risks of oral glucose tolerance test and pulmonary function test<br>Sections 4.1, 8.2 and 9.4: Added collection of tears as a biospecimen<br>Section 9.8: Added section on the mobile health platform<br>Section 9.9: Revision of data management section to include that data will also be collected from wearable devices.<br>Section 9.9.4: Added subsection on FISMA moderate environments<br>Section 10.1: Revision of definition of adverse event to align with revised NYULH IRB policy manual<br>Section 10.3: Revision of reporting requirements of reportable new information to align with revised NYULH IRB policy manual<br>Section 13.3: Added that group information sessions are allowed. |
| 8.0 | 7/07/2023 | Protocol Summary, Sections 6.2,12.2: Revised enrollment targets and effect sizes to align with OSMB recommendation<br>Sections 4.1, 19.2: Added risks of capnography<br>Sections 4.1, 19.4: Removed colonoscopy without biopsy<br>Sections 4.1, 19.4: Removed references to upper endoscopy<br>Sections 4.1, 19.4: Removed references to cardiac imaging with meta-iodobenzylguanidine (mIBG)<br>Section 6.1: Clarified that enrollment targets refer to participants that contribute data to the study protocol<br>Sections 7.5, 9.9.6: Clarified wording regarding removal of data after withdrawal of consent<br>Section 9.4: Corrected reference date used for acute reinfected participant biospecimen collections<br>Section 9.8: Clarified that participation in any element of MHP is optional for participants and included additional enrollment methods for MHP<br>Section 10.4: Included site staff in suicidal ideation alerts<br>Section 19.2: Added survey form about participation in Long Covid Treatment trials, revised wearable interval to continuous<br>Section19.3: Clarified that Mini International Neuropsychiatric Interview can be conducted at home visits; specified which pulmonary function tests are being conducted |

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| | | Throughout: Revised minor formatting and grammatical errors; changed pregnant women to pregnant participants |
| 9.0 | 9/21/2023 | Updated revision date for v8.0<br>Section 4.1: Added invasive cardiopulmonary exercise tests to list of Tier 3 tests.<br>Section 4.1: Added that participants 3 months postpartum or those who are breastfeeding cannot participate in select Tier 3 tests<br>Section 19.4: Added EMG and NCS as Tier 3 tests that cannot be performed by pregnant participants or participants 3 months postpartum<br>Section 19.4: Added invasive cardiopulmonary exercise tests to list of Tier 3 tests |
| 10.0 | 5/6/2024 | Section 3.3: Added scientific rationale and justification for all assessments that are more than minimal risk.<br>Section 4.1: Added guidance to exclude pregnant and post-partum participants from sleep tests for data quality reasons only<br>Section 4.1: Added minimal risk language surrounding electrocardiogram electrode placement.<br>Section 4.1: Added risk language for oral glucose tolerance test.<br>Section 4.1: Added risk language for echocardiogram with enhancing agents.<br>Section 4.1: Included invasive CPET under tests with local lidocaine administration.<br>Section 4.1: Updated radiation risk language to match consent form (now information sheet)<br>Section 4.1: Updated the type of contrast used for CT scans<br>Section 4.1: Added guidance for sites to follow ACR guidelines or local policy to determine gadolinium contrast administration.<br>Section 4.1: Added risk language for electromyography (EMG).<br>Section 4.1: Added risk language for cardiopulmonary exercise testing (CPET).<br>Section 4.1: Updated section reference for incidental findings.<br>Section 4.1: Removed safety exclusion criteria from all test risk sections and detailed them instead in Appendix 5.<br>Section 6.1 and Section 8.1: Included additional information on Tier 2 and Tier 3 test selection, including proportion of controls and availability at local sites.<br>Section 6.1: Included site responsibility to ensure participants do not meet safety exclusion criteria<br>Section 12.2: Corrected table number and reference<br>Section 13.3: Added information sheets to informed consent process for Tier 3 tests that are more than minimal risk. |

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| | | Section 13.3.1: Included information sheets to list of consent documents. Removed Tier 1 and Tier 2 consents as approved stand-alone documents.<br>Section 19.3: Aligned Tier 2 tests in name only to match existing SOPs.<br>Section 19.3: Confirmed that sites can use local policies to determine pregnancy status prior to any restricted testing<br>Section 19.4: Aligned Tier 3 tests in name only to match existing SOPs.<br>Section 19.4: Confirmed that sites can use local policies to determine pregnancy status prior to any restricted testing.<br>Section 19.5: Added Appendix 5 to detail clinical triggers and safety exclusion criteria of additional testing<br>Throughout: Revised minor formatting and grammatical errors |
| 11.0 | 8/2/2024 | Section 3.3: Added additional scientific justification for several tests. Removed IRB hold for bronchoscopy, right heart catheterization, colonoscopy, and invasive cardiopulmonary exercise testing.<br>Section 4.1: Removed risks associated with the Complete Eye Exam and included visual acuity testing under assessments with no appreciable risks. Added additional risks to lidocaine and procedural sedation sections. Corrected typo in radiation amount in gastric emptying study, added risks for bronchoscopy and right heart cardiac catheterization. Aligned risks for iCPET to standard CPET and updated right heart cardiac catheterization. Removed IRB hold for bronchoscopy, right heart catheterization, colonoscopy, and invasive cardiopulmonary exercise testing.<br>Section 6.1: Clarified how subjects are grouped into categories for Tier 2 and Tier 3 test assignments. Clarified when tests could be repeated, including those for participants with crossover infections.<br>Section 6.1.1: Provided new detail to clarify that sites should follow steps outlined in the MOP if unable to perform an assessment as expected.<br>Section 6.1.2: Provided new detail to clarify that any site variability that is inconsistent with the protocol must be reviewed by the IRB prior to implementation.<br>Section 8.2: Clarified study schedule for crossover participants.<br>Section 8.3: Clarified what constitutes an in-window study visit.<br>Section 9.4: Included biospecimen collections from Tier 3 tests.<br>Section 15.3: Included minimum and maximum reimbursement information for assessments and visits.<br>Section 19.1 (Appendix 1): Corrected minor errors in study schedule.<br>Section 19.2 (Appendix 2): Added confirmatory immunophenotyping assessments completed on previously collected samples.<br>Section 19.3 (Appendix 3): Separated combined assessments into individual component names for clarity. Ceased performance of renal ultrasound as of protocol v11.<br>Section 19.4 (Appendix 4): Clarified permissible repeat testing. Removed Complete Eye Exam as one of the Tier 3 tests (never conducted). Separated combined assessments into individual component names for |

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| | | clarity. Removed IRB hold for bronchoscopy, right heart catheterization, colonoscopy, and invasive cardiopulmonary exercise testing.<br>Section 19.5 (Appendix 5):<br>Updated test/procedure names to better reflect the acceptable clinical variation in the scope/content of procedures.<br>Provided additional detail regarding eligibility criteria for some Tier 2 and Tier 3 assessments.<br>Added a column providing additional information about procedures and the permissible clinical variation.<br>Provided additional detail regarding exclusion criteria for some Tier 2 and Tier 3 assessments.<br>Included comprehensive list of medications to be stopped prior to autonomic suite testing (Table 7).<br>Throughout: Changed term “mobile health” to “digital health.”<br>Throughout: Provided more specific names of procedures (e.g. “bronchoscopy” to “flexible bronchoscopy.”<br>Throughout: Revised minor formatting and grammatical errors. |
| 12.0 | 12/09/2024 | Section 3.3: Added additional scientific justification for several tests. Added laryngoscopy with stroboscopy and option for upper endoscopy along with scientific justification.<br>Section 4.1:<br>Added risks to autonomic testing.<br>Added risks to cardiopulmonary exercise testing.<br>Included laryngoscopy and upper endoscopy with biopsy in risks of lidocaine administration section.<br>Included upper endoscopy with biopsy in risks of sedation administration section.<br>Included risks of electrocardiogram in flexible bronchoscopy section.<br>Added risks of flexible laryngoscopy with stroboscopy.<br>Added risks to flexible bronchoscopy and right heart cardiac catheterization.<br>Added risks of upper endoscopy with biopsy.<br>Clarified risks of genetic testing to confirm that only whole genome studies performed in CLIA-approved labs will be eligible to return to participants if they have elected to receive these results.<br>Section 9.5: Included the network of biostatisticians for RECOVER (NBR) as personnel who are eligible to analyze data collected across RECOVER.<br>Section 9.7: Included further information about certification practices for several assessments that require either training or confirmation that the assessment is being performed as expected, including new Table 6. |

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| | | Section 9.9: Included additional software tools used to collect study data and additional laboratories that will be performing assays on RECOVER biospecimens.<br>Further clarified the data transfer and harmonization process and detailed how the fully de-identified data are made available to the public.<br>Added a new section (9.9.6) to clarify how investigators may be given access to RECOVER data.<br>Provided further information about InteleShare (formerly Ambra) in section 9.9.9<br>Section 13.4.3: Clarified that omics assays are not included under reporting of genetic testing as they are not performed in CLIA-certified laboratories.<br>Provided additional guidance on how site staff should proceed to return genetic results to participants if these results are available and their local laws determine that this is permissible.<br>Table 8: Added maximum reimbursement for flexible laryngoscopy<br>Section 19.2: Included survey question for change in voice<br>Section 19.3: Clarified that ENT exam is flexible laryngoscopy with stroboscopy<br>Ceased performance of Oral Glucose Tolerance Test as of protocol v12.0 approval.<br>Included flexible laryngoscopy with stroboscopy under tests that cannot be done in pregnant and post-partum participants.<br>Section 19.4: Ceased performance of Serum B12 and Methylmalonic acid as of protocol v12.0 approval.<br>Included colonoscopy with biopsy, with or without upper endoscopy with biopsy, under tests that cannot be done in pregnant and post-partum participants.<br>Section 19.5: Included eligibility, clinical exclusions, and additional information about flexible laryngoscopy with stroboscopy.<br>Revised eligibility criteria for electromyography, nerve conduction studies, skin biopsy, muscle biopsy, and autonomic testing (cardiovagal innervation testing, Valsalva test, and tilt table).<br>Added history of gastric surgery as a clinical exclusion for gastric emptying test based on data quality reasons only<br>Clarified exclusions for lumbar puncture.<br>Added permissible clinical variation and additional test information to skin biopsy.<br>Added option for post-CPET muscle biopsy<br>Added permissible clinical variation and additional test information to muscle biopsy.<br>Corrected a typo in eligibility criteria for cardiopulmonary exercise testing<br>Revised Intra-procedure stopping criteria for cardiopulmonary exercise testing |

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| | | Corrected a typo in eligibility criteria and additional information of invasive cardiopulmonary exercise testing.<br>Corrected a typo in eligibility criteria of flexible bronchoscopy. Added additional information about test procedures and hospitalization criteria.<br>Clarified exclusions for right heart catheterization and added details of permissible clinical variation.<br>Added option for upper endoscopy to colonoscopy; included safety exclusion criteria specific to upper endoscopy; added additional information about permissible clinical variation and test procedures.<br>Throughout: Revised minor formatting and grammatical errors. |
| 13.0 | 1/3/2025 | Bronchoscopy – a sample involving supraglottic suctioning was added |
| 14.0 | 2/7/2025 | Section 4.1: Added EndoPAT as a test with no appreciable risk as it was previously only captured under clinical examinations.<br>Section 4.1: Included all tests that include the use electrocardiogram.<br>Section 4.1: Clarified that the amounts of additional radiation listed are annual, not cumulative.<br>Section 6.1: Clarified that safety exclusion criteria are evaluated and documented by the site staff after the participant has already been assigned the test.<br>Section 9.4: Updated wording to reflect that OGTT performance ceased as of protocol v12.<br>Section 9.7: Clarified that only site staff without C-Level certifications require certification by the CSC Neurocognitive team.<br>Section 9.7: Included that sites performing the facility sleep test must also submit a deidentified scan as part of the certification process.<br>Section 13.3.1: Added that procedure specific information and instructions are provided to the participant after they have consented to testing.<br>Section 19.5: Removed bleeding disorder and blood thinning medications as safety exclusion criteria for flexible laryngoscopy with stroboscopy.<br>Section 19.5: Added fasting information to the additional information for fibroscan.<br>Section 19.5: Added exercise to activities to avoid to the additional information for EndoPat.<br>Section 19.5: Added a recent hematocrit to the additional information for cardiac MRI as this is needed for interpretation of the results.<br>Section 19.5: Modified the eligibility criteria for muscle biopsy to no longer require reduced cognitive function, sleep disturbance, or fatigue.<br>Section 19.5: Moved the restriction on sedation and anesthesia to the additional information section for lumbar puncture. Added clinical exclusions for participants who did not elect for biospecimen collection and those with possible elevated intracranial pressure, depressed mental status, or skin infection. |

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| | | Section 19.5: Added restrictions of alcohol, nicotine, caffeine, and food prior to testing to the additional information for Autonomic (cardiovagal innervation) testing.<br>Section 19.5: Standardized the wording surrounding fasting time for CPET and iCPET.<br>Section 19.5: Added allergy to lidocaine as a clinical exclusion criteria for iCPET (documented under right heart catheterization exlclusons).<br>Section 19.5: Added allergy to lidocaine as a clinical exclusion for bronchoscopy.<br>Section 19.5: Added allergy to lidocaine and abnormal renal function lab result as a clinical exclusion for right heart catheterization.<br>Section 19.5: Clarified symptoms and resolved the error in number of biopsies taken for both colonoscopy and upper endoscopy.<br>Table 9: Revised the name of the table to align with the SOP and updated the list of medications included.<br>Throughout: Updated version number in footer and version date in revision table that were not updated during v13 revisions.<br>Throughout: Minor formatting and grammatical errors. |

**Statement of Compliance**

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), any other applicable US government research regulations, and institutional research policies and procedures. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the study subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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Signature of Site Principal Investigator Date

List of Abbreviations

| | |
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| AE | Adverse Event/Adverse Experience |
| BMI | Body mass index |
| CFR | Code of Federal Regulations |
| COVID-19 | Coronavirus Disease 2019 |
| CRF | Case Report Form |
| DRC | Data Resource Core |
| DHHS | Department of Health and Human Services |
| DSMB | Data and Safety Monitoring Board |
| EDC | Electronic Data Capture |
| EHD | Electronic Health Database |
| HER | Electronic Health Record |
| ETL | Extract, Transform, Load |
| FISMA | Federal Information Security Modernization Act of 2002 |
| GCP | Good Clinical Practice |
| HIPAA | Health Insurance Portability and Accountability Act of 1996 |
| ICF | Informed Consent Form |
| ICMJE | International Committee of Medical Journal Editors |
| I2b2 Data Hub | Implementing Informatics from Bench to Bedside – Data Hub for all PASC datatypes harmonized into a common data model |
| IRB | Institutional Review Board |
| MOP | Manual of Operations and Procedures |
| N | Number (typically refers to subjects) |
| NHLBI | National Heart, Lung and Blood Institute |
| NIH | National Institutes of Health |
| OHRP | Office for Human Research Protections |
| OHSR | Office of Human Subjects Research |
| OSMB | Observational Study Monitoring Board |
| PASC | Post-Acute Sequelae of COVID-19 |
| PBC | PASC Biorepository Core |
| PHI | Personal Health Information |
| PI | Principal Investigator |
| PII | Personal Identifiable Information |
| REDCap | Research Electronic Data Capture |
| REDCap Central | Research Electronic Data Capture Central dataset |
| SAE | Serious Adverse Event |
| SOP | Standard Operating Procedure |
| US | United States |

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| UUID | Universally Unique Identifiers |
| WHO | World Health Organization |

# Table of Contents

# 1 Protocol Summary

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|---|---|
| Title | NIH RECOVER: A Multi-site Observational Study of Post-Acute Sequelae of SARS-CoV-2 Infection in Adults |
| Short Title | Understanding the Long-term Impact of COVID-19 in adults |
| Brief Summary | This is a combined retrospective and prospective, longitudinal, observational meta-cohort of individuals who will enter the cohort with and without SARS-CoV-2 infection and at varying stages before and after infection. Individuals with and without SARS-CoV-2 infection and with or without PASC symptoms will be followed to identify risk factors and occurrence of PASC. This study will be conducted in the United States and subjects will be recruited through inpatient, outpatient, and community-based settings. Study data including age, demographics, social determinants of health, medical history, vaccination history, details of acute SARS-CoV-2 infection, overall health and physical function, and PASC symptom screen will be reported by subjects or collected from the electronic health record using a case report form at specified intervals. Biologic specimens will be collected at specified intervals, with some tests performed in local clinical laboratories and others performed by centralized research centers or banked in the Biospecimen Repository. Advanced clinical examinations and radiologic examinations will be performed at local study sites with cross-site standardization. |
| Objectives | • Characterize the incidence and prevalence of sequelae of SARS-CoV-2 infection.<br>• Characterize the spectrum of clinical symptoms, subclinical organ dysfunction, natural history, and distinct phenotypes identified as sequelae of SARS-CoV-2 infection.<br>• Define the biological mechanisms underlying pathogenesis of the sequelae of SARS-CoV-2 infection. |
| Methodology | Ambi-directional longitudinal meta-cohort study (combined retrospective and prospective) with nested case-control studies. |
| Endpoint | Primary Endpoints: Presence of candidate PASC symptoms over time (incidence and prevalence).<br>Secondary Endpoints: Biological and recovery trajectories from SARS-CoV-2 infection; organ injury; incident clinical disease. |
| Study Duration | Four years |
| Subject Duration | Up to four years |

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| Title | NIH RECOVER: A Multi-site Observational Study of Post-Acute Sequelae of SARS-CoV-2 Infection in Adults |
| Population | Infected: Individuals at least 18 years of age meet WHO criteria for suspected, probable or confirmed SARS-CoV-2 infection on or after January 1, 2020.<br>Uninfected: Individuals at least 18 years of age who have never met any of the WHO criteria for suspected, probable or confirmed SARS-CoV-2 infection. |
| Number of subjects | 12,200 total subjects with SARS-CoV-2 infection and 2,680 total subjects without SARS-CoV-2 infection. |
| Statistical Analysis | A flexible study design is proposed to allow modifications to PASC case definition, tiered phenotyping assessments, comparator groups, and/or statistical plan after its initiation to optimize public health impact without undermining validity and integrity of study findings. Modifications in study design may be based on analyses of structured cohort data, unstructured cohort EHR data, and other cohort EHR data. |

## 2 Key Roles

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| Principal Investigators<br>Clinical Science Core | Stuart Katz, MD MS<br>Helen L. and Martin S. Kimmel Professor of Advanced Cardiac Therapeutics<br>Director Heart Failure Programs<br>NYU Grossman School of Medicine<br>Leon H. Charney Division of Cardiology<br>530 First Avenue, Skirball 9R<br>New York, NY 10016<br>212-263-3946<br><br>Andrea B. Troxel, ScD<br>Professor and Director, Division of Biostatistics<br>Department of Population Health<br>NYU Grossman School of Medicine<br>180 Madison Avenue, Suite 5-55<br>New York, NY 10016<br>Tel (646) 501-3654<br><br>Leora Horwitz, MD, MHS<br>Professor, Department of Population Health at NYU Grossman School of Medicine<br>Professor, Department of Medicine at NYU Grossman School of Medicine<br>Director, Center for Healthcare Innovation and Delivery Science<br>Director, Division of Healthcare Delivery Science<br>550 1st Avenue, Suite 1803<br>New York, NY 10016 |
| Contact Principal Investigator<br>Data Resource Core | Andrea S Foulkes, ScD<br>Director, Biostatistics, Massachusetts General Hospital<br>Professor of Medicine, Harvard Medical School<br>Professor, Department of Biostatistics, Harvard TH Chan School of Public Health<br>50 Staniford Street, Suite 560 \| Boston, MA 02114 \| +1 (617) 724-8208 |

# 3 Introduction, Background Information and Scientific Rationale

## 3.1 Background Information and Relevant Literature

COVID-19 is a global pandemic currently affecting the US, yet little is known about risk factors for illness, including more severe illness and indicators of recovery. Since its nascence in China's Wuhan province in late 2019, the outbreak has evolved with startling rapidity with approximately 177 million people infected with COVID-19, resulting in at least 3.8 million deaths globally.[1] COVID-19 positive cases are identified with COVID-19 polymerase chain reaction test or an antigen test using saliva, nasopharyngeal or bronchial samples.[1,2] Fever, chills, cough, shortness of breath, fatigue, muscle aches, loss of taste and/or smell, nausea, diarrhea, and other symptoms are typical of the acute phase of the disease.[3,4] COVID-19 multi-organ manifestations are now well-documented [5-8], and even after recovery from acute illness, more than 70% of those infected report a diverse array of persistent mild to severe symptoms and diseases, from fatigue and persistent loss of taste or smell to stroke, renal failure, myocarditis, neurological syndromes, COVID-associated thrombosis, and pulmonary fibrosis.[3,9-17] Persistent or new symptoms after COVID infection are now termed post-acute sequelae of SARS-CoV-2 (PASC). The underlying pathophysiology of persistent symptoms after COVID-19 infection is unknown but has been proposed to be attributable to viral persistence, complications from critical illness related to prolonged intubation, prolonged bed rest, and malnutrition or impacts of pandemic-related disruptions on health.[16]

The goal of this study is to identify, evaluate, and characterize the heterogeneity in the pace and extent of recovery after acute COVID-19 infection, the clinical course of PASC symptoms in subjects who have recovered from acute infection, and the risk factors associated with the severity of the clinical course of PASC. This prospective longitudinal observational cohort study will focus on the biological differences that distinguish those who recover quickly from those who develop PASC symptoms and the long effects of COVID-19 infection, while explicitly considering racial/ethnic disparities in risks and outcomes. Data acquired from this study will provide accurate and quantifiable measures for PASC symptoms in selected case and control populations and allow for comparisons among groups to provide clues on PASC progression and complete recovery.

## 3.2 Rationale and Study Significance

This research study will ascertain information about subjects who have recovered from COVID-19 infections and define and categorize the clinical spectrum and risk factors for PASC. Data generated from this study will also serve to understand the long-term effects of COVID-19 infection and treatment options for affected individuals.

## 3.3 Scientific Justification of Assessments

This research study includes assessments that are more than minimal risk. The scientific justification and rationale for these procedures are explained in further detail below in Table 1.

**Table 1 Rationale for assessments that are more than minimal risk**.

| Assessment | Rationale |
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| Oral glucose tolerance test | SARS-CoV-2 infection has been shown in small studies to cause new onset diabetes in previously non-diabetic subjects and worsening of glucose control in previously diabetic subjects.[18,19] Oral glucose tolerance test with glucose, insulin and c-peptide measurements is the gold standard test for understanding the underlying pathophysiology of glucose metabolism, including in patients with known diabetes,[20-28] and is therefore included in the protocol.[20,21,27,29-32] (performance ceased as of protocol v.12) |

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| Resting thoracic echocardiography with enhancing agent, if clinically indicated | Shortness of breath is a common symptom of PASC and can be worked up by noninvasive testing including echocardiography. Echo is a very safe procedure and will help determine if there is a cardiac component to shortness of breath. Cardiac events that occur during acute COVID or during PASC (e.g., myocardial infarction, myocarditis or other injury to the heart) are most safely seen on echocardiogram.[33,34] Further, pulmonary hypertension is a known complication of acute COVID that may contribute to PASC and can be screened for with echo.[35] The use of image enhancing agents in patients with poor image quality on regular echocardiography enhances the detection of myocardial viability and allows for diagnosis of coronary artery disease, myocardial infarction or microvascular dysfunction in patients for whom regular echocardiography is inadequate. |
| Brain MRI without gadolinium | A cardinal symptom of PASC is cognitive dysfunction. Brain MRI is necessary to examine whether there are visible cortical changes in people with PASC, such as cerebral atrophy, compared to people without PASC or prior infection. Preliminary studies show visible effects on brain MRI post-COVID.[36] |
| Brain MRI with gadolinium | Gadolinium contrast adds to the value of brain MRI by making it possible to identify areas of inflammation in the brain. Moreover, use of contrast improves image quality and interpretation.[37] |
| Cardiac MRI without gadolinium | SARS-CoV-2 infection is well known to cause cardiac inflammation, including myocarditis and pericarditis, and consequent dysfunction. Cardiac MRI is needed to identify residual cardiac dysfunction in people with PASC compared to those without PASC or prior infection. Preliminary studies show visible effects on cardiac MRI post-COVID.[38] |
| Cardiac MRI with gadolinium | Gadolinium is a standard component of cardiac MRI protocols, and is particularly helpful in distinguishing ischemic from non-ischemic cardiomyopathy, and in determining prognosis.[39] The current formulation of gadolinium in use (macrocyclic) is very safe, with reported contrast-related adverse events reported in <0.5% of tests.[40] |
| Flexible laryngoscopy with stroboscopy | Preliminary studies have shown that non-intubated patients with upper airway complaints following COVID have demonstrated dysphonia and laryngeal dysfunction.[41-43] As such, reports of chronic cough and/or change in voice should trigger laryngoscopy with stroboscopy to further examine the mechanism for these symptoms compared to those without PASC or prior infection.[44] |
| Gastric emptying study | Autonomic dysfunction is a cardinal manifestation of PASC.[45,46] Gastric emptying study is the gold standard test for diagnosing gastric autonomic dysfunction.[47] There are case reports of impaired gastric emptying post-COVID.[48] It is necessary to validate these with systematic assessment comparing those with PASC to without PASC or prior infection. |
| Nerve conduction study and electromyography | Patients with PASC report abnormalities in sensation and motor function that have been ill-characterized to date. Nerve conduction studies and electromyography are the gold standard tests for quantifying nerve and muscle function. As nerve conduction and electromyography have been established clinical procedures for many years, there are already historical normal values available. The added risk of performing these tests in control subjects is not warranted when their data are not needed to answer the research questions set by this protocol. |

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| Skin biopsy | Skin biopsy allows assessment of microthrombi, complement deposition, interferon activation, and antiviral proteins as well as the structure and integrity of small nerve fibers. Patients with acute COVID have marked abnormalities in all of these domains.[49] They have also been found to be disrupted in some PASC patients who present with autonomic dysfunction.[50] We therefore include skin biopsy in the protocol as the least invasive method of determining whether microthrombi or immune dysregulation contributes to PASC pathophysiology. Three samples are required to be obtained because small fiber neuropathy is patchy and single biopsy specimens are insufficient for analysis.[51] The laboratory being used for RECOVER requires three samples. Comparison to those without PASC or prior infection is necessary to determine whether subclinical changes are evident and/or whether changes are associated with symptoms. |
| Muscle biopsy | Post exertional malaise is one of the most common symptoms reported in PASC. Muscle biopsy is the only means of assessing skeletal muscle structure, metabolic disturbances, exercise-induced myopathy and tissue infiltration of amyloid-containing deposits. A recent landmark study of 26 PASC patients found muscle abnormalities in a matched set of biopsies pre and post exercise testing compared to 21 controls.[52] Similarly, two small autopsy case series comparing people with (total N=78) and without history of infection (total N=21) found significantly more muscle degeneration in those with SARS-CoV-2 exposure than those without.[53,54] These findings require validation in a larger sample. Therefore, we will conduct muscle biopsies in infected participants and, in a subset of those also qualifying for exercise testing, will conduct matched pre- and post-exercise biopsies. |
| Lumbar puncture with or without imaging | Patients with dementia and mild cognitive impairment have been demonstrated to have abnormalities in the CSF, such as abnormal levels of Tau, neurofilament light chain and cytokines. It is necessary to obtain CSF in participants with and without PASC to determine whether similar abnormalities are seen in PASC with cognitive dysfunction and to identify whether subclinical changes are present in those without PASC. |
| Autonomic Suite (tilt table testing, cardiovagal innervation testing, supine and upright plasma catecholamine testing) | Autonomic dysfunction is a cardinal manifestation of PASC.[45,46] The gold standard tests for cardiovascular autonomic dysfunction include tilt table testing, cardiovascular innervation testing (beat-to-beat variability during regular breathing and Valsalva) and catecholamine testing.[46]These tests are therefore necessary to include to determine the presence of autonomic dysfunction in those with PASC compared to those without PASC or prior infection. |
| Invasive cardiopulmonary exercise testing | Dyspnea, exercise intolerance and orthostatic intolerance are frequent symptoms of PASC. Invasive CPET allows for dynamic assessment of potential physiologic contributors to PASC including preload insufficiency, decreased oxygen extraction, mixed preload insufficiency with decreased oxygen extraction, exercise pulmonary hypertension, chronic pulmonary embolism, deconditioning, and ventilatory limitation. These diagnoses can only be made through invasive CPET, which combines exercise testing with direct cardiac measurements through cardiac catheterization. A small study of 37 PASC patients has already shown physiologic abnormalities in those with exercise intolerance compared to those without.[55] This is necessary to meet the study objective, to “define the pathophysiology of and mechanisms of post-acute sequelae, including direct and indirect causal effects of COVID-19 infection, and |

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| | potential modifiers (e.g., sex, age, and race/ethnicity)." We will minimize any potential risks towards participants by excluding high risk participants, performing this assessment only at expert centers, and ensuring that the site staff are trained on the most recent SOP. These sites will have additional oversight by the CPET Reading Center staff in ensuring safe and standardized assessments are completed. As invasive CPET has been an established clinical procedure for many years, there are already historical normal values available. Thus, we will be able to identify abnormal cardiopulmonary physiology in symptomatic participants without needing controls for comparison. The added risk of performing this test in control subjects is not warranted when their data are not needed to answer the research questions set by this protocol. |
| Flexible bronchoscopy | Several pulmonary complications of PASC including persistent infiltrates and endothelial changes can only be characterized pathologically via bronchoscopy. PASC patients safely tolerate bronchoscopy and it has been demonstrated to be diagnostic in several cases.[56] In addition, lung alveolar tissue has been found to be a viral reservoir in SARS-CoV-2 infection;[57] thus, deep pulmonary sampling is needed to find persistent infection. We will specifically study several types of participants: those with dyspnea and normal chest CT; those with dyspnea and abnormal chest CT; those with impaired diffusion capacity, regardless of chest CT findings; and asymptomatic controls (infected and uninfected). Including symptomatic participants with and without lung abnormalities on chest CT will allow us to compare specimens from a subgroup of patients with COVID-induced lung disease and from those without. The mechanisms by which SARS-CoV-2 infection causes structural change in the lungs are likely different than the mechanisms by which SARS-CoV-2 infection causes dyspnea without structural lung changes, meriting inclusion of both. Additionally, radiographic changes themselves vary and likely are related to different underlying mechanisms. Ground glass opacities more likely result from an ongoing inflammatory process such as viral persistence, whereas a traction bronchiectasis process is more likely to harbor other bacterial colonizers such as pseudomonas. Including asymptomatic infected controls will allow us to examine whether having had SARS-CoV-2 causes changes to respiratory epithelium independent of symptoms. Given that SARS-CoV-2 enters through the respiratory epithelium, we can use uninfected participants to examine whether there are characteristics of their epithelium that convey innate resistance to infection. In addition, uninfected and asymptomatic infected participants can be used as controls for comparisons of microbiome results and other assays for which normal values are not well established in the general population. To establish the distinction between upper and lower airway samples, we will retain a sample of subglottic secretions for comparison. Subglottic suctioning is a standard, required part of all bronchoscopies that removes secretions from the back of the throat to reduce the risk of pneumonia and other complications. Although these secretions are typically discarded during the procedure, we will retain one sample for reference. Bronchoscopy will include bronchoalveolar lavage and four bronchial brushings. Bronchial brushings are safe, simple, and less invasive than either lavage or biopsies. Bronchial brushing collects airway epithelial cells of high viability which can be used for RNA extraction,[58,59] immunohistochemistry,[60] protein extraction,[61] and studies of viral persistence.[62] The respiratory epithelium is affected by the local pulmonary environment.[63] Since lung changes are often localized, taking |

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| | brushings from multiple locations in the lower lobe (two each from two different subsegmental regions) is necessary to account for the potential heterogeneity of respiratory epithelium. Diagnostic yield for changes of epithelium increases with additional brushings, particularly when the atypical epithelial location is not evident visually. Additionally, the need for precision down to genomic level requires multiple samples to mitigate potential contamination. |
| Right heart catheterization | Dyspnea and exercise intolerance is a cardinal manifestation of PASC and often has not been explained by other noninvasive testing (Echo, PFT). We will perform right heart catheterization to identify the cause of dyspnea in symptomatic patients who do not have a clear cause on echocardiogram or PFT. Specifically, we are looking for diagnoses such as elevated pulmonary vascular resistance, pulmonary hypertension, or exercise induced pulmonary hypertension. Pulmonary hypertension is a known complication of acute COVID and may contribute to PASC.[35] This is necessary to meet the study objective, to "define the pathophysiology of and mechanisms of post-acute sequelae, including direct and indirect causal effects of COVID-19 infection, and potential modifiers (e.g., sex, age, and race/ethnicity)." We will minimize any potential risks towards participants by excluding high risk participants, performing this assessment only at expert centers, and ensuring that the site staff are trained on the most recent SOP. As right heart catheterization has been an established clinical procedure for many years, there are already historical normal values available. Thus, we will be able to identify pulmonary hypertension in symptomatic participants without needing controls for comparison. The added risk of performing this test in people without PASC or prior infection is not warranted when their data are not needed to answer the research questions set by this protocol. |
| Colonoscopy with biopsy with or without upper endoscopy with biopsy | The gastrointestinal tract has been found to be a reservoir for viral persistence in SARS-CoV-2 infection.[64,65] In addition, colonic microbiome has been postulated to play an important role in pathogenesis of and risk for PASC.[66,67] Therefore, evaluation of the gastrointestinal tract is crucial in identifying pathogenesis of PASC. Colonic biopsies will allow for detection of viral particles as well as evaluation of the intestinal mucosa with prolonged infection; these cannot be studied with stool samples. In addition, colonoscopy allows for collection of colonic microbiome sample via biopsy. Biopsy-derived microbiome samples are well-established to be substantially different from and superior to fecal microbiome samples.[68] Detection of viral particles has shown to be higher in the gastric and duodenal tissue than the colon,[64] and therefore upper endoscopy with biopsy as well as colonoscopy with biopsy will produce the highest yield for studies of viral persistence, among participants willing to undergo bidirectional endoscopy. Obtaining multiple biopsies is the norm during endoscopy and colonoscopy to ensure adequate sample for analysis. For example, the Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy (ASGE) recommends a total of 12 biopsies (2 each from six sites);[69] others advocate for 30 biopsies (5 each from 6 sites).[70] Similarly, for detection of inflammatory bowel disease, the ASGE recommends at least 10 ileocolonic biopsies (≥2 each from 5 sites); for pancolitis the recommendation is to take at least 33 biopsy samples.[69] It is necessary to obtain data from those without PASC to determine whether viral persistence is |

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| | differentially associated with symptoms, and from those without prior infection to identify whether microbiome is differentially altered in those with PASC. |

# 4 Potential Risks and Benefits

## 4.1 Known Potential Risks

This study includes patient-reported questionnaires, data extraction from electronic health records (EHR) and claims data, basic clinical examinations, blood draws, and radiology studies. We describe the risks of each in turn. Some patients (maximum of 20%) will be asked to undergo moderate risk procedures; these patients will be separately consented for those procedures. Clinical consent will be signed as per each local institution's requirements and policies for procedures that require clinical consent. These clinical consents will be administered in addition to the complete research consents, with all of the risks outlined, administered by the research team, for tests such as colonoscopy, endoscopy, flexible bronchoscopy, and right heart catheterization.

**Risks of survey completion:** While we anticipate no risk greater than that found in everyday life from survey completion, we understand that completing questionnaires about the COVID-19 experience could cause subjects to become upset or frustrated. To minimize risk, staff will be trained to let subjects know that they can stop the line of questioning at any point and ask for the subject to notify them if and/or when they are ready to resume the questioning.

Additionally, loss of confidentiality for the subjects' answers is another potential risk. Loss of confidentiality could result in damage to a patient's financial standing, employability, insurability, or reputation. The aforementioned risk is significantly minimized through the use of a secure, encrypted, password-protected database such as i2b2 Data Hub REDCap Central residing in a protected cloud environment. Minimal necessary access to the REDCap database will be granted only to study and research personnel. The REDCap Central database and the i2b2 Data Hub will meet compliance with FISMA-Moderate federal standards.

PHI that may link subjects to the data will be stored in REDCap Central. In the databases used for analyses, such as the i2b2 Data Hub, there will be a HIPAA-defined coded Limited Data Set. Universally Unique Identifiers (UUIDs) will be generated from PHI to maintain unique, non-duplicated subject identifiers across the analysis databases used in the study (see section 13.4.2).

Any PHI that may link subjects to the data will be stored in a separate location.

**Risks of digital health technology:** Use of commercial products and devices made by third party companies, including wearable fitness trackers, wearable sleep monitors, mobile apps, websites, web apps and types of computer software that permit screen sharing, record keystrokes, gain access to device files and/or use location tracking technology, may be associated with loss of privacy and risk of breach of confidentiality. These products and devices will only be used to collect study data with IRB approval and if the subject has agreed to all applicable Terms of Service and EULAs. Any newly created digital health platforms will be assessed and cleared before use in this study.

**The following protocol elements pose no appreciable risk:**

- Clinical examinations (e.g., vital signs, height and weight, physical examination, vision, capnography)
- Collection of urine, stool, tears, and saliva
- Home activity monitoring
- Sleep studies*
- Ultrasound studies
- EndoPAT

*Although these tests pose no risk appreciable risk to pregnant or post-partum participants, we will exclude pregnant participants and those within 3 months of delivery from completing both home and facility sleep studies as the data they contribute is not likely to be valid during this time and may skew study results.

**Electrocardiogram:** Electrode placement and/or shaving on the chest may cause discomfort such as redness or itching for some people. In addition to being performed independently, several study procedures (including the home and facility sleep studies, echocardiogram, tilt table test and cardiovagal testing, cardiopulmonary exercise testing, right heart catheterization, invasive cardiopulmonary exercise testing, flexible bronchoscopy, and cardiac MRI) include the use of electrocardiogram.

**Phlebotomy:** Having blood taken poses minimal risks like lightheadedness or feeling faint. Redness, pain, bruising, bleeding or (rarely) infection may occur at the site of a puncture during blood collection.

**Risk of COVID-19 Nasal Swab:** The nasal swab test may be uncomfortable and may cause a small proportion of people to gag, cough or have a nosebleed.

**Mental and behavioral screening:** The tests of memory, attention, and thinking may be frustrating or stressful for some people. Subjects may stop the questions at any time.

**6-minute walk test:** Some patients may get tired or short of breath or have palpitations during these tests.

**Pulmonary function tests (PFTs):** Some people may feel dizzy or lightheaded during the test. If patients are given the medicine albuterol during this test, they may have side effects. Side effects of albuterol include feeling nervous, shakiness, headache, sore throat or nostrils, and muscle aches. More serious, but less common, side effects of albuterol include a fast heartbeat or feeling like their heart is pounding.

**Oral glucose tolerance test:** Bruising (tenderness), bleeding, or infection may occur at the IV location. After drinking the sugary liquid, patients may have some side effects. Side effects may include feeling sick to their stomach (nausea), feeling sweaty, or feeling dizzy or like they might faint. People with diabetes may have high blood sugar after the test. They may need to take a dose of their regular diabetes medicine after the test if their blood sugar is high. In rare circumstances, participants may have a hyperglycemic event after this test, which may lead to hospitalization.

**Echocardiogram:** This test without contrast poses no appreciable risk. If patients are given contrast or enhancing agents during this test, they may have side effects. Side effects of contrast or enhancing agents may include headache, flushing, a drop in blood pressure, back or chest pain, nausea, or dizziness. Very rarely (approximately 1 in every 10,000 tests) participants may have an allergic reaction that includes itching, swelling, rash/hives, or difficulty breathing. Severe allergic reactions are even more rare (less than 1 in every 25,000 tests) but may cause respiratory distress or death. As contrast is given intravenously, the risks of administration include bleeding, bruising, or infection at the injection site. There may be a small risk of a reaction at the injection site. There may also be risks associated with the use of electrocardiogram (see above).

**Tilt table test and cardiovagal testing:** Bruising, bleeding, redness, pain, or a small chance of infection may occur at the IV location. Some patients may feel lightheaded, nauseous, sweaty or weak or may faint during these tests or may have other symptoms related to the tilting, such as a change in blood pressure or heart rate. If so, the patient will immediately be laid flat and monitored until improvement. There may also be risks associated with the use of electrocardiogram (see above).

**Local Lidocaine administration:** Several study procedures (including the skin and muscle biopsies, lumbar puncture, invasive cardiopulmonary exercise testing, flexible laryngoscopy with stroboscopy, upper

endoscopy with biopsy, flexible bronchoscopy, and right heart catheterization) include use of lidocaine locally, which can occasionally cause an allergic reaction. Symptoms of any allergic reaction can include redness, a rash, hives, itching, and/or difficulty breathing, swelling of the lips, tongue or face, or closing of the throat (anaphylaxis). Other risks may include a change in your heart rate (too fast or slow), convulsions (seizures) or feeling tired (lethargy). Serious adverse reactions, including anaphylaxis, seizures, or death, are extremely rare. To avoid these risks, we place strict limits on the total amount of numbing medicine (lidocaine) that can be used.

**Sedation administration:** Several study procedures (including flexible bronchoscopy, right heart cardiac catheterization, colonoscopy with biopsy, and upper endoscopy with biopsy) may include the use of procedural sedation. Institutions will provide procedural sedation in line with institutional policy including but not limited to personnel administering the sedation, intra-operative monitoring, selection of sedation agents, dosage limits and intervals, and credentialing. Each procedural sedation medication works slightly differently. Benzodiazepines (e.g. midazolam) relax the muscles, reduce anxiety and help participants forget the procedure. Opiates (e.g. fentanyl) reduce pain from the procedure. Propofol both helps participants relax and reduces pain. All procedural sedation medications may slow breathing, lower blood pressure, or cause transient cognitive impairment, dizziness or lightheadedness, fatigue, or allergic reactions. Symptoms of any allergic reaction can include redness**,** a rash, hives, itching, and/or difficulty breathing, swelling of the lips, tongue or face, or closing of the throat (anaphylaxis). These risks can be treated with oxygen, fluids, additional medications including reversal agents, or a lower dose of sedation. Serious adverse reactions, including anaphylaxis or death, are extremely rare. Additional risks depend on the class of sedative agent used. Opiates can cause nausea, vomiting, or constipation, while propofol can change the heart rate (too fast or slow), cause headache, blurry vision, or sweating.

**Radiation risk:** CT scans, x-rays (after flexible bronchoscopy), gastric emptying study, cardiac catheterization, invasive cardiopulmonary exercise testing and (potentially) lumbar puncture involve radiation. We estimate the additional amount of radiation that a patient will receive as a result of participating in this study will likely be a maximum of approximately 39 mSv over four years (if the participant undergoes all tests involving radiation), which is comparable to 13 times the yearly dose from natural environmental radiation in the US (3.1 mSv). This is well within the annual limits of 50 mSV set by the FDA for individuals participating in basic research studies. Radiation has been shown to cause cancer from exposures that are higher than the additional annual radiation dose received by participating in this study. According to the International Commission on Radiological Protection (ICRP), the increased risk of health effects, such as cancer, from radiation doses of this amount is either too small to be observed or nonexistent.

**CT Scans**: Some CT scans use iodinated contrast dye. Risks of contrast dye include allergic reaction and kidney damage. Kidney damage is usually mild and temporary.

**MRI/MRV/MRA:** MRI does not involve high-energy radiation but poses risk for people with metal implants, which may cause burns if the patient is not properly excluded from having the study. Patients may experience claustrophobia, difficulty breathing, hear loud noises, feel warm or hot, or experience tingling during the MRI. Very rarely, patients may experience burns even without metal in the body. Some subjects may experience nausea, dizziness, or have allergies to the gadolinium dye used to generate MRI contrast images. In rare situations, MRI dye can cause nephrogenic systemic fibrosis. Sites will follow the most current version of the American College of Radiology Manual on Contrast Media or local policies, whichever are more restrictive, to determine what (if any) pre-test testing is required, and if contrast can be safely administered, prior to completion of any test with gadolinium. There may also be risks associated with the use of electrocardiogram (see above).

**Nerve conduction study (NCS) and electromyography (EMG):** Either of these tests may cause mild patient discomfort, including tender muscles, because of the electrical signals (NCS) and tiny needles

(EMG) used in the studies. Rarely, bruising or bleeding may occur at EMG sites. EMG may contribute to asymptomatic bleeding; however, clinically significant bleeding is rare. There is a small risk of pneumothorax associated with EMG of the diaphragm and chest wall muscles. Some patients may feel lightheaded or weak or may faint during these tests.

**Flexible laryngoscopy with stroboscopy:** Risks of this test may include sneezing, gagging, coughing, or bleeding during the test. The risk of an injury or tear to the nose or throat is uncommon with flexible laryngoscopy but may still occur. Participants may experience a nosebleed, sore throat, or cough after the tube is removed. In addition, participants will be instructed not to eat or drink after the procedure until their gag reflex returns when the throat-numbing medication wears off. Laryngospasms are very rare and may occur in fewer than 1 out of 100 cases (<1%). The decongestants used are generally considered safe as they are available as over-the-counter medicines. Some participants may feel some burning, stinging, or dryness inside the nose, increased nasal discharge, sneezing, nervousness, nausea, dizziness, headache, difficulty falling asleep or staying asleep, or a change in heart rate (too fast or slow). There may also be risks associated with the use of local lidocaine administration (see above).

**Skin and muscle biopsies:** Risks of biopsy include bleeding, bruising, soreness, discoloration of the skin, or infection. Rarely, people may get a small scar where the biopsy was taken. There may also be risks associated with the use of local lidocaine administration (see above).

**Lumbar puncture:** Risks of lumbar puncture include bleeding, bruising, headache (3%) or infection, including low risk of developing brain infection. Very rarely, headaches may persist for a week or be sufficiently severe as to require return to a clinical setting for a blood patch for treatment. In extremely rare cases, bleeding can compress the spinal cord, requiring surgical removal of the clot. If imaging (fluoroscopy or CT) is required to conduct the study, the effective radiation dose is approximately 2.9 mSv, about the same as the yearly dose from natural environmental radiation in the US (3.1 mSv). There may also be risks associated with the use of local lidocaine administration (see above).

**Flexible bronchoscopy with lavage and brushings:** The main risk from fiberoptic bronchoscopy is post-procedure fever, which is transient and occurs in 5% of patients. Less frequent risks include low oxygen, tachycardia, hypo/hypertension, bleeding, infection, bronchospasm, throat irritation, coughing, or risk of injury to the mouth, nose, vocal cords, trachea, or part of the lung. Heart attacks may occur in participants who already have a history of heart disease. Pneumonia, abnormal cardiac rhythms, and pneumothorax are rare complications. Rarely, hospitalization may be required for aspiration pneumonia (hypoxia, cough, wheezing), arrhythmia, confusion, hemoptysis or pneumothorax. Hemodynamically unstable arrhythmia may require treatment with emergency defibrillation. Pneumothorax may require treatment with chest tube. Death is very rare and may occur in fewer than 1 out of 5,000 cases (0.02%). The bronchoscopy assessment consists of a supraglottic sample, bronchial brush biopsy and bronchoalveolar lavage. To minimize risk, no more than four bronchial brushings will be done (in the lower lobe of one side, in two different subsegmental locations; each brushing will be done in a different subsegmental bronchus), no more than 150 cc of fluid will be instilled during lavage (on the opposite side as the brushings), and lavage fluid will be administered in divided doses. In addition, participants will be instructed not to eat or drink after the procedure until their gag reflex returns when the throat-numbing medication wears off. There may also be risks associated with the use of sedation or local lidocaine administration (see above). A post-procedure chest X-ray is required after bronchoscopy. The effective radiation dose from this test is approximately 0.1 mSv, comparable to 3 percent of the yearly dose from natural environmental radiation in the US (3.1 mSv). There may also be risks associated with electrocardiogram (see above).

**Right heart cardiac catheterization:** Bruising or bleeding may occur at the catheter insertion site and, in rare cases, infection or occlusion from a blood clot at the insertion site may also occur. Participants may be asked to lie down for the test in a way that could be uncomfortable or might hurt later. If occlusion does occur during the procedure, it will be addressed by draining the blood clot. If the catheter is inserted in a vein in the neck, there are very low risks of vein or artery damage, vessel perforation, internal hemorrhage,

hemothorax, cardiac tamponade, air embolism, lung collapse, heart attack or stroke, nerve injury, damage to heart valves, dizziness, low oxygen, low blood pressure (hypotension) or high blood pressure (hypertension) which may result in in-patient hospitalization. The catheterization can lead to abnormal heart rhythms. In very rare cases, therapy may be necessary. Serious changes may require treatment with emergency defibrillation (application of electrical shock to the heart). There may also be risks associated with the use of sedation or local lidocaine administration (see above). Fluoroscopy is required to ensure correct catheter placement. The effective radiation dose from fluoroscopy is approximately 1.7 mSv, about half as much as the yearly dose from natural environmental radiation in the US (3.1 mSv). There may also be risks associated with the use of electrocardiogram (see above).

**Cardiopulmonary exercise testing:** Complications of exercise testing are rare. Major complications of exercise testing may include abnormal heart beats (arrhythmias); change in blood pressure (for example, too high or too low); heart attack (myocardial infarction); muscle, bone, or joint injury; or death. If the test causes abnormal heartbeats (palpitations), we may need to give the participant a special medicine or treatment. Serious changes may mean the participant may need to have emergency defibrillation (giving an electrical shock to the heart). These complications happen in less than 1 in every 5,000 to 10,000 tests, with death estimated at 1 in every 20,000 tests. There may also be risks associated with the use of electrocardiogram (see above).

**Invasive cardiopulmonary exercise testing:** With the exception of risks of sedation and discomfort from lying down during the test, neither of which apply to iCPET, the risks associated with iCPET include all those found in non-invasive cardiopulmonary exercise testing and in right heart catheterization (see above). In addition, there is risk of bruising, bleeding, occlusion or injury to the radial artery. The catheter in the wrist artery may very rarely (less than 1 in 500 cases) cause spasm of the artery, pooling of blood in the artery ("pseudoaneurysm"), connection to the vein ("AV fistula"), or nerve injury. There may also be risks associated with electrocardiogram (see above).

**Gastric emptying study:** The radioactive substance used in a gastric emptying study is not harmful, is not absorbed through the gastric tract but excreted. This test may cause diarrhea or lightheadedness. The amount of radiotracer used is usually between 0.5 to 1.2 mCi, which is a unit used to measure the amount of radiation. However, this amount can vary by about 20%, so it could be as low as 0.4 mCi or as high as 1.4 mCi. This amount is adjusted based on the participant's weight.

**Colonoscopy with biopsy:** People may experience nausea, vomiting, bloating or pain while doing the bowel preparation. Serious side effects of the procedure itself are very rare and include a puncture of the colon requiring antibiotics, hospitalization and possibly surgery to repair. After the test, participants may experience flatulence or bloody stool. Bruising, bleeding, or infection may occur at the IV location. There may also be risks associated with the use of sedation (see above).

**Upper endoscopy with biopsy:** Serious side effects of the procedure itself are very rare and include a puncture of the tissue requiring antibiotics, hospitalization and possibly surgery to repair. After the test, participants may experience bloody stool or bloody vomit. Bruising, bleeding, or infection may occur at the IV location. There may also be risks associated with the use of sedation or lidocaine (see above).

**Risk of incidental findings:** Tests performed by certified clinical laboratories may provide results that indicate a clinically significant or medically actionable condition might be present. In these circumstances, the Principal Investigator or other designated licensed medical professional at each site will determine if further testing is necessary and will contact participants as detailed in Section 13.4.3.1.

**Risk of genetic testing:** There is a risk of discovering potentially pathogenic genetic variants of uncertain clinical significance during the study. Results are defined as clinically actionable according to the standards and guidelines defined by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMB-AMP guidelines). Some whole genome sequencing studies may be performed

in a CLIA-approved lab. The consent form will inform the participant of the potential for actionable results and will ask participants whether they would like to be informed about the presence of actionable potentially pathogenic genetic variants. For participants who elect to be informed of these genetic results, the genetic variant discovered will be shared with the site PI or designated study personnel, who will use their local process and policies for re-identification of the participant and referral if needed for evaluation, which may include involvement of their local genetics team and/or the participant's cardiologist or other caregivers.

There is some chance that analysis of the relationship of genetic findings to outcomes could cause psychological distress. Some people involved in genetic studies feel anxious about the possibility of carrying (or their child carrying) an altered gene that may place them at risk or that might be passed on to subsequent generations.

### 4.2 Known Potential Benefits

There are no known potential benefits to this study to subjects, but there is a potential benefit to public health. Healthcare physicians may have a better understanding of how to meet the needs of COVID-19 survivors more effectively.

## 5 Objectives and Purpose

The purpose of this study is to characterize the spectrum of PASC symptoms and define the incidence, prevalence, and underlying mechanism of PASC in the adult across multiple sites in the United States. The specific aims are to:

- Characterize the incidence and prevalence of PASC, including clinical and biological features, severity, and distinct sub-phenotypes, following COVID-19 infection.
- Characterize the clinical course and recovery of sequelae over time and determine associated risk factors for PASC among COVID-19 infected individuals and compared them to uninfected individuals.
- Define the pathophysiology of and mechanisms of post-acute sequelae, including direct and indirect causal effects of COVID-19 infection, and potential modifiers (e.g., sex, age, and race/ethnicity).

We hypothesize that this study will elucidate risk factors(s) that contribute to PASC progression.

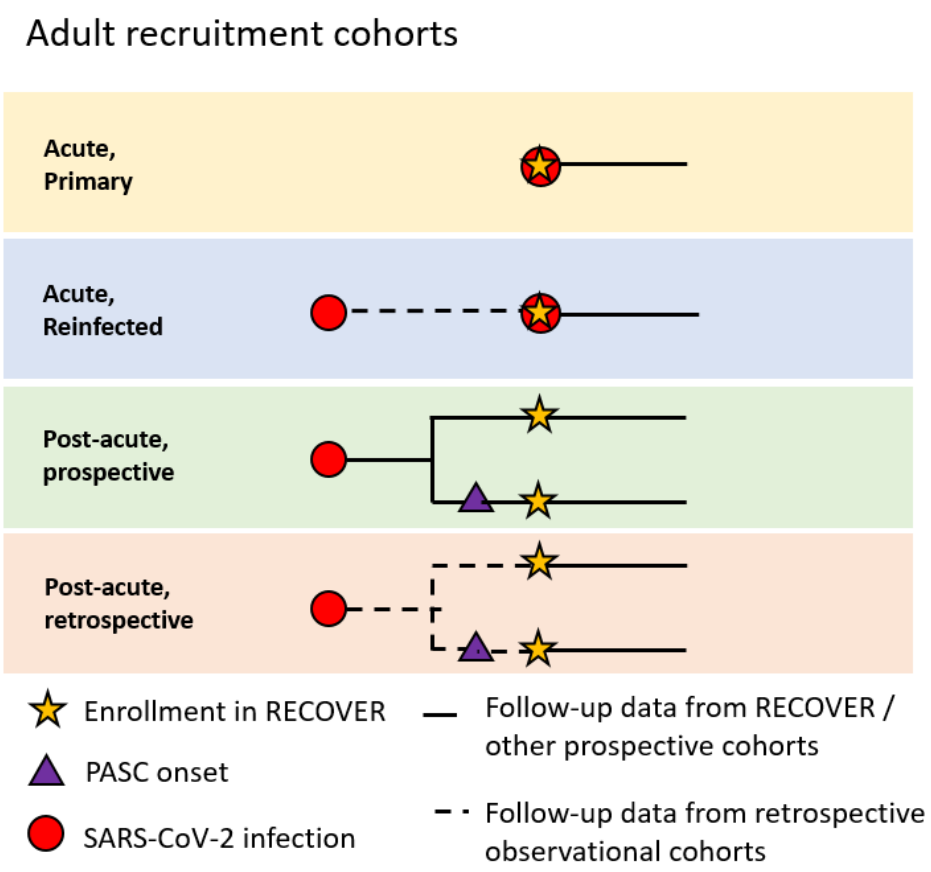
## 6 Study Design

### 6.1 Study Design

This is an ambi-directional longitudinal meta-cohort study (combined retrospective and prospective) to determine the clinical spectrum, progression, prevalence, and incidence of PASC (COVID-19). Individuals with and without COVID-19 infection will be enrolled at varying stages during and after first infection (Figure 1).

We will include subjects who meet our inclusion and none of the exclusion criteria and analyze the core set of questions on demographics, comorbidity, COVID-19 vaccination status, acute COVID-19 clinical course, current clinical condition/symptom, and outcomes as tiered testing. Participants who consent and contribute data to the study will count towards target enrollment numbers.

**Figure 1: Enrollment plan for people with infection**




Many of the participating health centers utilize electronic health record (EHR) systems. This facilitates the pooling of data to ask and answer numerous research questions applicable to the care of subjects with or at risk for COVID-19. Data will be entered into the secure HIPAA compliant REDCap database for analysis and storage.

A subset of participants will be asked to undergo Tier 2 (**Appendix 3**: Tier 2 questions, tests and procedures) and in some cases Tier 3 testing (**Appendix 4**: Tier 3 tests and procedures), if available at the local study site. In order to meet the sample size targets described in Section 12.2 below, participants are organized into four "buckets" based on their COVID-19 infection status and whether or not they present with relevant PASC symptoms. To ensure statistical power to differentiate between each protocol-specified bucket for analysis, this protocol specifically assigns Tier 2 and Tier 3 assessments to participants in each bucket. These assessments are assigned to the appropriate number of infected participants (Bucket 1) and uninfected participants (Bucket 2) who have these relevant symptoms. Then, they are assigned to participants without symptoms by randomly selecting the appropriate number of infected (Bucket 3) and uninfected (Bucket 4) participants. This process is shown in **Table 2**.

Table 2: Distribution of Participants by COVID-19 Infection History and Relevant Symptoms

| | **Infected subject** | **Uninfected subject** |
|---|---|---|
| **With relevant symptom(s)** | [Bucket 1] | [Bucket 2] |
| **Without relevant symptom(s)** | [Bucket 3] | [Bucket 4] |

All relevant symptoms are described in the corresponding procedure's SOP. As the symptoms that are most relevant to PASC are better understood, RECOVER's subject matter experts refine the list of relevant symptoms for each test. Once the target sample size (see Section 12.2) has been reached with adequate data quality within each bucket, we cease assigning the test to people in that bucket. This ensures that our total enrollment and procedure sample sizes are as small as can be in order to ensure usable data. In other words, this approach allows us to minimize the number of people that we ask to undergo each test and the risk of the study, while maintaining adequate statistical power.

We anticipate that approximately 30% of participants will undergo each Tier 2 test and a maximum of 20% of participants will undergo each Tier 3 test. For Tier 2 testing, a random sample of 5.6% of SARS-CoV-2 infected and 11.4% of uninfected participants will be asked to undergo each Tier 2 test. These percentages may be modified to ensure the correct distribution of PASC and control participants as testing accrues. In addition, some or all participants who meet test-specific clinical criteria set by subject matter experts will be offered the test so that we ensure an adequate number of participants likely to have a positive test for statistical analysis (see **Appendix 5**: Tier 2 and 3 eligibility, exclusion criteria for additional description of this process). Among the eligible participants, REDCap will assign some or all of those that meet clinical oversample eligibility criteria to the test (at adaptive proportions designed to keep the total number of participants assigned to the test at not more than 30%) and will randomly assign the test to some of the remainder as described above. As RECOVER follows an adaptive study design, the relevant symptoms for each test will be reviewed and updated periodically to ensure the proper proportion and populations are being selected. When relevant symptoms are changed for tests that also allow random assignment, they only affect the proportion of participants with various conditions assigned the test, but do not change the fact that any participant is still eligible and therefore do not change the risk of the study as a whole. Any participant may decline any Tier 2 or Tier 3 test if offered.

It is necessary to have uninfected participants undergoing the Tier 2 and Tier 3 tests so that we can conduct analyses to identify any significant worsening in outcomes among participants with PASC or SARS-CoV-2 infection versus control. It is necessary to have asymptomatic infected participants undergoing the Tier 2 and Tier 3 tests so that we can identify subclinical pathophysiologic changes. However, for the small subset of highest risk Tier 3 procedures, if there are well-established norms in control populations that can be used for comparison, the test will not be offered to uninfected controls and/or to asymptomatic previously infected participants (see **Appendix 5**: Tier 2 and 3 eligibility, exclusion criteria for details of which tests are only offered to symptomatic infected participants).

The REDCap database uses data entered by the sites to assign Tier 2 and Tier 3 assignments to participants at each study visit. REDCap excludes participants with recent SARS-CoV-2 infections, who have already completed the test within the eligible window, or who meet test-specific safety exclusion criteria from selection. After participants initially complete a Tier 2 test, they will be re-assigned this test approximately annually as a follow-up assessment. Minimal risk Tier 3 tests may also be re-assigned approximately annually. More than minimal risk Tier 3 tests will not be offered more than once, except in the case of participants who were enrolled as uninfected and become infected during the course of the study. After infection, these participants may be offered the opportunity to repeat any more than minimal risk Tier 3 tests done while uninfected, as pre- and post-infection data is highly valuable in identifying changes related to SARS-CoV-2 infection. The REDCap database has strict limitations in place so that participants are not assigned a test for which they are eligible if they have completed the same test within the previous four visit windows (approximately annually). This functionality is only effective if REDCap is updated in a timely fashion. Therefore, sites must update REDCap to indicate performance of a Tier 2 or 3 test within six weeks of test conduct.

Site staff are ultimately responsible for ensuring participants do not meet safety exclusion criteria after they have been selected for a test, as not all exclusion criteria are available in REDCap. Pre-test assessments that are conducted once participant is assigned to a test to determine safety exclusion criteria must then be documented in REDCap. SOPs for these tests make clear that ineligible participants might be assigned tests because not all exclusion information is known to REDCap. These safety exclusion criteria are also

available as checklists for site staff in the respective SOP. For all Tier 3 tests that are more than minimal risk, a licensed physician will review the exclusion criteria for that test and confirm that the subject does not meet any of the specified safety exclusion criteria.

Procedures in Tier 3 will only be performed concurrently when clinically appropriate. Complete information regarding how tests will be assigned will be in each assessment's SOP. Tier 2 and 3 procedure eligibility, exclusion criteria, and clinical variance affecting the risk/benefit assessment are found in **Appendix 5**: Tier 2 and 3 eligibility, exclusion criteria.

### 6.1.1 Tier 2 or 3 Procedure Feasibility

Our expectation is that all participating sites will conduct all Tier 2 and 3 tests and procedures. However, we understand that in practice this may not be the case. Sites that are unable to perform one of the study procedures must follow the CSC notification process outlined in the MOP.

### 6.1.2 SOP Modification Requests

Procedure-specific SOPs are developed to be consistent with the IRB-approved protocol. These SOPs are written to include all clinical variation permitted by the protocol. Any SOP adjustments that are inconsistent with the currently approved protocol will be submitted for IRB review as an overall protocol amendment before implemented.

Procedures in Tier 3 will only be performed concurrently when clinically appropriate. Complete information regarding how tests will be assigned will be in each assessment's SOP. Tier 2 and 3 procedure eligibility, exclusion criteria, and clinical variance affecting the risk/benefit assessment are found in **Appendix 5**: Tier 2 and 3 eligibility, exclusion criteria**.**

## 6.2 Characteristics of the Study Population

**Number of Subjects:** 14,880 subjects with and without existing subject records.

**Age of Subjects:** 18 years and above.

**Racial and Ethnic Origin:** Expected overall recruitment by race/ethnicity will be 53% non-Hispanic Whites, 16% non-Hispanic Blacks, 27% Hispanic/Latinx, and 4% Asian Americans, Native Hawaiians, Pacific Islanders, American Indians, and Native Alaskans with equal distributions between male and female populations.

**Severity of Illness:** Targeted distribution will be 25% hospitalized for COVID-19 infection; 75% not hospitalized.

**Pregnancy status:** Targeted enrollment will be 1,867 infected and 583 uninfected at the time they were pregnant.

## 6.3 Recruitment and Retention

### 6.3.1 Recruitment Strategy and Procedures

Recruitment of people with and without SARS-CoV-2 infection will be stratified to ensure adequate representation by sex and race/ethnicity as described in section 6.2. For patients with SARS-CoV-2 infection recruited during or after infection, priority will be placed on recruiting patients from lists of SARS-CoV-2 infected patients to ensure that (a) potential patients were identified prior to study enrollment to minimize bias associated with self-referral, and (b) enrollment response rates can be generated from known denominators. If lists are large, recruitment can be phased using random sampling and adequate "working of recruitment lists" to maximize generalizability. Attempts will be made to include a diversity of

sites of care (e.g. not only from a post-COVID clinic or only from patients cared for in academic medical centers) and severity of illness (i.e. not only from hospitalized patients). For patients without SARS-CoV-2 infection, patients will be randomly sampled and recruited from known lists of potential study subjects in similar communities, demographics, and sites of care as those being recruited into the SARS-CoV-2 positive cohort. Recruitment will be stratified to match the SARS-CoV-2 positive group in terms of racial/ethnic diversity, index time point, and proportion of patients not hospitalized, hospitalized but not in the intensive care unit, and those hospitalized in the intensive care unit.

For patients enrolled after acute infection, preference should be given to those who have data and/or biospecimens collected before or during the acute phase available to the investigators, though efforts should still be made in such cases to recruit an unbiased sample of such patients (for instance, by oversampling this group for underrepresented minority patients or those in communities not already well-represented in the cohort).

If the subject has provided prior consent to be contacted for research at their study site institution, the site study team may create a query in the local EHR system to identify potential subjects based on study entry criteria (age and history of prior diagnosis or testing related to COVID-19). Institutions may also partner with local health departments to obtain complete case lists of people with positive tests in a geographic area. A secure email will be sent to potential subjects to solicit interest in the study, with instructions to contact the study team if interested in participating. This email will be provided to the IRB for review and approval before use in the study. Once contacted by the potential subject, the study Principal Investigator or designated study staff members will provide additional IRB-approved information to the subject as described below and may schedule a study visit. The EHR query or public health department query may be repeated for the duration of the 4-year study. All query responses will be de-identified and retained per section 14.2. A waiver of authorization will be in place at participating sites so study teams can review patient medical records to identify potential subjects who meet eligibility criteria.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to site Principal Investigator or designated study team member.

Additional information sent to or available to subjects prior to enrollment may include videos, slide presentations, pamphlets, or website information that provide a general overview of the study and/or more details about the informed consent form. Subjects may view these materials prior to in-person discussion. All materials will be submitted to the IRB for approval prior to their use. Any recruitment information sent by email will utilize a secure encrypted email platform. Once potential subjects have been identified, the study team may need to notify the treating physician that they have patients eligible to participate.

If notification to the treating physician is necessary, one or more of the following methods will be used to notify the treating physician:

1. The treating physician will be given a list, advertisement, letters or oral script to use when contacting potential subjects
2. The treating physician and site Principal Investigator will send a letter to all potential subjects (letter must have both names)
3. If the treating physician agrees, the study team will directly contact potential subjects on behalf of TP by letter, phone, email, or an electronic medical record patient portal.

Once contact is made, approved recruitment language will be used to communicate the reason they are being contacted and subjects will be asked if they are interested in participating in this specific study. Should the potential subjects agree, the study team will provide the subjects with information regarding the next steps for participation.

Social media platforms, websites, conventional mass media (radio and print publications), flyers or other advertising may also be used for recruitment purposes. All recruitment materials or text for any of these platforms will be submitted to the IRB for approval prior to use.

### 6.3.2 Retention Strategy

Participant retention may be promoted by providing educational materials related to PASC symptom management, through newsletters or other periodic outreach, through personalized periodic contact (e.g., birthday greetings), by rollout of a mobile application, by creation of a patient web portal to provide access to study personnel for questions, and by using feedback received from participants to enhance participant experience. Additional efforts may be made to minimize attrition: 1) reminder calls for assessments, rescheduled if missed; 2) maintaining contact information (e.g., updating at each contact, obtaining alternate contacts, re-connecting in primary care), and maintaining ongoing contact with all participants during the study. All materials used for participant retention will be provided to the IRB for review and approval before use in the study.

Contacts with participants may include reminders for completion of study surveys, reminders for study appointments, a post-visit thank-you card or call, a newsletter, a birthday or greeting card, and a holiday or end-of-year card. We aim to design both culturally and religiously appropriate contact documents. Therefore, these contacts will be initiated by each site and will be conducted in the language of choice of the participant. In addition, because some religions (e.g., Jehovah's Witnesses) may not celebrate birthdays or holidays, specialized cards will be designed to accommodate these participants. Newsletters and cards sent to RECOVER respondents will be reviewed for health literacy to ensure comprehension among participants of varying literacy levels.

Participant response burden may be monitored in real-time during the study. If burden is found to be excessive, it may be reduced by altering the overall study data collection strategy, such as by increasing the interval of assessments to 6 months instead of 3 months; reducing the number of data elements collected (eliminating rare symptoms); pre-filling prior responses to reduce data entry time for patients; tightening criteria for Tier 2 and Tier 3 data collection; increasing the availability of home-based Tier 2/3 assessments; and/or increasing participant reimbursement. All such modifications will be approved by the IRB before implementation study wide. Individuals at risk of withdrawing from the study because of burden may, on a case-by-case basis, be offered: the option to skip an interval assessment (e.g. if traveling); the option to skip a survey instrument that is not the PASC symptom survey; the option to increase the interval of assessments to 6 months; home-based Tier 2/3 assessments where feasible; or, if no other strategy is acceptable, the option to return for a final assessment only.

Recruitment and retention data will be monitored on an ongoing basis to compare target versus actual recruitment rates by site (stratified by race/ethnicity, hospitalization status and acute infection at enrollment); compare the number of expected surveys completed and biospecimens collected to target; and to review participant retention reports indicating the number of participants active, completed, and lost to follow-up.

# 7 Study Enrollment and Withdrawal

## 7.1 Inclusion Criteria

- Patients will be eligible for inclusion if they are at least 18 years of age and have reached the age of majority in their state of residence.
- Infected individuals must have suspected, probable, or confirmed SARS-CoV-2 infection as defined by WHO criteria within 36 months of enrollment, or positive SARS-CoV-2 infection-specific antibody testing, to meet inclusion criteria.
- Participants must be willing to generally comply with the overall protocol (e.g. cannot agree to participate only for survey collection) and must expect to be available for the duration of the study.

**Adults with suspected SARS-CoV-2 infection**

An adult qualifies as having suspected SARS-CoV-2 infection if meeting criteria a, b or c below:

a) Patients who meet the following clinical criteria plus one of the epidemiological criteria:
**Clinical criteria**: Acute onset of fever and cough OR acute onset of any three of more of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status.
Note: Signs separated with a slash (/) are counted as one sign, in which the slash means "OR" (i.e., participants may have anorexia and/or nausea and/or vomiting; any combination of these symptoms counts as one of three symptoms needed to meet clinical criteria).

**Epidemiological criteria:**

i. Residing or working in an area with a high risk of transmission of virus: closed residential settings, humanitarian settings such as camp and camp-like settings for displaced persons; anytime within the 14 days before symptom onset; or
ii. Residing or travel to an area with community transmission* anytime within the 14 days before symptom onset; or
iii. Working in any health care setting, including within health facilities and within households or within the community; anytime within the 14 days before symptom onset.

*Note: For purposes of this protocol, we define "community transmission" as any county with at least substantial community transmission according to the CDC definition (50 or more cases per 100,000 in the past 7 days). If the participant has traveled to or from another country, use the most granular data available to determine transmission in that area. Historical data about community transmission levels can be found in the MOP.

b) A patient with severe acute respiratory illness: (acute respiratory infection with history of fever or measured fever of ≥38C°; and cough; with onset within the last 10 days; and requires hospitalization).
c) A person with a positive SARS-CoV-2 Antigen-RDT who is asymptomatic or meets some but not all clinical or epidemiologic criteria.

Note: A patient who meets criteria for suspected SARS-Cov-2 infection who had a negative test at the time does NOT qualify as an infected case.

**Adults with probable SARS-CoV-2 infection**

An adult qualifies as having probable SARS-CoV-2 infection if meeting any one of a-d below:

a) A patient who meets clinical criteria for suspected SARS-CoV-2 AND is a contact of a probable or confirmed case or linked to a COVID-19 cluster;
b) A suspect case with chest imaging showing findings suggestive of COVID-19 disease;
c) A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause;
d) Death, not otherwise explained, in an adult with respiratory distress preceding death AND was a contact of a probable or confirmed case or linked to a COVID-19 cluster (Not applicable to the RECOVER adult protocol)

Note: A patient who meets criteria for probable SARS-Cov-2 infection who had a negative test at the time does NOT qualify as an infected case.

**Adults with confirmed SARS-CoV-2 infection**

An adult qualifies as having confirmed SARS-CoV-2 infection if meeting any one of a-d below:

a) Any person with a positive Nucleic Acid Amplification Test (NAAT) OR positive SARS-CoV-2 antibody test*;
b) Any person with a positive SARS-CoV-2 Antigen-RDT (including home-administered rapid test) AND meeting either the probable case definition or suspect criteria A OR B;

c) An asymptomatic person with a positive SARS-CoV-2 Antigen-RDT (including home-administered rapid test) who is a contact of a probable or confirmed case;
d) Any person with a positive SARS-CoV-2 nucleocapsid protein antibody test OR a positive SARS-CoV-2 spike protein antibody test IF not vaccinated.

*This protocol modifies the WHO criterion a to add detectable SARS-CoV-2 antibody as a qualifying test

**Adults with no SARS-CoV-2 Infection**

- Does not meet WHO criteria for a suspected, probable, or confirmed case of SARS-CoV-2 infection, AND
- Has negative NAAT SARS-CoV-2 testing from a respiratory specimen performed at the time of enrollment/screening AND
- Has a negative SARS-CoV-2 nucleocapsid protein antibody and spike protein antibody test (spike only if not vaccinated) performed at the time of enrollment, AND
- Lives in the same communities or recruited from the same sources as those in the SARS-CoV-2 infected cohort.

Note: uninfected individuals may participate independent of their vaccination status

### 7.1.1 Change in Infection Status

Uninfected individuals who develop SARS-CoV-2 infection during the study period will be designated as SARS-CoV-2 infected at the time of infection and will be considered to have been enrolled prior to SARS-CoV-2 infection.

## 7.2 Exclusion Criteria

- Individuals who have not yet reached the age of majority
- Unable to provide consent
- Individuals in hospice care
- Any serious medical condition which would prevent long-term participation
- Individuals participating as children in the study NIH RECOVER-Pediatric: Understanding the long-term impact of COVID on children and families; individuals participating as caregivers are eligible for inclusion in the adult protocol
- Incarcerated individuals
- For adult cohort sites only: individuals who were pregnant at the time of their COVID infection or qualifying negative test, and whose pregnancy is ongoing.
- For adult cohort sites only: individuals who were pregnant at the time of their COVID infection or qualifying negative test, and whose pregnancy is completed and resulted in a live birth.
    - Individuals who were pregnant at the time of COVID or qualifying negative test and who did not have a live birth remain eligible for inclusion at adult cohort sites.
    - Individuals who were pregnant at the time of COVID or are currently pregnant, regardless of pregnancy outcome, remain eligible for inclusion at pregnancy cohort sites
- For pregnancy cohort sites only: pregnant participants with an estimated date of delivery on or after December 31, 2023.

Note that participation in other observational or intervention studies while participating in RECOVER is not an exclusion criterion.

## 7.3 Vulnerable Populations

### 7.3.1 Pregnant Participants

Data from pregnant participants will be included as part of the study as it is important to understand COVID-19 in all populations. The study cannot be conducted without the group because pregnant participants represent a portion of the population affected by COVID-19 and their responses to COVID-19

disease may be different from that of the general population. No inducements, monetary or otherwise, will be offered to terminate a pregnancy. Individuals engaged in the research will have no part in any decisions as to the timing, method or procedures used to terminate a pregnancy. Individuals engaged in the research will have no part in determining the viability of a neonate. Participants who were pregnant while they had COVID-19 will be offered the opportunity to enroll their infants (once born) in the NIH RECOVER-Pediatric: Understanding the long-term impact of COVID on children and families study if they are being enrolled at a site that also supports the pediatric study. Agreement to participate in the pediatric protocol is not required for participation in the adult protocol. Similarly, agreement to participate in the pediatric protocol does not require participation in the adult protocol. This option will only be available to the participants at the pregnancy sites in the RECOVER Network and is consistent with the pediatric protocol. At these sites, participants who had Covid while pregnant and gave birth before enrollment in RECOVER, will have their medical information during pregnancy collected and used in this protocol.

### 7.3.2 Prisoners

Long-term incarcerated individuals will not be enrolled in the study. If an enrolled participant becomes incarcerated, the IRB should be notified immediately. If the incarceration is short-term (i.e., less than 90 days), the participant can remain in the study.

### 7.3.3 Students and Employees

Students and employees recruited as research subjects are more vulnerable to coercion or undue influence. Students may feel their participation in research is necessary as part of their academic requirements, or that failing to participate will negatively impact their relationship and academic/professional opportunities with the instructor/investigator. Employees may feel unable to exercise free choice in their decision to participate, due to belief that their decision may affect (favorably or unfavorably) their performance evaluations, advancement opportunities, or other employment-related decisions. The appearance of coercion and undue influence of employees/students must be minimized in recruitment methods, including the informed consent process, and other procedures. The informed consent process must include a discussion stating that the subject's decision to participate will not impact the status of employment, academic status, and/or grades respective to the target subject population.

Students or employees will not be specifically targeted for participation in this protocol but will also not be excluded based on these protected statuses. Passive recruitment methods, such as those that require the employee/student to reach out regarding participation include, but are not limited to: an unassociated, non-supervisory recruiter; IRB-approved flyers; site-wide e-mails to specific groups that encourage those interested to reach out for information. All prospectively enrolled students or employees will be informed of the specific risks to privacy and confidentiality that may be compromised prior to signing consent. This information may include sensitive topics included in the RECOVER protocol including, but not limited to, comorbidities, mental health, sexual behavior, and/or drug/alcohol use.

## 7.4 Loss to Follow-up

Subjects may be considered lost to follow-up if they have missed at least three consecutive study visits and are not responsive to the site's contact attempts or offers to reduce participant burden (see **Retention Strategy**). For each missed visit, the site should attempt to contact the participant through at least three different methods at different times. Contact methods include, but are not limited to, email, phone calls, certified letters, and/or contacting their emergency contact. An IRB-approved appointment reminder letter can be used for this purpose. If a participant contacts or returns to the site even after multiple missed follow-up visits, they should be given the option to resume participation in the study. If a participant is lost to follow-up or declines future visits, their data will be retained for future use unless they provide written documentation to withdraw their consent (see Section 7.5); however, no additional data will be collected.

Should a study participant return after a period of no contact, study staff should conduct the closest visit based on their schedule.

### 7.5 Subject Withdrawal

Subjects are free to stop participating in the study at any time upon request. However, if the subject desires to withdraw consent from participation, this includes removal of collected data from prospective analyses and destruction of any unused biospecimens. The subject must provide a written notice of withdrawal of their consent (either via letter or e-mail) to the study site PI clearly stating that they wish all eligible data removed from prospective analyses and unused biospecimens (if collected) to be destroyed. Once the subjects withdraw their consent to participate, no more information will be collected. However, in cases when the data removal will affect the integrity of the study, all previously collected data will not be removed. Subjects will be informed about this during the consenting process. Data that have already been distributed to the i2b2 Data Hub will not be removed. Subjects may not re-enroll in the study once they have withdrawn their consent, and removal of data from prospective analyses cannot be reversed, nor destroyed biospecimens restored.

### 7.6 Premature Termination or Suspension of the Study or a Participant

This study or a participant may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for suspension or termination, will be provided by the suspending or terminating party to the NIH Sponsor and site investigators. If the study is prematurely terminated or suspended, the site PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor and/or IRB.

## 8 Study Schedule

### 8.1 Overview of Study Schedule

Data will be collected at baseline and then following the study schedule, which starts at time of first infection for those enrolled during first infection or after their first infection without an active infection at time of enrollment; at time of reinfection if enrolled during an acute infection; or at time of negative COVID-19 test, for those who are uninfected. Uninfected pregnant individuals will begin the study schedule on their delivery date. See **Table 3**. Collection will be tiered such that all enrolled patients will undergo Tier 1 data collection, and those with abnormal findings on Tier 1 collection may progress to more intensive, invasive, or costly Tier 2 and Tier 3 data collection. We anticipate that approximately 30% of participants will undergo each Tier 2 test and a maximum of 20% of enrollees will undergo each Tier 3 test for any given symptom, including both those with relevant symptoms and a random sample of 5.6% of SARS-CoV-2 infected and 11.4% of uninfected subjects without relevant symptoms. All patients will undergo at least one in-person visit annually, which can be provided at home if provisions are made for home blood and biospecimen collection.

**Table 3: Index date and study schedule date, by enrollment type**

| Enrollment Category | Index Date | Study Schedule Start Date |
|---|---|---|
| Acute Infected (First Infection) | **First** positive SARS-CoV-2 test result date or, if test not | **First** positive SARS-CoV-2 test result date or, if test not |

| | | |
|---|---|---|
| | available, date of COVID symptom onset | available, date of COVID symptom onset |
| Acute Infected (Reinfected) | **First** positive SARS-CoV-2 test result date or, if test not available, date of COVID symptom onset | **Most recent** positive SARS-CoV-2 test result date or, if test not available, date of COVID symptom onset |
| Acute Uninfected | Qualifying negative SARS-CoV-2 test result date | Qualifying negative SARS-CoV-2 test result date |
| Post-Acute Infected | **First** positive SARS-CoV-2 test result date or, if test not available, date of COVID symptom onset | **First** positive SARS-CoV-2 test result date or, if test not available, date of COVID symptom onset |
| Post-Acute Uninfected | Qualifying negative SARS-CoV-2 test result date.<br>For pregnancy sites **only**, index date is the prior delivery date | Qualifying negative SARS-CoV-2 test result date.<br>For pregnancy sites **only**, index date is the prior delivery date |

**Home Visits**

Some study testing or procedures may be conducted through home visits to alleviate travel burden for subjects. These home study visits will be conducted by members of the clinical research study staff or contracted clinical examiners (such as Exam One) and will follow COVID safety protocols for the duration of the visit. As only delegated staff will be performing these visits, responses and specimens collected will be strictly confidential. These study visits will be strictly for data and/or specimen collection and all research tests and procedures which may be performed through home visits are indicated in **Appendix 3**: Tier 2 questions, tests and procedures and **Appendix 4**: Tier 3 tests and procedures.

### 8.2 Baseline/Enrollment Visit

A core set of questions on demographics, habits, social determinants of health, comorbidity, medications, SARS-CoV-2 vaccination status, acute SARS-CoV-2 clinical course and current clinical condition/symptom inventory will be answered by the patient upon enrollment. Following completion of the core questions, a minimal physical examination will be performed, including collection of height, weight, vital signs (including orthostatic vital signs) and measurement of waist circumference. Additional clinical examinations will be performed at that time as indicated by patient responses to the symptom questionnaires. This visit will also include collection of blood, urine, saliva, tears, and stool both for initial clinical testing and banking (see below), and/or retrieval of specimens already banked for the patient during acute COVID. If enrollment occurs at time of acute COVID-19 and the patient is too ill to participate in the complete baseline assessment or assessment is not safe for research personnel, some patient-reported elements may be deferred until recovery but should then be completed as close to the acute infection as possible. Should a patient lose capacity to consent or participate in research during the study, all research activities will be paused. Research activities will only resume once a member of the clinical team caring for the patient deems the patient capable of consent or participation. See **Appendix 1**: Schedule of assessments for schedule of assessments. The study schedule resets for participants who were enrolled as uninfected and then sustain an on-study infection, such that the new index date will be the date of first infection. The corresponding “enrollment” date for this new index date is the first visit date following the new infection.

### 8.3 Follow-up Visits

A current symptom inventory (
will be collected at 90-day intervals, with further physical examination, blood/specimen and radiologic testing conducted in a subset per protocol (see **Appendix 3**: Tier 2 questions, tests and procedures and **Appendix 4**: Tier 3 tests and procedures). To minimize burden on participants and maximize flexibility and

retention, study visit windows are ±45 days from the target visit date. Participants have up to 12 weeks to complete Tier 2 or Tier 3 testing once assigned. See MOP for details of how tests are selected and conducted.

### 8.4 One-time Visits

Participants who develop COVID while enrolled in the study may be eligible for a one-time off-schedule study visit that will follow the protocol for month 0 (acute infection) visit, if the infection is identified within the acute infection window, and if it is the first repeat infection while in the study. See MOP for details of handling of on-study infections in previously uninfected vs infected participants.

## 9 Study Procedures/Evaluations

### 9.1 Tier 1 Assessments

Tier 1 assessments are listed in **Appendix** 2: Tier 1 topics, tests and procedures.

### 9.2 Tier 2 Assessments

Tier 2 assessments are listed in **Appendix 3**: Tier 2 questions, tests and procedures.

### 9.3 Tier 3 Assessments

Tier 3 assessments are listed in **Appendix 4**: Tier 3 tests and procedures.

### 9.4 Biospecimen Banking

The following biospecimens will be collected for banking at baseline, at 3 and 6 months after index date (or most recent infection date if acute reinfected) if enrolled during that time period, and then yearly, if the participant has consented to provide biospecimens (see **Table 4**):

- Nasal swabs in freeze medium
- Blood
    1. 2 x 8.0 mL CPT tubes – for peripheral blood mononuclear cell (PBMC) collection
    2. 2 x 8.5 mL SST tubes – for serum collection
    3. 1 x 2.5 mL PAXgene tube – for mRNA
    4. 2 x 2.7 mL Sodium citrate tubes – for plasma collection
    5. 1 x 10.0 mL EDTA tube – for plasma and white blood cells (WBCs) collection

The following additional biospecimens will be collected on a different schedule:

- Saliva (on enrollment only, if the participant has consented to genetic testing)
- Stool and urine (on enrollment and at year 2)
- Tears (schedule to be determined)

Patients who underwent the Oral Glucose Tolerance Test (ceased performance as of protocol v12) also consented to provide additional biospecimens for future research and had the following samples collected and sent to the biospecimen core. These samples were taken from the SST tubes that were collected for central lab assessments; no additional volume was collected solely for biospecimens.

- 4 x 1.4mL matrix tubes for PBC aliquoting

For patients undergoing Tier 3 invasive testing through additional consent, samples will whenever possible be collected and sent to the biospecimen core, including:

- Cerebrospinal Fluid (CSF)
- Biopsy specimens

- Bronchoalveolar lavage (BAL) and brushings

The following biospecimens will be collected for banking at the one-time on-study reinfection visit if the participant has consented to provide biospecimens:

- Nasal swabs in freeze medium
- Blood
    1. 2 x 8.0 mL CPT tubes – for peripheral blood mononuclear cell (PBMC) collection
    2. 2 x 8.5 mL SST tubes – for serum collection
    3. 1 x 2.5 mL PAXgene tube – for mRNA
    4. 2 x 2.7 ml Sodium citrate tubes – for plasma collection
    5. 1 x 10.0 ml EDTA tube – for plasma and white blood cells (WBCs) collection
- Stool and urine

If saliva was previously collected it does not need to be repeated for this one-time reinfection visit.

**Table 4: Biospecimen collection schedule**

| | **Study schedule time point** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Biospecimen** | Baseline | On Study Reinfection | 3M | 6M | 12M | 24M | 36M | 48M |
| Nasal Swab | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Blood | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Saliva | Yes* | Only if not previously collected* | No | No | No | No | No | No |
| Stool | Yes | Yes | No | No | No | Yes | No | No |
| Urine | Yes | Yes | No | No | No | Yes | No | No |

*if participant has agreed to genetic testing

### 9.5 Data Sources

Data sets obtained directly from the subject, or from the subject's EHR or existing registries, will be stored in REDCap Central, in the digital health platform and the i2b2 Data Hub, which will collect and harmonize subjects' data from disparate sources and make it available for reporting and analysis by RECOVER cores, the network of biostatisticians for RECOVER (NBR), coordinating centers, and OSMB as needed.

### 9.6 Data Collection

Demographic data points that will be collected centrally and included in the main study database include date of birth, zip code, sex assigned at birth, ethnicity, race, COVID-19 status, COVID-19 vaccination status, COVID-19 vaccine received if applicable, medical history during COVID-19 hospitalization, and/or treatment, pregnancy status, medical history, and health status before COVID-19. Sites will retain other PHI elements locally in a HIPAA-compliant manner for purposes of follow up and data retrieval. See **Table 5** for PHI elements that may be collected locally.

**Table 5. PHI that will be collected for this study.**

| **Protected Health Information (HIPAA Identifiers)** | |
|---|---|
| 1 | Names |
| 2 | Street Address |

| | |
|---|---|
| 3 | Any of the following: City, State, Zip Code |
| 4 | Date of Birth |
| 5 | For those 90 or older: Any element of date (including year) indicative of age, or recording actual age (i.e., rather than recording age as “90 or older”) |
| 6 | Telephone numbers |
| 7 | Fax numbers |
| 8 | Electronic mail addresses |
| 9 | Social security numbers |
| 10 | Medical record numbers |
| 11 | Health plan beneficiary numbers |

### 9.7 Site Certification and Quality Control

For some Tier 2 and Tier 3 procedures, reading center staff or other subject matter experts will be utilized to develop and implement standardized protocols and case report forms, train site study staff, monitor site performance for safety, analyze and interpret the readings/scans/tissue, and implement quality control and quality assurance programs. Certification processes described below will either require clinical calibration data collected routinely outside of RECOVER according to each site’s local processes, non-subject volunteers to who have been instructed to provide false answers for training purposes, or data from a RECOVER participant will act as the confirmation of the site’s ability to perform the assessment per protocol. Further details about each calibration process are included below in **Table 6**. Selection criteria for the reading centers will take into account the experience of the center in clinical interpretation and collaboration with investigators in multisite clinical research.

**Table 6: Certification process for select Tier 2 and Tier 3 tests**

| Test | Certification process |
|---|---|
| **NIH Toolbox** | To certify administrators at each site, site staff who do not meet C-Level certifications set by the NIH must complete a practice NIH toolbox exam on a non-subject volunteer and provide both a video of the administration and the scored results for review by the CSC Neurocognitive team. An authorization form must first be signed by both the non-subject volunteer test taker and site staff becoming certified acknowledging that the video recording of the administration will be uploaded to NYULH REDCap and only watched by CSC Neurocognitive Team staff members. The videos, authorization forms, and practice score sheets are stored in a secure NYULH REDCap project or a secure folder through NYULH OneDrive, depending on the size of the files. Site staff must complete these certification processes prior to administering this exam for the RECOVER protocol. |
| **Complete neurocognitive exam** | To certify administrators at each site, site staff must complete a practice neurocognitive exam on a non-subject volunteer and provide both a video of the administration and the scored results for review by the CSC Neurocognitive team. An authorization form must first be signed by both the non-subject volunteer test taker and site staff becoming certified acknowledging that the video recording of the administration will be uploaded to NYULH REDCap and only watched by CSC Neurocognitive Team staff members. The videos, authorization forms, and practice score sheets are stored in a secure NYULH REDCap project or a secure folder through NYULH OneDrive, depending on the size of the files. Site staff must |

| | |
|---|---|
| | complete these certification processes prior to administering this exam for the RECOVER protocol. |
| **Brain MRI** | To certify that the standard local radiology procedures at each site are aligned with the RECOVER SOP for Brain MRI, each site must submit the data from the first RECOVER participant who completes this assessment for review to the Brain MRI Reading Center. This data is submitted through a secure file system called InteleShare (previously AMBRA). The Brain MRI Reading Center will then confirm that the scanner at each site is within performance specifications, that the scan sequence parameters are consistent with the RECOVER Brain MRI SOP, and that the site is able to produce and transmit the imaging data to the Brain MRI Reading Center. Sites must complete these certification processes before continuing to administer this exam for the RECOVER protocol. |
| **Cardiac MRI** | To certify that the standard local radiology procedures at each site are aligned with the RECOVER SOP for Cardiac MRI, each site must either submit the data from the first RECOVER participant who completes this assessment or provide a recent (within 3 months) historical de-identified clinical scan for review to the Cardiac MRI Reading Center, provided it was completed by the same technologist and performed on the same scanner which is being certified. This data is submitted through a secure file system called InteleShare (previously AMBRA). The Cardiac MRI Reading Center will then confirm that the scanner at each site is within performance specifications, that the scan sequence parameters are consistent with the RECOVER Cardiac MRI SOP, and that the site is able to produce and transmit the imaging data to the Cardiac MRI Reading Center. Sites must complete these certification processes before continuing to administer this exam for the RECOVER protocol. |
| **CPET** | To certify that the standard local exercise procedures at each site are aligned with the RECOVER SOP for CPET, each site must provide a historical de-identified calibration exam for review to the CPET Reading Center. This calibration exam should follow the site's routine calibration steps and should be performed on the same equipment which is being certified. This data is submitted through a secure file system. The CPET Reading Center will then confirm that the equipment at each site is within performance specifications, that the test parameters are consistent with the RECOVER CPET SOP, and that the site is able to produce and transmit the exam data to the CPET Reading Center. Sites must complete these certification processes prior to administering this exam for the RECOVER protocol. |
| **MINI** | To certify administrators at each site, site staff must complete a training provided by the Harm Research Institute, which includes a standardized patient pre-recorded interview video, and provide the scored results for review by the CSC Neurocognitive team. If the initial scoring is not satisfactory, the site staff will then watch a second pre-recorded standardized patient video (created by the CSC) and provide these score sheets for review by the CSC Neurocognitive Staff to review. The practice score sheets are stored in a secure NYULH REDCap project or a secure folder through NYULH OneDrive, depending on the size of the files. Site staff must complete these certification processes prior to administering this exam for the RECOVER protocol. |
| **Facility sleep study** | To certify that the standard local polysomnography procedures at each site are aligned with the RECOVER SOP for the Facility Sleep Study, each site must complete an Equipment Survey (which includes the make and model |

| | |
|---|---|
| | of both the hardware and software) and submit a deidentified existing study prior to performing this assessment in RECOVER participants. This data is submitted through a secure file system. The Sleep Reading Center will then confirm that the equipment at each site is within performance specifications, that the test parameters are consistent with the RECOVER Facility Sleep Test SOP, and that the site is able to produce and transmit the exam data to the Sleep Reading Center. Sites must complete these certification processes prior to administering this exam for the RECOVER protocol. |

### 9.8 Digital Health

The Digital Health Platform (DHP) and Digital Health Data Repository (DHDR) teams will implement a digital health program to augment data collected by this study and to promote participant engagement and retention. Participation in any component of the digital health program is optional for participants.

The Digital Health Program includes the following components:

- **Mobile application** – A secure smartphone application developed and managed by the DHP team (CareEvolution) will be deployed to participants who elect to use it. This application will enable participants, at their sole discretion, to report a new COVID infection, to share data from their cell phones and/or wearable devices, such as a Fitbit or an iWatch, to share their electronic health records, and/or to track their symptoms. Prior to downloading the application, participants will be provided an IRB-approved digital health information sheet that describes the digital health program, including information about the vendor, terms of use, and how to stop using the app. The purpose of the information sheet is to ensure that the participant is informed on the key points of the program. Once the participant agrees to be part of the DHP, they will receive a link to download the application, either from the study staff (via email or, if they consented to received text messages, text message) or as a prompt in the REDCap survey. From there, the participant will be able to download the app, NOTE: Study participants previously will have signed a consent form that includes a description of data that may be collected by the DHP.
- **Digital Health Data Repository (DHDR).** The DHP team will transfer digital health data to the DHDR team (Sage BioNetworks). The DHDR team is responsible for harmonizing, curating and analyzing digital health data before it is transferred to the RECOVER Data Resource Core (DRC).

### 9.9 Data Management

There will be several tools to collect study data.

- The first is an electronic data capture (EDC) system called REDCap. This secure HIPAA compliant electronic data capture system will enable study coordinators to record participant data.
- The second is a secure app that participants will be able to download to their mobile devices. This mobile app will record information that participants provide and will enable the collection of wearable device data and the collection of electronic health data. The digital health data will be transferred to a platform managed by the DHDR team, where it will be harmonized and curated. Image data are stored in a secure cloud environment managed by a vendor called InteleShare (formerly Ambra). There is a process for de-identifying images that are stored in InteleShare.
- Biospecimen data is managed in a Laboratory Information Management System (LIMS) at the PBC.
- Omics data for each panel (whole genome sequencing, epigenetics, transcriptomics, proteomics, and metabolomics) originates at the respective TOPMed Core Laboratory. Quality control of the raw data will be performed by either the Channing Institute for Systems Biology or the TOPMed Informatics Resource Core (IRC).
- Assays originate at several laboratories, including Associated Regional and University Pathologists (ARUP), NYU Langone Health, La Jolla University, Cutaneous NeuroDiagnostics (CND), Integrated Microbiome Core (IMC), and Mayo Clinic BioPharma Diagnostics. Data from these labs flow into the DRC environment for harmonization with other study data.

The staff at these participating institutions may access some identifiers that have been already collected, such as participant ID, age (or an indication they are over 90 years old), and demographic information that is necessary to perform these assays.

The REDCap, biospecimen, omics, laboratory, and digital data will be transferred to the DRC, where they will be harmonized with additional data types. Study data are then transferred from the DRC to BioData Catalyst (BDC), which is a secure platform managed by NHLBI. From there, they are linked to a private data repository (RECOVER Data Gateway; RDG) maintained by NHLBI BDC. These data may contain participant identifiers (e.g., Participant ID, date of birth, visit and laboratory study dates, 5-digit zip code, or other data that is entered by study staff into free text fields) and are available only to RECOVER Consortium investigators via a controlled access process. Ultimately, these data are de-identified by the BDC Data Management Core (DMC) and made available to the public via controlled access on the NHLBI BDC platform.

All RECOVER tools for capturing and harmonizing data reside in FISMA-moderate compliant cloud environments. Access to this aggregated study database will be limited to HIPAA-certified investigators who have been approved for participation in this study.

If any information is shared with external interested site(s), data use agreements will be established.

### 9.9.1 Data Transfer from Enrolling Sites to DRC

Recruiting sites will not send any PHI to the DRC except date of birth (DOB) and zip code. Each site will collect and retain subject identifiers in HIPAA-compliant databases such as local REDCap. These will be linked to the central REDCap database through a participant ID (RpID). Recruitment sites may enter identifiers in REDCap Central. In order to allow future linkage of study data to other national datasets without transferring PHI to the DRC, each recruiting site may use the commercial application Datavant to generate a deidentified participant key (known as a hash, or token) via a privacy-preserving record linkage (PPRL) methodology embedded in the software. Datavant's de-identification engine performs two functions: (i) removal of PII (personally identifiable information) from a participant's record and (ii) generation of multiple encrypted tokens that can be substituted for RpIDs. These tokens are irreversible and specific for a given study site, which retains the ability to view PII from its own participants. Tokens can be used to link a participant's record in one repository with a record from the same participant in a different repository, ***without ever exposing the PII.*** Over years of implementations, QA testing protocols have shown that Datavant's technology generates tokens in a manner that facilitates linking of participant PHI across disparate data sources while maintaining privacy. The token generation process is summarized graphically in Figure **2**.

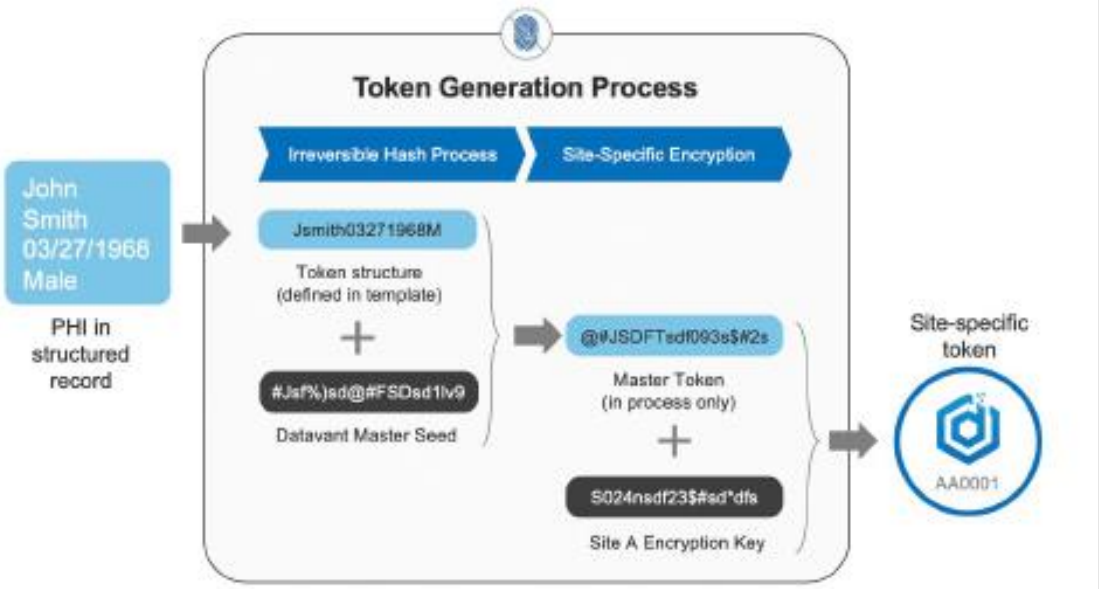



**Figure 2: Token generation process**

Sites will send RECOVER study data (surveys, clinical laboratory results, etc.) to the DRC via REDCap electronic case report forms (eCRFs). REDCap automatically generates a RECOVER Participant ID (RpID), and the only other PII associated with study data are the participant's date of birth and zip code. The eCRFs and PII are submitted to "REDCap Central," a FISMA-Moderate repository located at Harvard Medical School (HMS) and managed by HMS personnel. Summarizing, REDCap Central holds each RECOVER participant's study data and three PII data elements: DOB, ZIP Code and RpID.

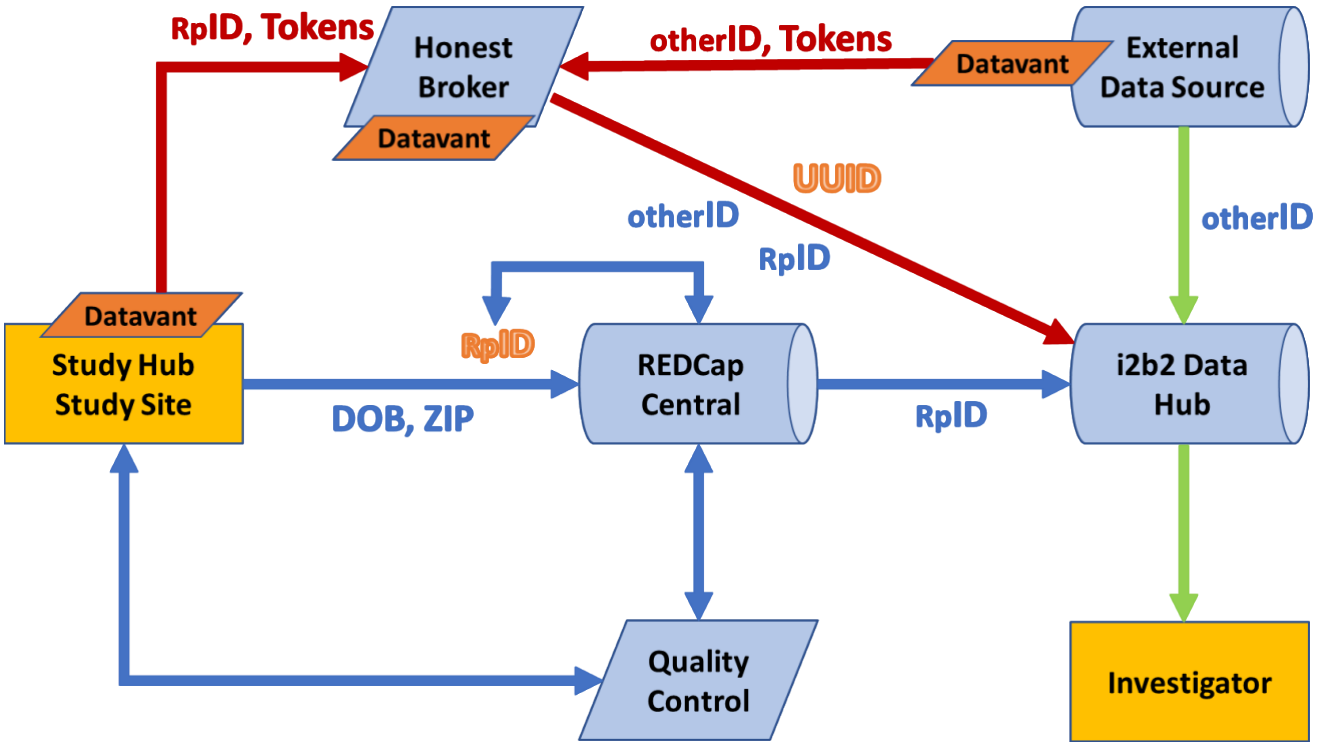



**Figure 3: Data transfer in RECOVER**

### 9.9.2 Data Transfer from PASC Biorepository Core (PBC) to and from DRC

### 9.9.3 Data Transfers

Data transfers within the DRC and with other RECOVER organizations (e.g., PBC, ACC, CSC, enrolling sites) are governed by appropriate agreements, such as Interconnection Security Agreements and Data Use Agreements.

### 9.9.4 FISMA moderate environments

All software that manages PHI in use as part of the study is hosted on secure, FISMA-moderate cloud environments. These environments leverage all the management and security systems, controls, change control methodologies, training documentation, and third-party security testing (e.g. penetration testing) and assessments (e.g. 3PAO reviews) that are required to obtain a FISMA authority to operate (ATO).

- **Mass General Brigham FISMA Azure cloud environment** – Hosts i2b2, which enables data harmonization, REDCap Central, and all statistical analysis tools.
- **Harvard Medical School FISMA AWS cloud environment** – REDCap and statistical analysis tools, such as R, SAS, and SQL Server have been hosted at Harvard Medical School. These tools will transition to the Mass General Brigham FISMA cloud environment in 2023 and at this point the Harvard Medical School FISMA cloud environment will be retired from the RECOVER study. Note: the HMS FISMA AWS cloud environment has not been used since July 2023.
- **Digital Health Platform (DHP) FISMA cloud environment** – Hosts the digital app and the coordinator console.
- **Digital Health Data Repository (DHDR) FISMA cloud environment** – Hosts the digital health data pipelines and the data portal that enables investigators to access digital health data.

### 9.9.5 Data Storage
Data will be stored in a cloud infrastructure. To comply with the government's Cloud Smart policy, all PHI will live within a FISMA Moderate cloud environment that has received an ATO from NHLBI based on review by NHLBI's cybersecurity office.

### 9.9.6 Data Access
Data will be made available to investigators via two mechanisms, both hosted in the BioDataCatalyst (BDC) environment managed by NHLBI. The first of these two environments, RECOVER Data Gateway@BDC, is accessible only to RECOVER Consortium investigators. It may contain identified data (date of birth, 5-digit zip code, dates, and possibly other identified data entered by enrollment sites into free-text entry fields). Access is managed by a Data Access Committee that is managed by NHLBI.

The second of these two environments, RECOVER@BDC, is accessible to people who are not formally affiliated with the RECOVER Consortium, with both controlled access and public access. The public access only provides aggregate counts. Controlled access provides individual-level data that have been de-identified. Access is managed by a separate Data Access Committee that is managed by NHLBI.

### 9.9.7 Data Removal
When participants withdraw consent from the study and ask for their data to be destroyed, their data will be marked as removed for future analyses in REDCap using standard REDCap functionality. The record, including its participant ID, will persist, as will records of ICFs that were signed, and the consent withdrawal record. All other data will be removed from REDCap. It will not be possible for participants to re-enroll once this data is removed.

Per the study protocols, data that are stored in the i2b2 Data Hub that have already been used for research will not be removed at the time of consent withdrawal.

When the study ends, all data will be de-identified in REDCap Central. The data in the i2b2 Data hub will persist in its existing de-identified form.

### 9.9.8 Data Integrity
Detailed Quality Control programs will be deployed to ensure and audit data integrity. The DRC team has extensive experience standing up and maintaining operational i2b2 instances (e.g., the MGB Biobank Portal, operational since 2015), including QC controls.

### 9.9.9 Security Management
Data will be stored in cloud infrastructure. To comply with the government's Cloud Smart policy, all PHI will live within a FISMA Moderate cloud environment that has received an ATO from NHLBI, which includes network firewalls and systems for access control, change control, continuous monitoring, and training. A System Security Plan, which will be reviewed and approved by NHLBI as part of the ATO, describes the cybersecurity and IT management plan in detail.

A secure, cloud-based platform called InteleShare will be used to manage images. InteleShare allows institutions to share de-identified medical images directly, without the need for physical media. All RECOVER personnel interacting with either end of the platform's upload-download workflow will be required to complete dedicated training to ensure every protection of participant information is maintained.

# 10 Safety and Adverse Events

## 10.1 Definitions

**Unanticipated Problems Involving Risk to Subjects or Others**
Any event, incident, experience, outcome, or new information that meets **all** of the following criteria:

- unexpected in nature, severity, or frequency given the information provided in research-related documents and characteristics of the subject population being studied; and
- is related or possibly related to participation in the research; and
- suggests that the research caused harm to subjects or others or places subjects or others at increased risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Please refer to 10.3 for reporting requirements.

**Adverse Event**
An **adverse event** (AE) is defined as any physical and psychological harm occurring to subjects during the course of participating in research, whether or not it is related to participation in the research (**excluding** symptoms, signs and co-morbidities already captured in the PASC symptom, co-morbidity, laboratory results or procedure results forms), or an adverse consequence of a study-related procedure. An AE can be any unfavorable or unintended event that is temporally related to the research.

Known manifestations of acute and post-acute SARS-CoV-2 infection will be recorded as endpoints on the PASC symptom, co-morbidity, laboratory results or procedure results forms in REDCap, rather than as AEs or SAEs, even if occurring in uninfected individuals.

**Serious Adverse Event**
Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal, OR
- life-threatening, OR
- requires or prolongs hospital stay, OR
- results in persistent or significant disability or incapacity, OR
- a congenital anomaly or birth defect, OR
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the participant, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered a serious adverse event.

**Preexisting Condition**
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period and is not captured in existing RECOVER study forms as detailed above.

**Post-study Adverse Event**
At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

### 10.2 Recording of Adverse Events in REDCap

At each contact with the participant, the investigator will seek information on adverse events. Information on all adverse events will be recorded immediately in the adverse event form in REDCap Central, unless the event is captured on another form in REDCap Central. Related signs, symptoms, and abnormal diagnostic procedures results should be recorded as a single event/diagnosis in REDCap Central. Note that while AEs

and SAEs should be recorded in REDCap whether related to the study or not, only related events need prompt IRB reporting (see Section 10.3).

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study participation is not the cause. Thus, only events that are related to study participation need to be followed until resolution or stabilization.

### 10.3 Reporting New Information to the IRB

Federal regulations require timely filing of unanticipated problems posing risks to subjects or others to the local IRB. These events are:

- unexpected, AND
- related or possibly related to study participation, AND
- serious or involve risks to subjects or others

This section also describes the NYULH IRB reporting requirements for other types of events, though investigators at participating sites are responsible for meeting any additional local requirements and/or those of the relevant sIRB. The following categories of events are considered reportable and require a submission to the IRB using the Reportable New Information e-submission form.

1. New or Increased Risk

Information arising from the study that indicates a new or increased risk or safety issue. For example:

- New information (e.g., an interim analysis, safety monitoring report, publication in the literature, sponsor report, or investigator finding) that indicates an increase in the frequency or magnitude of a previously known risk or uncovers a new risk.
- Protocol violation that harmed subjects or others, or that indicates subjects or others might be at increased risk of harm.
- Complaint of a subject that indicates subjects or others might be at increased risk of harm or at risk of a new harm.

2. Unexpected Harm to a Subject or Other Individual

Any harm experienced by a subject or other individual(s) that, in the opinion of the investigator, is unexpected and related or possibly related to the research procedures. Harms can include psychological, economic, legal, and other non-physical harms.

- A harm is “unexpected” when its specificity or severity is inconsistent with risk information previously reviewed and approved by the IRB in terms of nature, severity, frequency, and characteristics of the study population
- A harm is “probably related” to the research procedures if, in the opinion of the investigator, the research procedures more likely than not caused the harm

Examples of harm include:

a. Death of a Research Subject. Investigators are required to report deaths of research participants to the IRB if the death was unanticipated and related or probably related to participation in the study.

b. Adverse Events. Only Unanticipated Adverse Events that are related to the research need to be reported to the IRB. As described above, RNI includes events that may increase risks or cause harm.

3. Non-Compliance

Non-compliance with federal regulations governing human research, NYU Langone Health’s HRP policies, or with IRB requirements or determinations, or allegations of such non-compliance.

4. Audits
External audits, inspections, or inquiries by a federal agency and any resulting reports (e.g., FDA Form 483).

5. Reports
Written reports of study monitors, reports to/from a study sponsor or other information that indicates a change to the risks or potential benefits of the research.

6. Researcher Error
Failure to follow the protocol due to the action or inaction of the investigator or research staff.

7. Breach of Confidentiality
Breach of subject or patient confidentiality, data breach, or data incident. Any unauthorized disclosure of subject's personally identifiable information.

8. Unreviewed Change
Any change in the IRB-approved study protocol that was taken without prior IRB review to eliminate immediate hazard to subjects must be reported. This would include protocol violations and deviations.

A protocol violation refers to an accidental or unintentional change to the IRB-approved protocol that harmed subjects or others, or that indicates subjects or others may be at increased risk of harm. Examples: subject received the wrong dose of study medication.

9. Incarceration
Investigators must report to the IRB when a subject who is enrolled in a study that is not IRB-approved to involve prisoners becomes incarcerated and the study team plans to continue study activities with prisoners while incarcerated.

10. Complaint
Complaints made by a subject that are related to the study and either indicate increased risk and/or that cannot be resolved by the research team must be reported.

11. Suspension or Termination
Principal Investigators must report premature suspension or termination of the research by the sponsor, investigator, or institution.

**When to Report Events**

**Report promptly, but no later than 5 calendar days** from the time the investigator becomes aware of the event:

- **Unanticipated Problems Involving Risk to Subjects or Others** (see Section 10.1 for definition), if the event requires immediate intervention to prevent serious harm to subjects or others, or the subject suffered serious harm.
- **Death of a Participant**, if it is unexpected and related to a study procedure

**For all other reportable events listed above, report promptly, but no later than 10 calendar days** from the time the investigator becomes aware of the event:

The IRB will accept other reports when the Principal Investigator is unsure whether the event should be reported. The Principal Investigator should first contact IRB Operations by email or telephone to determine if the reporting is necessary under this Policy.

Events that do not meet the above criteria for immediate reporting should be summarized and reported to the IRB at the time of continuing review.

**Reporting Process**
The reportable events noted above will be reported to the IRB using a Reportable New Information submission and will include a description of the event with information regarding reporting category above, follow-up/resolution, and need for revision to consent form and/or other study documentation. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

### 10.4 Participant Mental Status

Survey questions may ask about the mental status of participants, including suicidal ideation. The CSC and site staff will receive an alert when a response suggesting possible thoughts of suicidality is submitted. Upon receipt, CSC staff will communicate with site study staff to confirm accuracy of the response and verify appropriate measures are taken to ensure participant safety.

## 11 Study Oversight

### 11.1 Monitoring Board

Oversight of data and safety is provided by a RECOVER Observational Safety Monitoring Board (OSMB) appointed by the National Heart, Lung and Blood Institute (NHLBI). A charter of the OSMB will be submitted for IRB review before starting enrollment. The OSMB will meet at least twice a year to review data on AEs, unanticipated events, patient-reported outcomes, data quality, and study recruitment as described in the committee charter, and make recommendations about study conduct to the NHLBI. As the Adult PASC Investigator Consortium study does not involve any interventions, an early stopping rule for efficacy or futility is not indicated.

### 11.2 Data Safety Monitoring Plan

The Data and Safety Monitoring Plan for this trial will follow recommended monitoring principles for an observational study of a vulnerable population. Oversight of data and safety is provided by the RECOVER Observational Safety Monitoring Board appointed by NHLBI. The OSMB will be composed of experts in longitudinal research (adult and pediatric populations), clinical experts in adult and pediatric manifestations of COVID-19, biostatistics, bioethics, and patient/caregiver representatives. The OSMB will also appoint ad hoc members with subspecialty expertise in the diverse array of clinical manifestations of PASC. The OSMB will meet at least twice a year to review data on AEs, adverse reactions, suspected adverse reactions, unanticipated events, patient-reported outcomes, data quality, and study recruitment, and make recommendations about study conduct to the NHLBI. As the PASC study does not involve any interventions, an early stopping rule for efficacy or futility is not indicated.

After each OSMB meeting, the OSMB determination letter and a summary report of adverse events will be prepared within 30 days and will be distributed by NHLBI staff to each principal investigator and study coordinator for review. The summary report will contain the following information:

- A statement that an OSMB review of outcome data, adverse events, and information relating to study performance across all centers took place on a given date.
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent.
- A statement that a review of recent literature relevant to the research took place.
- The OSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the OSMB recommends changes to the protocol or informed consent, the rationale for such changes and any relevant data will be provided.

- A statement that if safety concerns are identified, the NHLBI Program Official will communicate these promptly to the investigators.

# 12 Statistical Considerations

The analysis will integrate retrospective and prospective data on individuals at multiple stages, including pre-infection, acute infection, and long-term progression and recovery. Characterizing the incidence and prevalence of PASC will be achieved by estimating the incidence of PASC phenotypes among subjects with COVID-19 infection, compared with uninfected individuals followed over the same time.

To characterize the clinical course, recovery, and risk factors of PASC, PASC positive individuals will be compared with infected PASC negative individuals and uninfected individuals to characterize patterns. To define the pathophysiology and mechanisms of PASC, we will estimate the direct and indirect effects of COVID-19 infection and its severity on the development of PASC. Longitudinal data models such as generalized estimating equations, generalized linear mixed models, and functional principal component analysis will be used to characterize patterns of outcomes and develop analysis models.

## 12.1 Hypotheses to be Tested

The following hypotheses will be addressed in the analytic approach to Aim 1:

- Hypothesis 1a: Clinically meaningful PASC cases and sub-phenotypes will be discernable based on a combination of candidate PASC symptoms, and potentially other clinical features.
- Hypothesis 1b: Cumulative incidence of long-term sequelae differs between SARS-CoV-2 infected and uninfected individuals, overall and stratified by hospitalization.
- Hypothesis 1c: Incidence rate of long-term sequelae differs between SARS-CoV-2 infected and uninfected individuals, overall and stratified by hospitalization
- Hypothesis 1d: The point prevalence of long-term sequelae differs between SARS-CoV-2 infected and uninfected individuals, overall and stratified by hospitalization.
- Hypothesis 1e: The severity of long-term sequelae differs between SARS-CoV-2 infected and uninfected individuals, overall and stratified by hospitalization.

The following hypotheses will be addressed in the analytic approach to Aim 2:

- Hypothesis 2a: The risk of PASC among infected individuals will vary by: demographic and clinical characteristics, vaccine status at the time of infection, severity of disease (including hospitalization and admittance to ICU), drug exposures, pregnancy during acute infection and calendar time.
- Hypothesis 2b: The rate of recovery from PASC among individuals with PASC will vary by: (i) demographic and clinical characteristics and vaccine status at the time of infection, and (ii) severity of disease (including hospitalization and admittance to ICU), drug exposures, and pregnancy during acute infection.

The following hypotheses will be addressed in the analytic approach to Aim 3:

- Hypothesis 3a: PASC and PASC sub-phenotypes will be associated with concurrent and subsequent abnormal laboratory results and other clinical and subclinical features.
- Hypothesis 3b: The association between PASC and PASC sub-phenotypes with concurrent and subsequent abnormal laboratory results and other clinical and sub-clinical features will be modified by demographic factors, including sex, age and race/ethnicity.
- Hypothesis 3c: Recovery from PASC will be associated with laboratory results and other clinical and subclinical features.
- Hypothesis 3d: The association between SARS-CoV-2 infection and PASC and PASC sub-phenotypes will be mediated by abnormal laboratory results and other clinical and sub-clinical features.

## 12.2 Sample Size Determination

The following factors were considered in determining the sample size

- Expected frequency of PASC in the SARS-CoV-2 infected population. Population estimates from studies with selection bias currently range from 25-70%; we used 25% as a conservative estimate.
- The desire for the cohort distribution to include a diverse population, with overrepresentation from non-Hispanic Black, Hispanic and Asian populations relative to general population prevalence to account for a higher rate of SARS-CoV-2 infection and PASC in these populations
- The desire to be able to perform subgroup analyses based on characteristics such as age, sex, race/ethnicity, pregnancy, vaccination status and combinations thereof.

**Table 7** shows minimal detectable effect sizes for the key study questions both for the full study sample (Tier 1 N=14,880; Tier 2 N=4,464 and Tier 3 N=2,976) and for subgroups of 25% thereof. These sample sizes for Tier 2 and Tier 3 assume that 25% of infected subjects will have abnormal symptoms. It is further assumed that all infected subjects with abnormal symptoms will progress to Tier 2 (N=3,050) and 5.6% of infected subjects without symptoms will progress to Tier 2 (N=512). Among uninfected subjects, 26.8% (N=718) will progress to Tier 2. 68% of infected subjects with abnormal symptoms will progress to Tier 3, and 3.8% of infected subjects without symptoms will progress to Tier 3. Among uninfected subjects, 18.2% will progress to Tier 3. The infected cohort is expected to include 1,867 pregnant individuals and the uninfected cohort is expected to include 583 pregnant individuals.

**Table 7**: Sample size calculations

| **Tier** | **Comparison groups** | | **Effect of interest** | **Assumptions** | **Min detectable ES*** | |
|---|---|---|---|---|---|---|
| | | | | | Full sample | 25% subgroup (e.g. inpatients, Hispanic individuals, etc.) |
| 1 | Infected | Uninfected | Difference in risk of PASC between infected and uninfected | Risk of PASC in infected: 25% | 3.50% | 6.80% |
| 1 | Infected w/ RF | Infected w/out RF | Risk difference for PASC in infected with RF vs. without RF | Prevalence of RF: 20%, risk of PASC in infected, RF+: 30% | 3.90% | 7.60% |
| 1 | Pregnant infected | Non-pregnant infected | Risk difference for PASC in pregnant infected versus non-pregnant infected | Risk of PASC in non-pregnant infected: 25% | 4.10% | 8.00% |
| 1 | PASC+ w/ RF | PASC+ w/out RF | Difference in proportion who recover from PASC for those with and without a risk factor | Prevalence of RF: 20%, probability of recovery in PASC+ w/out RF: 0.50 | 8.70% | 17.20% |
| 2 | PASC+ | | Precision of rate of feature | Rate of 50% (conservative) | ± 2.3% | ± 4.7% |
| 2 | Infected | Uninfected | Difference in proportion with a features in between infected | Rate of 50% with feature in infected | 7.90% | 15.60% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | and uninfected individuals | | | |
| 2 | PASC+ | PASC- | Difference in proportion with a feature, PASC+ vs. PASC-, infected only | Rate of 50% with feature in PASC+ (conservative) | 10.00% | 19.70% |
| 3 | PASC+ | | Precision of rate of feature | Rate of 50% (conservative) | ± 2.8% | ± 5.7% |
| 3 | Infected | Uninfected | Difference in proportion with a feature, infected vs. uninfected | Rate of 50% in PASC+ (conservative) | 9.50% | 18.80% |
| 3 | PASC+ | PASC- | Difference in proportion with a feature, PASC+ vs. PASC- | Rate of 50% in PASC+ (conservative) | 12.10% | 23.40% |

Only infected participants are included in analyses that consider PASC+ alone, or compare PASC+ with PASC-.
*ES: effect size; RF: risk factor

## 12.3 Statistical Methods

### 12.3.1 Methods of Data Collection

Structured data elements will be collected remotely through a mobile or web-based platform, by telephone with study personnel, by home visit by study personnel, or (if no other option) by return of written questionnaire by postal mail.

Biospecimen collection will be handled following the RECOVER Adult Standard Collection Workflow Manual of Operating Procedures or RECOVER Adult Local Collection Workflow Manual of Operating Procedures and associated reference materials. Either central or local processing is acceptable if procedures are followed.

Off-protocol clinically obtained samples including cerebrospinal fluid, bronchoalveolar lavage specimens, procedural biopsies, and surgical pathology specimens will be tracked and either transferred from study site biorepository to the central RECOVER biorepository or linked by the patient ID to the institutional RECOVER biorepository for future access.

## 12.4 Strategies for Study Modifications

This protocol is designed to be pragmatic and flexible in design. We will undertake the following procedures to guide protocol modifications over time:

1) The frequency of PASC will be monitored in real-time during the study. If the incidence or prevalence is found to be higher or lower than planned, recruitment strategies will be altered to deliberately undersample/oversample PASC cases.
2) Subject response burden will be monitored in real-time during the study. If burden is found to be excessive, it will be reduced by altering the data collection strategy, such as by increasing the assessment interval; reducing the number of data elements collected; increasing the availability of home-based assessments; and/or increasing subject reimbursement.
3) Free text responses to interval assessments will be monitored in real-time during the study. If a new symptom or outcome is being reported at a frequency >15% by subjects, the symptom will be added to the data collection tool.
4) Data elements may be modified based on ongoing analysis by DRC; data elements that are not informative to PASC definition models may be removed, with substitution by new data elements.

5) PASC definition will be revised in an iterative manner based on existing PASC data, medical literature, and feedback from patient representatives, subjects, and the scientific community. Updated PASC definitions may be used to implement a strategy to modify deeper phenotyping.
6) Tier 2 and Tier 3 assessments will be evaluated for futility at pre-specified intervals; protocol assessments will be adjusted accordingly and may include elimination of some assessments and introduction of other new assessments.

## 12.5 Overview of Analytic Approach to Aims

The complete analytic approach to aims is detailed in the statistical analysis plan, included as a separate attachment.

## 12.6 Data Management Plan

### 12.6.1 Data Sources

There are three primary sources of data for the RECOVER cohort studies initiative:

- Prospective observational cohort studies
  - Adult cohort sites
  - Pediatric cohort sites
  - Pregnancy cohort sites
  - Autopsy cohort sites
- RECOVER biorepository core
- Data repositories
  - Imaging
  - Pathology
  - Other

### 12.6.2 Data Categories

RECOVER study data may be divided into two broad categories: structured and unstructured. Structured data can be simple (e.g., surveys/lab tests) or complex (e.g., sleep studies). REDCap will be used to capture structured data electronically. REDCap will include PHI data, which will be used to create the universally unique identifiers (UUIDs), that will enable centralized coordination of biospecimen collection information and other data types.

#### 12.6.2.1 Data Types

RECOVER study data may be divided into at least 12 different operational data types.

1. Patient questionnaires (in-person or submitted online; Structured)
2. Clinical site historical data (extant data; Structured->Complex)
3. Clinical site historical data (extant data; Unstructured)
4. EHR repository data (Structured->Complex)
5. Lab test results (Structured->Complex)
   i. Hospital
   ii. Commercial
   iii. Home
6. RECOVER biorepository inventory data (Biospecimens, slides; Structured->Complex)
7. Neuropsychological assessment data (Complex/Structured)
8. Functional assessment data (e.g. exercise testing, pulmonary/liver/kidney function; Structured->Complex)
9. Digital health/wearable devices and computer software (i.e. fitness trackers, sleep monitors, Zio patch)
10. Advanced imaging data (CT and MRI; Unstructured)

11. Vaccination status data (Structured)
12. Physical exam and physiological testing data (e.g. PFTs, tilt table; Structured->Complex)

### 12.6.3 Electronic Data Capture (EDC) Methods

A uniform electronic data capture system will be used. Details will be found in the manual of operations.

### 12.6.4 Source Documents and Access to Source Data/Documents

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the study.

The study electronic case report form (eCRF) is the primary data collection instrument for the study. However, in some cases paper CRFs may be completed by subjects who cannot access surveys online or prefer paper. Data from these CRFs will be transferred into the eCRF by study staff. All data requested on the eCRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, insert "N/D." If the item is not applicable to the individual case, insert "N/A." Records will be retained of the date and time of any changes to data entered after initial completion.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

## 13 Ethics/Protections of Human Subjects

### 13.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46.

### 13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB-approved; a determination will be made regarding whether previously consented subjects need to be re-consented.

### 13.3 Informed Consent Process

When the enrollment visit is completed in person, informed consent will be obtained and documented in writing before participation in study procedures. When the enrollment visit is completed remotely, electronic

consent will be used. Study sites will identify potential subjects in their available recruitment pools (extant cohorts, clinical cohorts, acute cohorts, and/or post-acute cohorts). Once potential subjects are identified through standard processes by the study team, the research study will be explained in lay terms to each potential research subject in their preferred language, either one-on-one or in a group session. If through a group information session, no patient identifiers will be collected during the session. In addition, if using a group session, potential subjects must have an opportunity to meet individually with study staff after the group session to ask questions and provide written consent in a private setting. The option to use group information sessions will help with (1) meeting recruitment goals in a timely manner; (2) overcoming the challenges of patients not showing up for screening appointments; and (3) scheduling more than one subject visit per time slot.

The overall common consent document(s) will include:

1) consent for participation in all minimal risk RECOVER Tier 1, 2 and 3 activities;
2) consent for sharing identifiable data with the secure REDCap Central database;
3) consent to obtain and link data from electronic health records, regional health information exchanges, claims data and the National Death Index;
4) consent for sharing of deidentified data and specimens through RECOVER databases and specimen repositories (in addition to other NIH-designated repositories).
5) optional collection of biospecimens
6) If biospecimens are collected, additional optional collection of genetic sample for testing and optional consent for return of genetic information

Separate consent will be obtained by the pediatric study team from a person who was pregnant while infected with COVID-19 for the infant to participate in the pediatric study, if applicable.

The potential subject will provide informed consent before undergoing any study procedures. The consent process may be conducted by telephone, video conference (e.g., Webex), or in person. The investigator or suitable designee will conduct a meeting with the study candidate all the required elements of informed consent and to address all questions about the study. Comprehension of the study procedures and risks will be confirmed with standardized questions to the subject. Subjects will be provided information on how to contact an appropriate individual for pertinent questions about the research and their rights and whom to contact in the event that they sustain a research-related injury.

Documentation of consent will be recorded electronically via REDCap or an equivalent system. Subjects will be sent the link to the consent form via encrypted email, and subjects will be given the phone number of a study team member to call after they have reviewed the consent. The study team member will then explain the consent to the subject and ask if the subject has any questions. The subject will then electronically sign the informed consent document. Study personnel will verify identification before sanctioning an individual's electronic signature. An electronic or printed signed copy will be provided to the subject and a copy of the subject's consent to participate will be kept on a password-protected and secure drive at each study site.

Before participating in any activities that are more than minimal risk, subjects will be asked to sign a procedure-specific information sheet. The information sheet will provide a description of the procedure, attendant risks, and potential benefits. Research staff will review the information sheets with participants to provide full information about risks and benefits and will ensure that all of the participants' questions have been answered prior to signing. Separate procedural clinical consent may be obtained by the local clinical team at the relevant time for any activities that are more than minimal risk, as per local institutional policy. Research consent via information sheet must occur prior to procedural clinical consent and clinical consent can never be used instead of research consent.

Every site's eConsent link will be sent to the IRB for review before use in the study. Language consistency with the IRB-approved consent must be reviewed and approved by the IRB before eConsent is initiated.

If a subject is unable to provide an electronic signature during a remote visit, he or she will be required to sign a paper copy of the informed consent in the presence of a witness. The signature and date of the witness will also be required on the paper copy. A separate record of the required elements of the ICF process will be documented in the subject's study record.

### 13.3.1 Consent and Other Informational Documents Provided to Subjects

Consent forms and information sheets describing in detail the study intervention, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention. The procedure-specific information sheets for more than minimal risk testing will provide a description of the procedure and its attendant risks. The following consent materials are currently approved for use:

- Main informed consent form adult subjects, covering all Tier 1 and all minimal risk Tier 2 and Tier 3 procedures
- Procedure-specific information sheets for more than minimal risk procedures
- Procedure-specific restrictions and instructions are communicated via the relevant appointment reminder template or pre- and post- procedure instructions after participant consents to testing.

Materials such as videos, slide presentations and scripts may be used to aid in the informed consent process. All materials will be submitted to the IRB for approval prior to their use.

### 13.3.2 Posting to ClinicalTrials.gov

The proposed study is posted on clinicaltrials.gov.

## 13.4 Subject and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Investigators in this research will take all reasonable measures to protect the confidentiality of the medical records of patients and their families. Measures to protect confidentiality are as follows:

### 13.4.1 Storage of Study Materials

Investigators will take all reasonable measures to protect the confidentiality of the study subjects through the measures used in all PASC studies, including storage of study materials in locked, secure locations accessible only to study investigators, knowledge of the subject's name only at the local institution, use of a UUID with no personal identifiers in the study database, and use of secure password protected computer access and encrypted transmission of patient information.

### 13.4.2 Hashed Identifiers

A unique subject hashed identifier, called a universal unique identifier (UUID), will be assigned to each study participant. The hashed identifier is a universal subject ID that allows researchers to share data specific to a study participant without exposing personally identifiable information (PII) and at the same time

be able to match participants across labs, databases, or research studies, while preventing multiple different identifiers for the same subject.

### 13.4.3 Reporting of Genetic Testing

Clinically actionable results of whole genome sequencing performed during this study in a CLIA certified lab may be returned to the subject, if the subject has indicated on the consent form that s/he would like results returned. Results of whole genome sequencing or other omics assays performed in research labs will not be returned to participants. Clinically actionable means findings or results that would prompt clinical action by the subject's medical provider because there is an established medical/therapeutic intervention, preventative approach, or other actions (e.g., changes in medication) available that could have the potential to change the clinical course of the subject's disease or provide important pharmacogenetic information that is likely to impact future care. Clinical genetic testing targeting known disease-associated variants will not be performed. There is a reasonable possibility that no findings will result from this research effort. If this research does result in incidental findings, local laws dictate if and how these results may be provided to the participants as they are not a result of direct clinical testing and should not be used as a clinically actionable result. If any findings are detected and determined to be legally appropriate to return to the participant, the enrolling site will submit a return of results letter to the IRB for review at that time. Additionally, if findings are detected, it may be years before any utility of these findings is realized. Further, if samples are "anonymized" prior to release to other investigators for future research, it may not be possible to trace the results back to the subject.

#### 13.4.3.1 Reporting of Clinically Actionable Findings

Tests performed by certified clinical laboratories that are analytically valid and either clinically significant or medically actionable will be recorded in the participant medical record and will be reviewed by the Principal Investigator or other designated licensed medical professional at each site. If the Principal Investigator or licensed designee determine that the result is clinically significant or medically actionable, the participant will be contacted by telephone or in-person to explain the test findings within one week of the return of the test results. The participant will also be advised to follow up with their primary care physician. The results of incidental findings will be shared with subjects consistent with state and local regulation. Any additional testing ordered by the primary care physician will be paid by the participant or their insurance company.

### 13.4.4 Certificate of Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

To help us protect the privacy of subjects participating in the RECOVER cohort study, a Certificate of Confidentiality is issued by the National Institutes of Health (NIH). With this Certificate, the researchers of this study cannot be forced to disclose information that may identify a subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceeding. The Certificate

cannot be used to resist a request for information from the United States government when it is used for evaluating federally funded study projects or for information that must be disclosed to meet the requirements of the Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent a subject or his/her family from voluntarily releasing information about the subject's involvement in this research. If an insurer, employer, or other person obtains a subject's or family's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

This study is a genome-wide association study and will comply with the NIH Policy for Sharing of Data Obtained in NIH Supported or Conducted GWAS, which calls for investigators funded by the NIH for GWAS to 1) share de-identified genetic (genotypic and phenotypic) data through a centralized NIH data repository; and 2) submit documentation that describes how the institutions have considered the interests of the research subjects, such as privacy and confidentiality. Submission of data to the NIH GWAS repository will be consistent with the permissions and limitations delineated on the study consent signed by study subjects. Information from DNA analyses and clinical studies or medical records may be placed into a central data repository in the future, such as the National Center for Biotechnology Information repository. Data and samples will be de-identified before submission to this or any other central repository. Note that "true" genetic testing in a certified laboratory for the purpose of diagnosing subjects' predisposition to conditions they don't currently know they have will not be performed.

### 13.5 Research Use of Stored Human Samples, Specimens or Data

- Intended Use: Samples and data collected under this protocol may be used to study mechanisms and clinical manifestations of SARS-CoV-2 infection. It is anticipated that DNA testing will be performed in the future.
- Storage: Access to stored samples will be limited with policies and procedures requiring multiple reviews prior to release of any samples for analysis. Samples and data will be stored using UUID codes assigned by the investigators until the aliquots are used up. Only investigators will have access to the samples and data.
- Tracking: Data will be tracked using i2b2 at the PASC Investigator Consortium Data Resource Core. Each specimen will be labeled and tracked with a UUID.
- Disposition at the completion of the study: All stored samples will be sent to the RECOVER biorepository at Mayo Clinic. Study subjects who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

### 13.6 Future Use of Stored Specimens

Data collected for this study will be stored at the RECOVER biorepository at Mayo Clinic. After the study is completed, de-identified, archived data will remain at the biorepository, under the supervision of PI Dr. Mine Cicek, for use by other researchers including those outside of the study as determined by the policies and procedures of the RECOVER ancillary studies committee. The specimens will be retained indefinitely or until used up by future analyses. Permission to transmit data to the RECOVER Mayo Clinic biorepository will be included in the informed consent as an individual opt in component of the overall consent. Specific consent opt in will also be included for permission to conduct future genetic testing. Participant responses to these parts of the consent will be individually tracked in REDCap. Subjects who do not provide consent for biorepository or for genetic analyses will not have samples sent to the biorepository. Only individuals authorized by Mayo will have access to the samples. Samples will be identified by code and only the Data Resource Core will have access to the linking key between subject ID and subject identity.

With the subject's approval and as approved by the NYU sIRB and consortium central IRBs, de-identified biological samples will be stored at the RECOVER biorepository at Mayo Clinic. These samples could be used for research into the causes of long-term sequelae of SARS-CoV-2 infection, its complications and other conditions for which individuals with co-morbid conditions may be at increased risk, and to improve treatment. The RECOVER biorepository at Mayo may also be provided with a UUID that will allow linking the biological specimens, following study completion, with the phenotypic data from each subject, maintaining the masking of the identity of the subject.

During the conduct of the study, an individual subject can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed as samples will be fully anonymized and cannot be traced back to the subject.

When the study is completed, access to study data and/or samples will be provided through the RECOVER biorepository at Mayo Clinic as determined by the policies and procedures of the RECOVER investigator consortium ancillary studies committee and NIH Sponsor.

# 14 Data Handling and Record Keeping

## 14.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the study staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the subject's official electronic study record.

Clinical data and clinical laboratory data will be entered into the REDCap Central database, and then a HIPAA defined coded limited data set with UUID linkage and stored in the i2b2 data system (i2b2 Data Hub) provided by the RECOVER Data Resource Core (DRC). A central instance of REDCap will be used for capture of structured data. The data capture system meets Federal data security requirements and includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. At the end of the study, all identifiers will be removed from the central REDCap database. Identifiers may remain in the local site research database if the participant has provided consent for contact for future research.

## 14.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close out or 5 years after final reporting/publication. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

## 14.3 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol or MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations as soon as possible, but in all cases within 10 working days of identification of the protocol deviation. Certain protocol

deviations can be determined by periodic reports developed by the DRC so do not have to be reported separately (e.g. missed follow-up visits or missed procedures). All deviations associated with change in risk to subjects or compromise of scientific integrity of the study must be addressed in study source documents, reported through REDCap, and then reported by the Clinical Science Core to the RECOVER program scientific directors at NIH, and where relevant to the RECOVER DRC. Protocol deviations that impact risk or scientific integrity will be reported to the OSMB at 6-month intervals. Protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

### 14.4 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

## 15 Study Finances

### 15.1 Funding Source

This study is financed through a grant from the Other Transactional Authority (OTA) of the US Federal Government. The study is overseen by the National Institutes of Health, National Heart Lung and Blood Institute (NHLBI).

### 15.2 Costs to the Subject

There are no costs to the subject related to participation in the study. The OTA grant will pay for all study related procedures and costs.

### 15.3 Subject Reimbursements or Payments

Sites will offer patients a nominal reimbursement for participation in the remote interval assessments (amounts to be determined by each enrolling site) and more substantial reimbursement for participation in each more invasive or time-consuming Tier 2 and Tier 3 tests (amounts to be determined by each enrolling site). The payment amount criteria will be determined locally by the sites based on a combination of the complexity of the study; inconvenience to the participant; discomfort associated with the assessment; degree of risk, time, effort, and commitment needed from participants; engagement of vulnerable populations; and geographical location of site. The allowable ranges of subject reimbursements are listed below in Table 8.

**Table 8 Maximum amount of subject reimbursement by assessment or visit**

| **Visits/Assessment** | Minimum | Maximum |
|---|---|---|
| Baseline Visit | $30.00 | $200.00 |
| Tier 1 Office Visits | $20.00 | $125.00 |
| Follow-Up Questionnaires | $10.00 | $50.00 |
| Biospecimen Collection | $25.00 | $50.00 |

| | | |
|---|---|---|
| Travel reimbursement | N/A | As per receipts received |
| Tier 2 laboratory tests (Hepatitis B, Hepatitis C, ACTH, Cortisol) | $20.00 | $150 |
| Oral glucose tolerance test | $25.00 | $100 |
| 6-minute walk | $10.00 | $100 |
| Rehabilitation exam | $25.00 | $100 |
| Pulmonary Function Tests (PFTs) | $25.00 | $150 |
| Vision screen (Snellen chart) | $25.00 | $100 |
| Fibroscan | $25.00 | $200 |
| Flexible laryngoscopy with stroboscopy | $25.00 | $150 |
| UPSIT smell test | $10.00 | $100 |
| Neuropathy exam | $10.00 | $100 |
| Electrocardiogram | $20.00 | $100 |
| MINI | $25.00 | $100 |
| Echocardiogram | $20.00 | $200 |
| NIH Toolbox | $25.00 | $100 |
| Chest CT | $25.00 | $200 |
| Home Polysomnography | $25.00 | $125 |
| Audiometry | $25.00 | $100 |
| EndoPAT | $25.00 | $100 |
| Tier 3 laboratory tests (Vitamin B12, Methylmalonic acid) | $20.00 | $150 |
| Nerve conduction study (NCS) | $40.00 | $500 |
| Electromyography (EMG) | $40.00 | $500 |
| Complete Neurocognitive Testing | $50.00 | $100 |
| Skin biopsy | $25.00 | $500 |
| Muscle biopsy | $40.00 | $700 |

| | | |
|---|---|---|
| Lumbar puncture | $40.00 | $700 |
| Facility based sleep study | $50.00 | $500 |
| Tilt table test | $40.00 | $250 |
| Cardiovagal Innervation | $40.00 | $300 |
| Cardiopulmonary Exercise Test | $50.00 | $350 |
| Gastric emptying study | $50.00 | $350 |
| Brain MRI, with and without gadolinium contrast | $30.00 | $350 |
| Cardiac MRI, with and without gadolinium contrast | $30.00 | $350 |
| Bronchoscopy | $80.00 | $700 |
| Right heart catheterization | $100.00 | $700 |
| Invasive Cardiopulmonary Exercise Test | $100.00 | $700 |
| Colonoscopy | $80.00 | $700 |
| Upper endoscopy | $50.00 | $700 |

# 16 Study Administration

## 16.1 Study Leadership

The scientific leadership for the study and oversight of sites participating in the study is provided by the RECOVER Clinical Science Core (CSC) at the NYU Grossman School of Medicine. The RECOVER CSC collaborates with the RECOVER DRC for data management and data storage at the RECOVER biorepository at Mayo Clinic for biospecimen storage. The activity of the RECOVER Cores is overseen by a Steering Committee composed of the Core PIs, NIH Scientific Program leads, and Chairs of RECOVER Consortium study committees, an Executive Committee composed of NIH Institute leadership and Centers for Disease Control leadership, and an OSBM composed of experts in longitudinal observation studies, epidemiology, bioethics, and biostatistics. The Steering Committee, Executive Committee and OSMB will meet at a minimum of twice yearly.

# 17 Conflict of Interest Policy

All recipient institutions and investigators in the PASC consortium will comply with the requirements of 42 CFR 50, Subpart F, "Responsibility of Applicants for Promoting Objectivity in Research for which PHS Funding is Sought" (FCOI Regulation), as implemented in the 2011 Final Rule for grants and cooperative agreements.

The requirements promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct, or reporting of research funded under PHS grants or cooperative agreements will be free from bias resulting from any conflicting financial interest of an investigator. An

"investigator" is someone defined as the PD/PI and any other person, regardless of title or position who is responsible for the design, conduct, or reporting of research funded by PHS, or proposed for such funding which may include, for example, collaborators or consultants.

Each Institution shall maintain an up-to-date, written, enforced policy on financial conflicts of interest that complies with the regulation and make the policy available via a publicly accessible Web site.

These FCOI requirements do not apply to Federal employees or Federal agencies. Federal agencies have their own set of rules governing financial conflicts of interest for employees.

When submitting a grant application, the signature of the Authorized Organization Representative (AOR) will certify each PASC Consortium applicant institution's compliance with the requirements of 42 CFR 50, Subpart F, including that:

- There is in effect at the Institution an up-to-date, written and enforced administrative process to identify and manage Financial Conflicts of Interest (FCOI) with respect to all research projects for which NIH funding is sought or received;
- The Institution shall promote and enforce Investigator compliance with the regulation's requirements including those pertaining to disclosure of Significant Financial Interests;
- The Institution shall identify and manage FCOIs and provide initial and ongoing FCOI reports to the NIH consistent with this subpart;
- When requested, the Institution will promptly make information available to the NIH/HHS relating to any Investigator disclosure of financial interests and the Institution's review of, and response to, such disclosure, whether or not the disclosure resulted in the Institution's determination of an FCOI;
- The Institution shall fully comply with the requirements of the regulation.

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# 19 Appendices

## 19.1 Appendix 1: Schedule of assessments

| | Time After Index Date | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| eCRF | Baseline | 3m | 6m | 9m | 12m | 15m | 18m | 21m | 24m | 27m | 30m | 33m | 36m | 39m | 42m | 45m | 48m |
| Enrollment | A | | | | | | | | | | | | | | | | |
| Tier 1-2 Consent | A | | | | | | | | | | | | | | | | |
| Identity | A | | | | | | | | | | | | | | | | |
| Visit | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| Comorbidities | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| COVID Treatment* | B | | | | | | | | | | | | | | | | |
| Medications | B | | | | | | | | | | | | | | | | |
| Change in Medications | | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| Demographics | C | | | | | | | | | | | | | | | | |
| PASC Symptoms | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |
| Vaccine | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |
| SDoH | C | | | | | | | | | | | | | | | | |
| SDoH Follow-Up | | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |
| Alcohol/Tobacco | C | | | | | | | | | | | | | | | | |
| Alcohol/Tobacco Follow-Up | | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |
| Disability | C | | | | | | | | | | | | | | | | |
| Pregnancy | C | | | | | | | | | | | | | | | | |
| Pregnancy Follow-Up | | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |
| Tier 1 Office Visit | A | | A | | A | | | | A | | | | A | | | | A |
| Biospecimens | A | A | A | | A | | | | A | | | | A | | | | A |
| Lab Results | A | A | A | | A | | | | A | | | | A | | | | A |
| Tier 2/Tier 3 Tests | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |

**Legend**

**A** Completed by research coordinator
**B** Completed by research coordinator with review/validation by participant
**C** Completed by participant
* COVID Treatment not collected on uninfected controls.
The 39-48m schedule will be repeated for subjects who are more than 48 months after infection while the study is still ongoing.

## 19.2 Appendix 2: Tier 1 topics, tests and procedures (see CRFs for specific questions and data fields)

All Tier 1 questions, tests and procedures may be done using home visits.

All Tier 1 questions, tests and procedures may be done in pregnant populations. This includes the 30 second sit-to-stand, active standing test and capnography procedures which are no more than minimal risk. As the 30 second sit-to-stand and active standing test procedures involve sitting and standing for brief periods of time while blood pressure is taken, it does not pose a risk to pregnant individuals.

| Category | Element | Interval |
|---|---|---|
| Demographics | Name and contact information (retained locally) | At enrollment and every 3 months thereafter |
| Demographics | Alternate contacts (retained locally) | At enrollment and every 3 months thereafter |
| Demographics | Date of birth | Once, on enrollment |
| Demographics | Race and ethnicity | Once, on enrollment |
| Demographics | Biological sex | Once, on enrollment |
| Demographics | Gender identity | Once, on enrollment |
| Demographics | Sexual orientation | Once, on enrollment |
| Demographics | Marital status | At enrollment and every 3 months thereafter |
| Social determinants | Education | Once, on enrollment |
| Social determinants | Number of people in household | Once, on enrollment |
| Social determinants | Homelessness | At enrollment and every 3 months thereafter |
| Social determinants | Description of living place | At enrollment |
| Social determinants | Community cohesion | Once, on enrollment |
| Social determinants | Primary language | Once, on enrollment |
| Social determinants | Fluency in English | Once, on enrollment |
| Social determinants | Birthplace | Once, on enrollment |
| Social determinants | Financial insecurity | At enrollment and every 3 months thereafter |
| Social determinants | Employment | At enrollment and every 3 months thereafter |
| Social determinants | Income in 2019 | Once, on enrollment |
| Social determinants | Access to health care | Once, on enrollment |
| Social determinants | Health insurance | At enrollment and every 3 months thereafter |
| Social determinants | Loss of insurance because of COVID pandemic | Once, on enrollment |
| Social determinants | Hunger Vital Sign | Once, on enrollment |
| Social determinants | Discrimination | Once, on enrollment |
| Social determinants | Social support | Once, on enrollment |
| Social determinants | Alcohol and substance use | At enrollment and every 3 months thereafter |
| Baseline disability | Baseline disability | Once, on enrollment |

| Category | Element | Interval |
|---|---|---|
| Acute COVID | Diagnosis method | Once, on enrollment |
| Acute COVID | Site and level of care for initial infection | Once, on enrollment |
| Acute COVID | Treatments received for initial infection | Once, on enrollment |
| Long COVID | Participation in Long COVID Treatment Trial(s) | At enrollment and every 3 months thereafter |
| Pregnancy | Pregnancy status | At enrollment and every 3 months thereafter |
| Pregnancy | Pregnancy outcomes | At enrollment and every 3 months thereafter where relevant |
| Vaccination | Vaccination status and vaccine details | At enrollment and every 3 months thereafter |
| Comorbidity | Immunocompromised condition and specific types | At enrollment and every 3 months thereafter |
| Comorbidity | Rheumatologic, autoimmune or connective tissue disease and specific types | At enrollment and every 3 months thereafter |
| Comorbidity | Diabetes and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Gastrointestinal, liver, or kidney disease and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Active cancer or cancer treatment and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Dementia or cognitive impairment and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Central nervous system infection, inflammatory disease or demyelinating disease and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Seizure disorder | At enrollment and every 3 months thereafter |
| Comorbidity | Neuromuscular disease and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Movement disorder and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Cardiovascular disease and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Stroke or bleed and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Asthma | At enrollment and every 3 months thereafter |
| Comorbidity | Chronic obstructive pulmonary disease | At enrollment and every 3 months thereafter |
| Comorbidity | Other chronic lung disease | At enrollment and every 3 months thereafter |
| Comorbidity | Use of oxygen at home | At enrollment and every 3 months thereafter |

| Category | Element | Interval |
|---|---|---|
| Comorbidity | Anxiety, depression or PTSD | At enrollment and every 3 months thereafter |
| Comorbidity | Schizophrenia or bipolar disorder | At enrollment and every 3 months thereafter |
| Comorbidity | Other mental health disorder | At enrollment and every 3 months thereafter |
| Comorbidity | Sickle cell anemia | At enrollment and every 3 months thereafter |
| Comorbidity | Chronic pain syndrome or fibromyalgia | At enrollment and every 3 months thereafter |
| Comorbidity | Myalgic encephalomyelitis/chronic fatigue syndrome | At enrollment and every 3 months thereafter |
| Comorbidity | POTS or other form of dysautonomia or autonomic dysfunction and specific type | At enrollment and every 3 months thereafter |
| Comorbidity | Obesity | At enrollment and every 3 months thereafter |
| Comorbidity | Gynecologic problems | At enrollment and every 3 months thereafter |
| Comorbidity | Thyroid problems | At enrollment and every 3 months thereafter |
| Comorbidity | Blood or blood clotting problems | At enrollment and every 3 months thereafter |
| Comorbidity | Transplant and type | At enrollment and every 3 months thereafter |
| Comorbidity | Headache problems | At enrollment and every 3 months thereafter |
| Comorbidity | Sleep problems | At enrollment and every 3 months thereafter |
| Comorbidity | Allergy problems | At enrollment and every 3 months thereafter |
| Comorbidity | Infections | At enrollment and every 3 months thereafter |
| Comorbidity | Other health problems | At enrollment and every 3 months thereafter |
| Medications | Complete medication list | At enrollment and every 3 months thereafter |
| Symptoms | Overall health status | At enrollment and every 3 months thereafter |
| Symptoms | Social function | At enrollment and every 3 months thereafter |
| Symptoms | Physical function | At enrollment and every 3 months thereafter |
| Symptoms | Fatigue and fatigue details | At enrollment and every 3 months thereafter |
| Symptoms | Post-exertional malaise (e.g., feeling exhausted after walking) | At enrollment and every 3 months thereafter |

| Category | Element | Interval |
|---|---|---|
| Symptoms | Weakness in limbs | At enrollment and every 3 months thereafter |
| Symptoms | Fever, chills, sweats or flushing | At enrollment and every 3 months thereafter |
| Symptoms | Loss of or change in smell or taste | At enrollment and every 3 months thereafter |
| Symptoms | Pain in any part of body and site of pain | At enrollment and every 3 months thereafter |
| Symptoms | Headache details | At enrollment and every 3 months thereafter |
| Symptoms | Chest pain details | At enrollment and every 3 months thereafter |
| Symptoms | Shortness of breath or trouble breathing and details | At enrollment and every 3 months thereafter |
| Symptoms | Cough | At enrollment and every 3 months thereafter |
| Symptoms | Change in voice | At enrollment and every 3 months thereafter |
| Symptoms | Palpitations, racing heart, arrhythmia, skipped beats | At enrollment and every 3 months thereafter |
| Symptoms | Swelling of lower legs and details | At enrollment and every 3 months thereafter |
| Symptoms | Gastrointestinal symptoms and details | At enrollment and every 3 months thereafter |
| Symptoms | Bladder problems and details | At enrollment and every 3 months thereafter |
| Symptoms | Nerve problems and details | At enrollment and every 3 months thereafter |
| Symptoms | Problems with anxiety, depression, stress, or trauma-related symptoms like nightmares or grief | At enrollment and every 3 months thereafter |
| Symptoms | Depression screen and assessment | At enrollment and every 3 months thereafter |
| Symptoms | Suicidality screen and assessment | At enrollment and every 3 months thereafter |
| Symptoms | Anxiety screen and assessment | At enrollment and every 3 months thereafter |
| Symptoms | Stress | At enrollment and every 3 months thereafter |
| Symptoms | Problems thinking or concentrating and details | At enrollment and every 3 months thereafter |
| Symptoms | Problems with sleep and details | At enrollment and every 3 months thereafter |
| Symptoms | Faint, dizzy, "goofy," difficulty thinking soon after standing up from a sitting or lying position and details | At enrollment and every 3 months thereafter |
| Symptoms | Color changes in your skin, such as red, white or purple and details | At enrollment and every 3 months thereafter |

| Category | Element | Interval |
|---|---|---|
| Symptoms | Skin rash | At enrollment and every 3 months thereafter |
| Symptoms | Changes in sweating | At enrollment and every 3 months thereafter |
| Symptoms | Excessively dry eyes | At enrollment and every 3 months thereafter |
| Symptoms | Excessively dry mouth | At enrollment and every 3 months thereafter |
| Symptoms | Excessive thirst | At enrollment and every 3 months thereafter |
| Symptoms | Vision problems (blurry, light sensitivity, difficulty reading or focusing, floaters, flashing lights, "snow") and details | At enrollment and every 3 months thereafter |
| Symptoms | Problems with hearing (hearing loss, ringing in ears) and details | At enrollment and every 3 months thereafter |
| Symptoms | Hair loss | At enrollment and every 3 months thereafter |
| Symptoms | Problems with teeth or gums | At enrollment and every 3 months thereafter |
| Symptoms | Change in menstruation or menopause and details | At enrollment and every 3 months thereafter |
| Symptoms | Changes in desire for, comfort with or capacity for sex | At enrollment and every 3 months thereafter |
| Change in symptoms since infection | Changes in symptoms reported before and after index date | Once, at second visit or most recent visit if implemented after |
| Post-COVID utilization | Hospitalization since COVID or last assessment | At enrollment and every 3 months thereafter |
| Post-COVID utilization | Emergency department visit since COVID or last assessment | At enrollment and every 3 months thereafter |
| Clinical assessment | Height, weight, BMI | 0, 6 months after infection then yearly |
| Clinical assessment | Waist circumference (cm) | 0, 6 months after infection then yearly |
| Clinical assessment | Seated vital signs (blood pressure, heart rate, respiratory rate, oxygen saturation) | 0, 6 months after infection then yearly |
| Clinical assessment | 30 second sit to stand | 0, 6 months after infection then yearly |
| Clinical assessment | Active standing test | 0, 6 months after infection then yearly |
| Clinical assessment | Capnography | 0, 6 months after infection then yearly |
| Clinical assessment | Wearable with continuous remote monitoring for ECG, RR, SpO2, sleep fragmentation, actigraphy | Continuous |

| Category | Element | Interval |
|---|---|---|
| Laboratory study | Comprehensive metabolic panel with cystatin-C | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Complete blood count with differential | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Lipid panel | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Hemoglobin A1c | 0, 3, 6 months after infection then yearly |
| Laboratory study | Coagulation panel | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | D-dimer | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Troponin | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | NT-pro BNP | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Thyroid panel | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | 25-hydroxy vitamin D | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Urinalysis | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | Urine microalbumin and creatinine | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | hsCRP | 0, 3, 6 months after infection then yearly if abnormal |
| Laboratory study | SARS-CoV-2 spike and/or nucleocapsid antibody | On enrollment for uninfected controls |
| Laboratory study | SARS-CoV-2 NAAT | On enrollment for uninfected controls |
| Laboratory study | SARS-CoV-2 spike and nucleocapsid antibodies | Once |
| Laboratory study | T-cell reactivity assays | Once |

### 19.3 Appendix 3: Tier 2 questions, tests and procedures

These questions may be asked of or tests may be conducted on ~30% of patients, including those meeting test-specific criteria plus a random sample of those not meeting criteria. Tests and procedures will occur not more than once a year for a maximum of four times, if indicated.

(+Questions, tests and procedures which may be completed at home visits.)

| Category | Element |
|---|---|
| Clinical assessment | Home polysomnography (sleep test)+ |
| Clinical assessment | 6 minute walk test+ |
| Clinical assessment | Neurologic exam for neuropathy |
| Clinical assessment | Rehabilitation exam: Hand-held dynamometry maximum isometric grip strength |
| Clinical assessment | Rehabilitation exam: Hand-held dynamometry maximum isometric quadriceps strength |
| Clinical assessment | Rehabilitation exam: Timed Up & Go Test (TUGt) |
| Clinical assessment | Flexible laryngoscopy with stroboscopy |
| Clinical assessment | Complete psychiatric structured interview (Columbia-Suicide Severity Rating Scale (C-SSRS), Mini International Neuropsychiatric Interview (MINI), PCL-5, if eligible, and PG-13-R, if eligible)+ |
| Clinical assessment | Vision testing+ *(performance ceased as of protocol v11.0)* |
| Clinical assessment | University of Pennsylvania Smell Identification Test (UPSIT)+ |
| Clinical assessment | NIH Toolbox (oral reading recognition test age 3+ v2.1, picture vocabulary test age 3+ v2.1, auditory verbal learning test (Rey) 8+ v2.0, Flanker inhibitory control and attention test age 12+ v2.1, pattern comparison processing speed test age 7+ v2.1, picture sequence age 7+ v2.1)+ |
| Laboratory study | Anti-nuclear antibody+ |
| Laboratory study | Anti-CCP+ |
| Laboratory study | Rheumatoid factor+ |
| Laboratory study | EBV+ |
| Laboratory study | Anti dsDNA antibody+ |
| Laboratory study | Ro antibody+ |
| Laboratory study | La antibody+ |
| Laboratory study | Smooth muscle antibody+ |
| Laboratory study | RNP antibody+ |
| Laboratory study | ACTH+ *(performance ceased as of protocol v11.0)* |
| Laboratory study | Cortisol+ *(performance ceased as of protocol v11.0)* |
| Laboratory study | Hepatitis B testing+ *(performance ceased as of protocol v11.0)* |
| Laboratory study | Hepatitis C testing+ *(performance ceased as of protocol v11.0)* |
| Laboratory study | Cytokine panel (IL2 receptor; IL 1beta, 2, 4-6, 8, 10, 13, 17; interferon gamma, TNF alpha)+ |
| Laboratory study | ICAM-1+ |
| Laboratory study | Oral glucose tolerance test (OGTT), Insulin, and C-Peptide *(performance ceased as of protocol v12.0)* |
| Laboratory study | Fecal WBC |
| Laboratory study | Fecal SARS-CoV-2 viral load (viral RNA and/or antigen) + |
| Radiology | Volumetric non contrast chest CT |
| Radiology | Dual energy non-contrast and contrast chest CT |
| Radiology | Resting thoracic echocardiography with enhancing agent, if clinically indicated |
| Radiology | Renal ultrasound *(performance ceased as of protocol v11.0)* |
| Radiology | Fibroscan |
| Procedure | Electrocardiogram |

| Category | Element |
|---|---|
| Procedure | Pulmonary function tests (spirometry with and/or without bronchodilator, DLCO) |

Pregnant Participants:

Pregnant participants and participants within 3 months of delivery may **not** undergo the following Tier 2 procedures:

- Volumetric non contrast chest CT
- Dual energy non-contrast and contrast chest CT
- Home polysomnography (sleep test)
- Flexible laryngoscopy with stroboscopy

Sites will follow local policies to determine pregnancy status prior to completion of any test restricted for pregnant participants. Pregnant and post-partum participants may undergo all other Tier 2 procedures.

### 19.4 Appendix 4: Tier 3 tests and procedures

These questions and tests may be performed on a maximum of 20% of subjects. Tier 3 tests and procedures that are more than minimal risk (indicated with an asterisk) will not be performed more than once for the entire duration of the study, except for participants who cross over from uninfected to infected status. Those participants can undergo a more than minimal risk Tier 3 test twice: once while uninfected and once while infected. Eligibility and exclusion criteria for each assessment and test are specified in the manual of operations and **Appendix 5**: Tier 2 and 3 eligibility, exclusion criteria**.**

(*Greater than minimal risk) (+Questions, tests and procedures which may be completed at home visits.)

| **Category** | **Element** |
|---|---|
| Clinical assessment | Audiometry |
| Clinical assessment | Tinnitus sound matching |
| Clinical assessment | Complete neurocognitive testing+ |
| Clinical assessment | EndoPAT testing |
| Laboratory study | Serum protein immunofixation electrophoresis+ |
| Laboratory study | Serum B12 + *(performance ceased as of protocol v12.0)* |
| Laboratory study | Methylmalonic acid+ *(performance ceased as of protocol v12.0)* |
| Laboratory study | CPK, aldolase, myositis panel+ |
| Laboratory study | (CSF) cell count, glucose, protein |
| Laboratory study | (CSF) GFAP, Neurofilament light chain |
| Laboratory study | (CSF) INFg, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL17A, TNFa |
| Laboratory study | (CSF) AB40, AB42, PTau 217 |
| Laboratory study | (Stool) Microbiome |
| Laboratory study | (Stool) SARS-CoV-2 RNA |
| Laboratory study | Fecal calprotectin+ |
| Laboratory study | Anti-Mullerian hormone |
| Radiology | Brain MRI without gadolinium* |
| Radiology | Brain MRI with gadolinium* |
| Radiology | Cardiac MRI without gadolinium* |
| Radiology | Cardiac MRI with gadolinium* |
| Radiology | Gastric emptying study* |
| Procedure | Nerve conduction study* |
| Procedure | Electromyography* |
| Procedure | Skin biopsy* |
| Procedure | Muscle biopsy* |
| Procedure | Lumbar puncture with or without imaging* |
| Procedure | Facility-based polysomnography (sleep test) |
| Procedure | Autonomic testing: Tilt table testing, with supine and upright plasma catecholamine testing* |
| Procedure | Autonomic testing: Heart rate variability (cardiovagal innervation testing) |
| Procedure | Autonomic testing: Valsalva test |
| Procedure | Cardiopulmonary exercise testing (CPET) |
| Procedure | Invasive cardiopulmonary exercise testing* |
| Procedure | Flexible bronchoscopy with bronchoalveolar lavage and brushings* |
| Procedure | Right heart catheterization* |
| Procedure | Colonoscopy with biopsy, with or without upper endoscopy with biopsy* |

The following procedures may be performed under sedation:

- Flexible bronchoscopy with bronchoalveolar lavage and brushings
- Right cardiac catheterization
- Colonoscopy with biopsy

The type of sedation used will be in accordance with institutional protocol.

Pregnant Participants:

Pregnant participants and participants within 3 months of delivery may **not** undergo the following Tier 3 procedures:

- Brain MRI with gadolinium
- Cardiac MRI with gadolinium
- Gastric emptying study
- Skin biopsy
- Muscle biopsy
- Lumbar puncture
- Tilt table testing
- Cardiopulmonary exercise testing (non-invasive or invasive)
- Flexible bronchoscopy with bronchoalveolar lavage and brushings
- Right heart catheterization
- Colonoscopy with biopsy, with or without upper endoscopy with biopsy
- Electromyography and nerve conduction study
- Facility-based polysomnography (sleep test)

Participants who are breastfeeding may not undergo the following Tier 3 procedures:

- Gastric emptying study

Sites will follow local policies to determine pregnancy status prior to completion of any test restricted for pregnant participants. All clinical assessments and laboratory studies may be done in pregnant populations.

## 19.5 Appendix 5: Tier 2 and 3 eligibility, exclusion criteria, and additional information about test procedures and permissible clinical variation

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| Home polysomnography | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Snoring or sleep apnea<br>• Moderate to severe sleep disturbance<br>• Abnormal sleep duration<br>• Waking too early after sleep onset<br>• Moderate to severe fatigue | • Sleep pressure machine (e.g. PAP, CPAP, BiPAP) or dental device at home to sleep<br>• Use of home oxygen<br>• Pregnant or < 3 months post-partum. (excluded for data quality, not for safety) | N/A |
| Six-minute walk test | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Persistent cough<br>• Post-exertional malaise<br>• Hypoxia at rest<br>• Mechanical ventilation during acute COVID<br>• Decreased oxygen for extended sleep duration without sleep apnea | • Unstable angina in the previous month<br>• Myocardial infarction in the previous month | Permissible clinical variation:<br><br>Relative contraindications, as per local site clinician assessment of participant:<br>• Elevated heart rate (> 120 bpm) at rest<br>• Elevated blood pressure (SBP > 180 mmHg; DBP > 100 mmHg) at rest<br><br>Additional information about test procedures:<br><br>Intra-procedure stopping criteria<br>• Chest pain<br>• Intolerable dyspnea<br>• Leg cramps<br>• Staggering<br>• Diaphoresis or pale/ashen appearance |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | | • SpO2 < 80% when using continuous oximetry |
| Neurologic exam for neuropathy | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ALL of the following:<br>• Numbness, tingling, burning, or pins and needles sensations<br>• Moderate to severe neuropathy | N/A | N/A |
| Rehabilitation exam: Hand-held dynamometry maximum isometric grip strength | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Post-exertional malaise<br>• Hypoxia at rest<br>• Reduced physical function<br>• Moderate to severe inability to carry out everyday physical activities | N/A | Permissible clinical variation:<br>• A variety of equipment options are provided for selection as per local availability |
| Rehabilitation exam: Hand-held dynamometry maximum isometric quadriceps strength | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Post-exertional malaise<br>• Hypoxia at rest | N/A | Permissible clinical variation:<br>• A variety of equipment options are provided for selection as per local availability |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | • Reduced physical function<br>• Moderate to severe inability to carry out everyday physical activities | | |
| Rehabilitation exam: Timed Up & Go Test (TUGt) | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Post-exertional malaise<br>• Hypoxia at rest<br>• Reduced physical function<br>• Moderate to severe inability to carry out everyday physical activities | N/A | N/A |
| Flexible laryngoscopy with stroboscopy | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Change in voice<br>• Persistent cough | • History of intubation for COVID or any other indications since COVID infection, excluding brief surgical procedures.<br>• Severe throat infections (e.g. epiglottitis or croup)<br>• Contraindication or known allergy to lidocaine<br>• Contraindication or known allergy to decongestants<br>• Pregnant or < 3 months postpartum | Additional information about test procedures:<br>• Participants must fast for at least 6 hours prior to procedure and one hour after |
| Complete psychiatric structured interview | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | N/A | Additional information about test procedure:<br>• Remote administration of the MINI may be performed ONLY if the |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| (C-SSRS, MINI, PCL-5, PG-13-R) | | ANY of the following:<br>• Positive suicidality screener<br>• Positive depression screener<br>• Positive anxiety screener<br>• Positive PTSD screener<br>• Experiencing persistent grief | | patient has been assessed by a clinician to not be at elevated risk of harm.<br>• If the C-SSRS is positive for medium or high risk, the participant must meet with the site's medical or mental health professional prior to conducting the MINI to determine appropriateness of the interview. Should the clinician determine the MINI to be inappropriate due to severity of symptoms, it should be rescheduled at a later time. The main risk of concern is suicidal thinking and urges. |
| Vision testing | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ALL of the following:<br>• Reduced visual functioning | N/A | N/A |
| UPSIT | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ALL of the following:<br>• Altered smell and/or taste | N/A | N/A |
| NIH Toolbox | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | N/A | Permissible clinical variation: |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | ALL of the following:<br>• Reduced cognitive functioning | | • Specific components may be performed remotely if no option for in person testing |
| ACTH | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Post-exertional malaise<br>• Anorexia<br>• Feeling faint, dizzy, or goofy or having difficulty thinking soon after standing<br>• Orthostatic hypotension<br>• Postural orthostatic tachycardia<br>• Elevated potassium<br>• Decreased sodium | • Systemic glucocorticoids (e.g. hydrocortisone, prednisone, methylprednisolone, dexamethasone) | N/A |
| Cortisol | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Post-exertional malaise<br>• Anorexia<br>• Feeling faint, dizzy, or goofy or having difficulty thinking soon after standing<br>• Orthostatic hypotension<br>• Postural orthostatic tachycardia<br>• Elevated potassium<br>• Decreased sodium | • Systemic glucocorticoids (e.g. hydrocortisone, prednisone, methylprednisolone, dexamethasone) | N/A |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| Hepatitis B testing | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Elevated liver enzymes<br>• Abnormal FIB-4 index | N/A | N/A |
| Hepatitis C testing | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Elevated liver enzymes<br>• Abnormal FIB-4 index | N/A | N/A |
| Oral glucose tolerance test, Insulin, and C-Peptide<br>(Testing ceased with protocol v.12) | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Elevated fasting glucose without diabetes<br>• Elevated HbA1c without diabetes<br>• History of type 2 diabetes | • Type 1 diabetes<br>• Fasting blood glucose > 200 mg/dL OR < 50 mg/dL<br>• History of bariatric surgery (such as, Roux-en-Y Gastric Bypass (RYGB), vertical sleeve gastrectomy (VSG)), Nissen fundoplication or any major upper GI surgery removing parts of stomach or proximal small intestine<br>• Subjects who are on insulin therapy | Additional information about test procedures:<br>• Participants hold all anti-diabetic agents (oral and injectable diabetes medication) on the day of OGTT. Participants may take their diabetes medication once the test has been completed. Subjects may not eat or drink for at least 8 hours prior to OGTT test (water is permitted and the patient may take non-diabetes medications with water). |
| Volumetric non contrast chest CT ***or*** Dual energy non- | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | • *For contrast chest CT:* Severe kidney disease, allergy to iodinated contrast, shellfish allergy, declining contrast use | Permissible clinical variation:<br>• A variety of CT scanner models are permissible for use. The sites should consult the Reading Center |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| contrast and contrast chest CT | | ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Persistent cough<br>• Post-exertional malaise<br>• Hypoxia at rest<br>• Mechanical ventilation during acute COVID<br>• Decreased oxygen for extended sleep duration without sleep apnea | • Pregnant or < 3 months post-partum. | if their CT scanner model is not listed in the SOP. The Reading Center will assist the site to define a protocol specific to their model. Special attention should be placed to the dose parameters (tube current, kVp and dose modulation settings) to minimize the radiation dose exposure to the participants according to the institution's radiation safety standards |
| Resting thoracic echocardiography with or without ultrasound enhancing agent | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Elevated cardiac markers<br>• ECG findings indicating ischemia<br>• Moderate to severe shortness of breath<br>• Palpitations<br>• Chest pain<br>• Decreased oxygen for extended sleep duration without sleep apnea | N/A | Permissible clinical variation:<br>• This protocol does not include the use of contrast; it is preferred that contrast is not used within this protocol. If a site cannot ensure that contrast will not be given during the procedure, it must notify the CSC, consent the participant using the Echo with Contrast Information Sheet and note any use of contrast in REDCap. |
| Fibroscan | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Elevated liver enzymes<br>• Abnormal FIB-4 index | N/A | Additional information about test procedures:<br>• Participants may not eat or drink for at least 3 hours prior to fibroscan (medications may be taken with sips of water or other clear liquids).<br>Permissible clinical variation: |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | | • A variety of FibroScan machines are permissible for use. |
| Electrocardiogram | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Palpitations<br>• Moderate to severe shortness of breath<br>• Dizziness<br>• Elevated cardiac markers<br>• Abnormal heart rate | N/A | N/A |
| Pulmonary function tests (spirometry with and/or without bronchodilator, DLCO) | 2 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath<br>• Moderate to severe fatigue<br>• Persistent cough<br>• Post-exertional malaise<br>• Hypoxia at rest<br>• Mechanical ventilation during acute COVID<br>• Decreased oxygen for extended sleep duration without sleep apnea | N/A | Additional information about test procedures:<br>• Participants must hold short-acting bronchodilators for four hours prior to procedure and long-acting bronchodilators for 12 hours prior to procedure |
| Audiometry | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | N/A | N/A |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | ANY of the following:<br>• Feeling faint, dizzy, or goofy or having difficulty thinking soon after standing<br>• Problems with hearing | | |
| Tinnitus sound matching | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Feeling faint, dizzy, or goofy or having difficulty thinking soon after standing<br>• Problems with hearing<br>AND<br>• Ringing in ears | N/A | N/A |
| Complete Neurocognitive Testing | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Abnormal NIH Toolbox results | N/A | N/A |
| EndoPAT | 3 | All participants at sites with equipment are eligible for this test through random assignment. | N/A | Additional information about test procedures:<br>• Participants must avoid exercise, caffeine, food and smoking for 4 hours prior to procedure |
| Serum B12 | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as | N/A | N/A |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Neuropathy on exam | | |
| Methylmalonic acid | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Neuropathy on exam | N/A | N/A |
| Brain MRI ***or*** Brain MRI with gadolinium | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Abnormal NIH Toolbox or other cognitive test scores<br>• Headaches<br>• Abnormal autonomic testing<br>• Newly diagnosed PTSD, psychosis, prolonged grief, depression, or anxiety<br>• Tinnitus<br>• Newly diagnosed stroke<br>• Reduced smell functioning<br>• Mechanical ventilation during acute COVID | • Known contraindication to MRI examination (i.e., severe claustrophobia, pacemaker, defibrillator, neuro-stimulator, ferro-magnetic or unknown aneurysm clip, 3T MR incompatible metal implant of any kind or potentially dangerous foreign metal objects in the body such as bullets, shrapnel, metal slivers, etc.)<br>• Severe kidney disease depending on type of gadolinium used, or allergy to gadolinium<br>• Pregnant or < 3 months postpartum | Permissible clinical variation:<br>• Apart from the absolute exclusion for retained metal, MRI exclusions are primarily dependent on the guidelines/practices of the local imaging site. Prior to scheduling participant for procedure, sites should follow local policy regarding any required pre-test labs. Sites that do not have a local policy that outlines pre-test requirements should follow American College of Radiology guidance.[54]<br>• Choice of gadolinium-based contrast agent is dependent on local site availability. The MRI contrast agent and dosing administration use for the study |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | | should be followed per local site procedures/policies. If a site has multiple contrasts available, the site should contact the MRI reading center regarding contrast selection and should use one contrast agent for the course of the study. |
| Cardiac MRI ***or*** Cardiac MRI with gadolinium | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Elevated cardiac markers<br>• Reduced ejection fraction<br>• Pericardial effusion<br>• Abnormal global longitudinal strain | • Known contraindication to an MRI examination (i.e., severe claustrophobia, pacemaker, defibrillator, neuro-stimulator, ferro-magnetic or unknown aneurysm clip, 3T MR incompatible metal implant of any kind or potentially dangerous foreign metal objects in the body such as bullets, shrapnel, metal slivers, etc.)<br>• Severe kidney disease depending on type of gadolinium used, or allergy to gadolinium<br>• Pregnant or < 3 months postpartum | Permissible clinical variation:<br>• Apart from the absolute exclusion for retained metal, MRI exclusions are primarily dependent on the guidelines/practices of the local imaging site. Prior to scheduling participant for procedure, sites should follow local policy regarding any required pre-test labs. Sites that do not have a local policy that outlines pre-test requirements should follow American College of Radiology guidance.[54]<br>• Choice of gadolinium-based contrast agent is dependent on local site availability. The MRI contrast agent and dosing administration use for the study should be followed per local site procedures/policies. If a site has multiple contrasts available, the site should contact the MRI reading center regarding contrast selection and should use one contrast agent for the course of the study.<br>Additional information about test procedures: |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | | • A recent hematocrit (either 30 days before or 15 days after) the test is required for best interpretation of results. |
| Gastric emptying study | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br>ANY of the following:<br>• Persistent gastrointestinal symptoms<br>• Abnormal autonomic test | • Pregnant, breastfeeding, or < 3 months postpartum<br>• History of gastric surgery (such as, Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG)), or any major upper GI surgery removing parts of stomach or proximal small intestine (excluded for data quality, not for safety) | Permissible clinical variation:<br>• Participants with intolerance to the standard meal can have oatmeal instead<br>• Participants can do the test supine if unable to tolerate upright imaging<br><br>Additional information about test procedures:<br>• Participants must fast for at least 4 hours prior to procedure<br>• Prokinetic agents and medications that delay gastric emptying should be stopped 2 days prior to the test<br>• GLP-1 agonists should be held for one dose prior to procedure<br>• Clinical staff will review medication list to determine if any others need to be held |
| Electromyography (EMG) | 3 | ***Only participants who meet the eligibility criteria as follows may be asked to complete this assessment:*** | • Pacemaker<br>• Participants with lymphedema at site of testing<br>• Pregnant or < 3 months post-partum | Permissible clinical variation:<br>• Specific muscles to be tested depend on local site clinician |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | ANY of the following:<br>• Neuropathy or myopathy on exam | | examination as per standard EMG protocols.<br>• Total number of limbs tested for EMG may range from 1-3 limbs depending on examination and clinical appropriateness.<br><br>Additional information about test procedures:<br><br>Intra-procedure stopping criteria:<br>• Stop if participant cannot tolerate the procedure, requests that the test be terminated, or becomes vasovagal. |
| Nerve Conduction Study (NCS) | 3 | ***Only participants who meet the eligibility criteria as follows may be asked to complete this assessment:***<br><br>ANY of the following:<br>• Neuropathy or myopathy on exam | • Pacemaker<br>• Participants with lymphedema at site of testing<br>• Pregnant or < 3 months post-partum | Permissible clinical variation:<br>• Specific nerves to be tested depend on local site clinician examination as per standard NCS protocols.<br>• Total number of nerves tested may range from 1-13 depending on examination and clinical appropriateness.<br><br>Additional information about test procedures:<br><br>Intra-procedure stopping criteria: |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | | • Stop if participant cannot tolerate the procedure, requests that the test be terminated, or becomes vasovagal. |
| Skin biopsy | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Neurologic exam for neuropathy<br>• Orthostatic hypotension<br>• Postural orthostatic tachycardia<br>• Symptoms of dysautonomia<br>• Feeling faint, dizzy, or goofy or difficulty thinking soon after standing | • Taking blood thinning medication unless prescribing provider confirms they can be held for 7 days prior to and 1 day post procedure. These medications include:<br>○ Direct oral anticoagulants (e.g. rivaroxaban, dabigatran, apixaban or edoxaban)<br>○ Vitamin K antagonists (e.g. warfarin)<br>○ Low molecular weight heparins (e.g. bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g. clopidogrel, ticagrelor, or prasugrel)<br>○ Aspirin > 325 mg daily (Aspirin ≤ 325mg may be included without medication hold)<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures), including those with the following | Permissible clinical variation:<br>• Side of biopsy should be selected based on presence of symptoms or, if none, the non-dominant side<br><br>Additional information about test procedures:<br>• Three biopsies are obtained: one each from the posterior cervical, distal leg and distal thigh regions<br>• Each of the three biopsies taken should be approximately 3 mm |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | abnormalities (use most recent result within the prior year):<br>○ INR > 1.3 times upper limit of normal<br>○ PT > 13 seconds<br>○ aPPT > 36 seconds<br>○ Platelets < 125,000 /mL<br>• Contraindication or known allergy to lidocaine<br>• Patients who have a history of poor wound healing or who are prone to keloid formation<br>• Pregnant or < 3 months post-partum. | |
| Muscle biopsy | 3 | ***Only infected participants who meet the eligibility criteria as follows may be asked to complete this assessment***:<br><br>ANY of the following:<br>• Moderate to severe muscle weakness or pain<br>• Moderate to severe post-exertional malaise<br><br>Participants who also qualify for CPET will be offered the option to do a pre-CPET muscle biopsy and a post-CPET muscle biopsy at the same site | • Taking blood thinning medication unless prescribing provider confirms they can be held for 7 days prior to and 1 day post procedure, including<br>○ Direct oral anticoagulants (e.g. rivaroxaban, dabigatran, apixaban or edoxaban)<br>○ Vitamin K antagonists (e.g. warfarin)<br>○ Low molecular weight heparins (e.g. bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g. clopidogrel, ticagrelor or prasugrel)<br>○ Aspirin | Permissible clinical variation:<br>• Side of biopsy is at clinician discretion<br>• A 5 or 6 mm Bergstrom-style biopsy needle is permissible depending on local equipment availability<br><br>Additional information about test procedures:<br>• Participants must fast for 4 hours prior to procedure (medications with sips of water are acceptable)<br>• One biopsy is obtained from the vastus lateralis; multiple passes at same biopsy site are permissible to obtain weight of at least 150 mg (approximately a 5 mm biopsy)<br>• If participant agrees to pre/post-CPET biopsy, a second biopsy |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | ○ Non-steroidal anti-inflammatory medications (e.g. ibuprofen, ketorolac, naproxen, diclofenac, indomethacin, celecoxib, meloxicam)<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures), including those with the following abnormalities (use most recent result within the prior year):<br>○ INR > 1.3 times upper limit of normal<br>○ PT > 13 seconds<br>○ aPPT > 36 seconds<br>○ Platelets < 125,000/mL<br>• Contraindication or known allergy to lidocaine<br>• Patients who have a history of poor wound healing or who are prone to keloid formation<br>• Pregnant or < 3 months post-partum. | sample is obtained from the same leg and same muscle post-CPET |
| Lumbar puncture with or without imaging (fluoroscopy or ultrasound guidance) | 3 | Only infected participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Headaches<br>• Leptomeningeal, dural, or parenchymal enhancement | • Participants opting out of the CSF biospecimen collection for central processing<br>• Any intracranial space-occupying lesion with mass effect, posterior fossa mass or cerebral herniation as determined by clinical examination (e.g., papilledema, depressed | Permissible clinical variation:<br><br>• Use of fluoroscopy or ultrasound guidance is at discretion of site clinician and should consider patient factors.<br><br>• Choice of positioning (left or right lateral, or upright) and intercostal space (L3-4 or L4-5) is at |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | • Abnormal NIH Toolbox or other cognitive test score | mental status, focal neurologic findings), brain MRI or head CT<br>• Taking blood thinning medication, unless prescribing provider confirms they can be held for 7 days prior to and 1 day post procedure. These medications include:<br>○ Direct oral anticoagulants (e.g. rivaroxaban, dabigatran, apixaban or edoxaban)<br>○ Vitamin K antagonists (e.g. warfarin)<br>○ Low molecular weight heparins (e.g. bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g. clopidogrel, ticagrelor or prasugrel)<br>○ Aspirin > 81 mg (Aspirin ≤ 81 mg may be included without medication hold)<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures), including those with the following abnormalities (use most recent result within the prior 30 days): | discretion of site clinician and should consider patient factors.<br><br>Additional information about test procedures:<br><br>• Sedation or anesthesia is not permitted<br>• Up to three attempts are permissible.<br><br>Intra-procedure stopping criteria:<br>○ Stop procedure if participant experiences lower extremity neurologic symptoms. |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | ○ INR > 1.4 times upper limit of normal<br>○ Platelets < 100,000/mL<br>• Skin infection at the planned puncture site<br>• Allergy to lidocaine, iodine (may use chlorhexidine as an alternative), or latex (may use latex free gloves as an alternative)<br>• Pregnant or < 3 months post-partum | |
| Facility-based polysomnography | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Qualifies for home polysomnography and uses home oxygen or CPAP<br>• Central sleep apnea<br>• Moderate sleep disturbance<br>• Acting out dreams<br>• Restless legs<br>• Moderate to severe insomnia<br>• Decreased oxygen for extended sleep duration without sleep apnea | • Pregnant or < 3 months post-partum (excluded for data quality, not for safety) | Permissible clinical variation, depending on local equipment availability and patient factors:<br><br>• Sites may use 3 or 6 EEG sites<br>• Submental electromyography may be measured by 1-3 chin EMGs<br>• ECG may use 2 or 3-lead precordial placement<br>• Airflow may be measured via nasal pressure flow, PAP flow or oronasal thermocouple<br>• Snoring vibration may or may not be measured<br>• Leg movements may be measured by 1-2 EMG signals per leg<br>• Minimum and desired sampling rates (Hz) are provided in the SOP |
| Autonomic testing: Heart rate variability | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | • Pregnant or < 3 months post-partum<br>• Known clinically severe left ventricular outflow obstruction, critical mitral stenosis, or | Additional Information:<br><br>• Certain medications interfere with the results. Participants are advised that optimal results will be |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| (cardiovagal innervation testing) | | ANY of the following:<br>• Neuropathy on exam<br>• Orthostatic hypotension<br>• Postural orthostatic hypotension<br>• Symptoms of dysautonomia<br>• Feeling faint, dizzy, or goofy or difficulty thinking soon after standing | patients in whom low organ perfusion pressures may compromise end artery supplied tissue, as in severe proximal coronary artery and cerebrovascular stenosis | obtained if the medication can be stopped for a prespecified amount of time prior to procedure, but only with the prescribing physician's approval. See **Table 9** for a detailed list.<br>• Participants are advised no alcoholic drinks 14 hours before the scheduled study.<br>• Participants are advised no nicotine (cigarettes) or caffeine (tea, chocolate, coffee and caffeinated soft drinks) three hours before study.<br>• Participant to avoid eating at least 3 hours before testing. |
| Autonomic testing: Valsalva test | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Neuropathy on exam<br>• Orthostatic hypotension<br>• Postural orthostatic hypotension<br>• Symptoms of dysautonomia<br>• Feeling faint, dizzy, or goofy or difficulty thinking soon after standing | • Pregnant or < 3 months post-partum<br>• Known clinically severe left ventricular outflow obstruction, critical mitral stenosis, or patients in whom low organ perfusion pressures may compromise end artery supplied tissue, as in severe proximal coronary artery and cerebrovascular stenosis | Additional Information:<br>• Certain medications interfere with the results. Participants are advised that optimal results will be obtained if the medication can be stopped for a prespecified amount of time prior to procedure, but only with the prescribing physician's approval. See Table 9 for a detailed list. |
| Autonomic testing: Tilt table testing with upright and supine | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows: | • Pregnant or < 3 months post-partum<br>• Known clinically severe left ventricular outflow obstruction, critical mitral stenosis, or | Additional Information:<br>• Certain medications interfere with the results. Participants are advised that optimal results will be |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| catecholamine metabolites | | ANY of the following:<br>• Neuropathy on exam<br>• Orthostatic hypotension<br>• Postural orthostatic hypotension<br>• Symptoms of dysautonomia<br>• Feeling faint, dizzy, or goofy or difficulty thinking soon after standing | patients in whom low organ perfusion pressures may compromise end artery supplied tissue, as in severe proximal coronary artery and cerebrovascular stenosis | obtained if the medication can be stopped for a prespecified amount of time prior to procedure, but only with the prescribing physician's approval. See Table 9 for a detailed list. |
| Cardiopulmonary exercise testing | 3 | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ALL of the following:<br>• Shortness of breath<br>• Non-reduced ejection fraction | • Acute myocardial infarction (3-5 days) or unstable angina<br>• Uncontrolled symptomatic arrhythmias<br>• Active endocarditis<br>• Acute myocarditis or pericarditis<br>• Symptomatic severe aortic stenosis<br>• Acute pulmonary embolism or DVT<br>• Suspected dissecting aneurysm<br>• Uncontrolled asthma<br>• Uncontrolled pulmonary edema<br>• Room air desaturation to <85%<br>• Acute illness (e.g. infection) or orthopedic injury that is anticipated to affect exercise performance<br>• Mental impairment leading to inability to cooperate<br>• History of exercise-induced ventricular arrhythmia | Permissible Clinical Variation:<br>• Cycle ergometry is the preferred exercise modality for the RECOVER study. For CPET laboratories that do not perform cycle ergometry an alternative treadmill ergometry exercise protocol has been devised.<br><br>Additional information about test procedures:<br>• Participants should fast for at least two hours prior to procedure<br><br>Intra-procedure stopping criteria<br>• Definitive ischemic ECG changes with associated chest pain<br>• Complex ectopy (i.e. ventricular tachycardia)<br>• Mobitz 2 second degree or third-degree atrioventricular heart block<br>• Marked hypertension (systolic BP > 240 mmHg, diastolic BP > 120 mmHg) |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | • Neuromuscular impairment that precludes incremental exercise on the ergometer being used to conduct the study<br>• Pregnant or < 3 months post-partum | • Severe oxygen desaturation, SpO2<80% when accompanied by signs of severe hypoxia within 2 minutes of unloaded pedaling<br>• Neurologic compromise such as mental confusion or loss of coordination<br>• Inability to coordinate a regular cadence of >40 cycles/minute |
| Invasive cardiopulmonary exercise testing | 3 | ***Only infected participants who meet the eligibility criteria as follows may be asked to complete this assessment:***<br>ALL of the following:<br>• Shortness of breath<br>• Non-reduced ejection fraction<br>. | All exclusions for CPET and right heart catheterization apply, plus:<br>• Inability to exercise on cycle ergometer (treadmill is not permitted for iCPET)<br>• Allergy to lidocaine | Additional information about test procedures:<br>• All additional information for CPET and right heart catheterization applies, plus:<br>• Participants should fast for at least two hours prior to procedure<br>• Procedure site may involve right or left internal jugular or brachial vein only, not femoral vein.<br>• In addition, a right or left radial arterial catheter is placed<br>• Side of placement is at discretion of the local clinician and should take into account patient factors<br>Intra-procedure stopping criteria<br>• All intra-procedure stopping criteria for CPET and right heart catheterization apply, plus:<br>• Markedly abnormal resting supine hemodynamic values (i.e. PVT > 10 WU, PCWP > 40 mgHg) |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| Flexible bronchoscopy with bronchoalveolar lavage and brushings, with or without (IV) procedural sedation | 3 | All participants are eligible for this test through random assignment.<br><br>Participants with the following characteristics (as outlined in the applicable SOP) will be oversampled for assignment as follows:<br><br>ANY of the following:<br>• Moderate to severe shortness of breath with normal chest CT<br>• Moderate to severe shortness of breath with abnormal chest CT | • Lack of safe discharge plan (needs adult companion at time of release) or reliable post-procedure contact<br>• Age > 70 years<br>• Age 60-70 years with ASA > 2<br>• Resting room air oxygen saturation < 90% or < 88% at high altitude<br>• Hypoxia requiring > 2L nasal cannula at rest<br>• Untreated obstructive sleep apnea<br>• Severe pulmonary hypertension (mPAP ≥ 35)<br>• Systolic blood pressure > 180 mmHg or MAP < 50<br>• Known elevated intracranial pressure<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures), including those with the following abnormalities within 30 days of procedure:<br>  ○ PTT > 1.5 times upper limit of normal<br>  ○ INR > 1.3 times upper limit of normal<br>  ○ Platelets < 100,000/mL<br>• Any of the following abnormal results within 30 days of procedure: | Permissible Clinical Variation:<br><br>• Choice of transoral or transnasal approach is at local clinician discretion and should consider patient factors.<br>• Delivery of topical lidocaine (gargle, aerosol, spray or instillation) is at local clinician discretion and should consider patient factors.<br>• Use of procedural sedation is at local clinician discretion and should consider patient factors.<br>• If procedural sedation is selected, personnel administering sedation, choice of sedation agents, dosage limits and intervals, and credentialing must be managed according to local institutional policy, but may not exceed limits indicated in the SOP.<br>• The choice of left or right side for specimen collection is at clinician discretion and should be tailored to chest CT findings<br>• Monitoring during and post-procedure must be conducted in accordance with local institutional policy.<br><br>Additional information about test procedures<br>• Participants must fast for 6 hours prior to procedure |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | ○ Creatinine > 2 mg/dL<br>○ ECG with unstable ischemic disease<br>• Taking blood thinning medication, unless prescribing provider confirms they can be held for 7 days prior to and 1 day post procedure. These medications include:<br>○ Direct oral anticoagulants (e.g. rivaroxaban, dabigatran, apixaban or edoxaban)<br>○ Vitamin K antagonists (e.g. warfarin)<br>○ Low molecular weight heparins (e.g. bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g. clopidogrel, ticagrelor, prasugrel)<br>○ Aspirin > 81 mg daily (Aspirin ≤ 81 mg may be included without medication hold)<br>• Active cardiac ischemia or unstable angina<br>• FVC < 50% or FEV1 < 50%<br>• BMI > 50 kg/m$^2$ | • A total of 4 bronchial brushings are collected from either the left or right lower lobe<br>• A Supraglottic Sample Is Taken<br>• A post-procedure chest X ray is required<br><br>Intra-procedure stopping criteria:<br>• Bronchoscopy will be stopped if the following abnormalities are detected for longer than one minute:<br>○ Systolic blood pressure < 90 or > 160 mmHg,<br>○ Respiratory rate > 25 breaths or < 8 breaths per minute<br>○ Persistent heart rate < 60 or > 120 beats per minute<br>○ Irregular heart rhythm<br>○ EtCO2 > 50 mmHg or an absent ETCO2 waveform and/or oxygen saturation (SaO2) below 90% despite oxygen supplementation<br>• If a participant is uncomfortable or agitated during the procedure despite safe maximal doses of procedural sedation, the procedure can be aborted by the operating physician<br>• Additionally, the procedure can be stopped at any point at the discretion of the investigator performing the bronchoscopy. |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | | • Hospitalization within 6 weeks of procedure<br>• Non-elective intubation within last 6 months<br>• Active upper respiratory infection within 2 weeks of procedure<br>• Active tuberculosis<br>• Contraindication to sedation<br>• Allergy to lidocaine<br>• Pregnant or < 3 months post-partum. | Hospitalization criteria:<br>○ Significant cough persisting beyond 2 hours after completion of procedure<br>○ Persistent hypoxia <90% at end of monitoring time<br>○ Persistent tachycardia >130 bpm at end of monitoring time<br>○ Hemodynamically unstable arrhythmia<br>○ Unexpected altered mental status during or after procedure<br>○ Significant hemoptysis >50 ml<br>○ Requirement for bronchodilator every 2 hours on more than 3 occasions<br>○ Pneumothorax |
| Right heart catheterization<br>- with or without procedural sedation | 3 | Only infected participants who meet the eligibility criteria as follows may be asked to complete this assessment:<br><br>ANY of the following:<br>• Possible pulmonary hypertension on PFT, echocardiogram, or CPET or heart failure with preserved ejection fraction<br>• Moderate to severe shortness of breath with normal echocardiogram and normal PFT at sites not | • Lack of safe discharge plan (needs adult companion at time of release)<br>• Unable to lie flat or still for one hour<br>• Taking blood thinning medication, unless prescribing provider confirms they can be held for 7 days prior to and 1 day post procedure. These medications include:<br>○ Direct oral anticoagulants (e.g., | Permissible Clinical Variation:<br><br>• Choice of procedure site (right or left internal jugular vein, femoral vein or brachial vein) is at local clinician discretion, and should consider patient factors<br>• Choice of sheath size (5-9F) is at local clinician discretion, and should consider patient factors<br>• Use of procedural sedation is at local clinician discretion. |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
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| | | performing RECOVER invasive cardiopulmonary exercise testing | rivaroxaban, dabigatran, apixaban or edoxaban)<br>○ Vitamin K antagonists (e.g., warfarin)<br>○ Low molecular weight heparins (e.g., bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g., clopidogrel, ticagrelor, prasugrel)<br>○ Aspirin > 325 mg daily (Aspirin ≤ 325 mg may be included without medication hold)<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures)<br>• Any of the following labs with abnormalities within 21 days of procedure:<br>○ INR > 1.3 times upper limit of normal<br>○ PT > 13 seconds<br>○ aPPT > 36 seconds<br>○ Platelets <150,000/mL<br>○ Hemoglobin <11 g/dL<br>○ BUN > 60 mg/dL<br>○ Creatinine > 2 mg/dL | • If procedural sedation is selected, personnel administering sedation, choice of sedation agents, dosage limits and intervals, and credentialing must be managed according to local institutional policy, but may not exceed limits indicated in the SOP.<br>• Monitoring during and post-procedure must be conducted in accordance with local institutional policy.<br>Additional information about test procedures<br>• Participant must fast for 6 hours prior to procedure<br>• Results of screening labs are determined to be abnormal through both the local reference ranges and clinician discretion<br>Intra-procedure stopping criteria:<br>• ECG signifying current myocardial injury or potentially lethal arrhythmias<br>• Systemic hypotension (e.g., mean arterial blood pressure <50 mmHg)<br>• Extreme hypertension (e.g., mean arterial pressure > 110 mmHg)<br>• Syncope or pre-syncope or lightheadedness<br>• Peripheral oxyhemoglobin saturation level <80% |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | ○ Bicarbonate > 32 mEq/L<br>○ Sodium, potassium, or chloride outside normal reference range<br>• Severe/symptomatic aortic stenosis, mechanical or bioprosthetic valve replacement, or active tricuspid endocarditis<br>• Severe peripheral vascular disease at site of proposed catheterization<br>• Frequent arrhythmias (including atrial fibrillation or frequent ventricular arrhythmias) that are not rate controlled<br>• Stroke or syncope in the last 30 days before procedure<br>• Thrombosis within heart or lungs<br>• Right atrial/ventricular thrombus<br>• Pulmonary embolism within the last 6 months before procedure<br>• Type 1 diabetes<br>• Type 2 diabetes requiring >30 units of basal insulin/day<br>• Known severe pulmonary hypertension with mPAP > 30<br>• Symptoms consistent with unstable coronary artery syndrome<br>• New York Heart Failure Class III or greater | |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | • Current fever<br>• Active infection<br>• Contraindication to sedation<br>• Allergy to lidocaine<br>• Pregnant or < 3 months post-partum | |
| Colonoscopy with biopsy and potential polypectomy with procedural sedation, with or without upper endoscopy with biopsy | | All participants are eligible for this test through random assignment. Participants with relevant symptoms (as outlined in the applicable SOP) will be oversampled for assessment as follows:<br><br>ANY of the following:<br><br>• Participants with a score of 11 or more on the RECOVER PASC score (change/loss of smell/taste, post-exertional malaise, chronic cough, brain fog, thirst, palpitations, chest pain, fatigue, change in sexual desire or capacity, dizziness,shortness of breath, and sleep apnea)[71]<br>• Persistent gastrointestinal symptoms | • Lack of safe discharge plan (needs adult companion at time of release)<br>• Myocardial infarction or stroke within last 6 months<br>• Abdominal surgery/bowel injury within last 3 months<br>• Known or suspected abdominal infection, or colonic perforation/toxic megacolon/fulminant colitis<br>• History of colectomy<br>• Taking blood thinning medication, unless prescribing provider confirms they can be held for 7 days pre and 1 day post procedure, including<br>  ○ Direct oral anticoagulants (e.g. rivaroxaban, dabigatran, apixaban or edoxaban)<br>  ○ Vitamin K antagonists (e.g. warfarin)<br>  ○ Low molecular weight heparins (e.g. bemiparin, certoparin, dalteparin, enoxaparin, nadroparin, | Permissible Clinical Variation:<br><br>• Choice of preparation agent is at local clinician discretion and should consider patient factors (e.g. diabetes)<br>• If procedural sedation is selected, personnel administering sedation, choice of sedation agents, dosage limits and intervals, and credentialing must be managed according to local institutional policy, but may not exceed limits indicated in the SOP.<br>• General anesthesia is not permitted.<br>• Choice of 2.8 mm or 3.2 mm biopsy forceps size is at clinician discretion based on equipment availability and patient factors.<br>• Clinical polypectomy or biopsy may be performed (not for RECOVER analysis) if clinically warranted<br>• Monitoring during and post-procedure must be conducted in accordance with local institutional policy.<br><br>Additional information about test procedures |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | parnaparin, reviparin or tinzaparin)<br>○ Heparin<br>○ Anti-platelet agents (e.g. clopidogrel, ticagrelor, prasugrel)<br>○ Non-steroidal anti-inflammatory medications (e.g. ibuprofen, ketorolac, naproxen, diclofenac, indomethacin, celecoxib, meloxicam)<br>○ Aspirin > 81 mg (aspirin ≤ 81 mg may be included without medication hold)<br>○ Other aspirin-containing medications (e.g. Alka Seltzer, Excedrin)<br>• Iron supplements<br>• Intrinsic bleeding disorder (liver failure, uremia, other known bleeding disorder, history of excessive bleeding during procedures), including those with the following abnormalities (use most recent result within the prior year):<br>○ Hemoglobin < 10 g/dL<br>○ INR > 1.3<br>○ PT>13 seconds<br>○ aPTT>36 seconds<br>○ Platelets < 125,000 /mL | • Participants must alter food and liquid intake pre-procedure according to bowel preparation instructions; medications may be taken 2 hours or more before procedure<br>• Colonoscopy involves 6 total biopsies (1 double bite) from each of three locations: terminal ileum, proximal ascending colon and descending colon<br>• Upper endoscopy involves 4 biopsies (1 double bite) from each of two locations: the stomach and duodenum<br><br>Intra-procedure stopping criteria:<br>• Significant hemodynamic changes such as significant decrease in heart rate, hypotension, hypoxia, or tear in the intestines<br>• At the discretion of endoscopist or anesthesiologist |

| Procedure name(s) | Tier | Eligibility | Exclusion criteria | Additional information about test procedures and permissible clinical variation |
|---|---|---|---|---|
| | | | • Severe immunosuppressive medications including:<br>  ○ Chemotherapy<br>  ○ Cytotoxic therapy<br>  ○ High dose steroids (e.g., daily prednisone ≥ 10 mg)<br>  ○ Biologic agents<br>• End stage disease including:<br>  ○ Renal, lung, liver, or heart failure<br>• Any other conditions with significant limitations to life expectancy<br>• Inability to lay flat or lay still for 1 hour<br>• Contraindication to sedation<br>• Contraindication to lidocaine (upper endoscopy only)<br>• Pregnant or < 3 months post-partum | |

Table 9 Medications that should be stopped (if possible) prior to Autonomic Test Suite

| Medication | Days to stop | Medication | Days to stop | Medication | Days to stop | Medication | Days to stop |
|---|---|---|---|---|---|---|---|
| **A** | | **D** | | Lofexidine (Lucemyra) | 3 | Scopolamine | 3 |
| Abilify (Aripiprazole) | 7 | Darbid | 7 | Lomotil | 3 | Sectral | 1 |
| Acebutolol | 1 | Darifenacin (Enablex) | 4 | Lonamin | 4 | Serevent (Salmeterol) | 2 |
| Aclidinium (Tudorza Pressair) | 1 | Desipramine | 7 | **M** | | Seroquel | 2 |
| Acyclovir | 5 | Desloratadine(Clarinex) | 6 | Marezine | 4 | Sertraline | 5 |
| Adalat | 1 | Desoxyn | 3 | Maxair (Pirabuterol) | 1 | Serzone | 7 |
| Adderall | 3 | Desvenlafaxine (Pristiq) | 3 | Meclizine | 2 | Silodosin (Rapaflo) | 5 |
| Adipex | 4 | Desyrel | 7 | Mepenzolate | 2 | Sinequan | 5 |
| Akineton | 4 | Detrol | 2 | Mestinon | 1 | Solifenacin (Vesicare) | 7 |
| Akovaz (Ephedrine) | 1 | Dexmethylphenidate (Focalin) | 1 | Metaproterenol | 2 | Solabegron | 1 |
| Albuterol | 1 | Dexchlorpheniramine | 4 | Methyldopa | 1 | Solrimaferol (Sunosi) | 2 |
| Aldomet | 1 | Dicyclomine | 2 | Methylphenidate | 3 | Spiriva (Tiotropium) | 7 |
| Alfuzosin (Uroxatral) | 2 | Diltiazem | 2 | Metoclopramide | 3 | Strattera | 7 |
| Allegra | 2 | Dimenhydrinate (Dramamine, Triptone, Dimetabs) | 2 | Metoprolol | 5 | Sudafed | 2 |
| Alupent | 1 | Dimetabs | 2 | Midodrine (Proamatine) | 1 | Sunosi | 2 |
| Amantidine | 7 | Dimetane | 5 | Mirabegron(Myrbetriq) | 5 | Surmontil (Trimipramine) | 7 |
| Ambrisentan | 2 | Dimetapp | 2 | Milnacipran (Savella) | 2 | Symax | 2 |
| Aminophylline | 1 | Diovan | 3 | Mirapex | 7 | Symmetrel | 7 |
| Amitriptyline | 7 | Diphenhydramine | 2 | Modafinil (Provigil) | 4 | Synacort | 1 |
| Amlodipine | 4 | Dipivefrin | 3 | Myrbetriq (mirabegron) | 5 | Sunosi | 2 |
| Anafranil | 7 | Disopyramide (Norpace) | 1 | **N** | | **T** | |
| Anaspaz | 1 | Ditropan | 2 | Nadolol | 5 | Tagamet | 1 |
| Anisotropine | 2 | Donnatal | 5 | Nebivolol (Bystolic) | 7 | Tamsulosin (Flomax) | 4 |
| Antivert | 2 | Doxazosin | 5 | Nefazodone | 7 | Tasosartan | 5 |
| Apresoline | 2 | Doxepin | 7 | Nifedipine | 3 | Tavist | 4 |
| Arformoterol (Brovana) | 6 | Dramamine | 2 | Nisoldipine (Sular) | 4 | Telmisartan | 5 |
| Aripiprazole (Abilifly) | 7 | Duvadilan | 1 | Norflex | 2 | Tenormin | 5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Artane | 3 | Dyphylline (Lufyllin) | 1 | Normadyne | 2 | Terbutaline | 1 |
| Asenapine (Saphris) | 4 | **E** | | Norpramin | 7 | Tetrabenazine (Xenaline) | 2 |
| Aranidipine | 1 | Effexor | 5 | Nortriptylline | 7 | Theo-dur | 1 |
| Arformoterol (Brovana) | 5 | Elavil | 5 | Norvasc | 4 | Theophylline | 1 |
| Astelin (Azelastine) | 7 | Ellipta (umeclidinium bromide) | 3 | **O** | | Thorazine | 7 |
| Atarax | 2 | Enablex (Darifenacin) | 4 | Olanzapine | 3 | Tiotropium (Spiriva) | 7 |
| Atenolol | 5 | Ephedrine (Akovaz) | 1 | Olmesartan | 3 | Tizanidine (Zanaflex) | 1 |
| Atomoxetine | 5 | Eprosartan | 2 | Olopatadine HCL (Patanol) | 2 | Tudorza (Pressair Aclidinium) | 1 |
| Atrovent | 2 | Ergoloid mesylate (hydergine, gerimal) | 1 | Ondansetron | 1 | Tofranil | 5 |
| Aventyl | 7 | Escitalopram | 7 | Optimine | 3 | Tolterodine | 2 |
| Axid | 1 | Excedrin | 1 | Orphenadrine | 3 | Topiromate/Topomax | 4 |
| Azatadine | 3 | **F** | | Oxybutynin | 2 | Toprol | 2 |
| Azelastine (Astelin) | 7 | Famotidine | 1 | Oxytrol | 2 | Toprol XL | 5 |
| Azelnidipine | 5 | Fanapt (Iloperidone) | 5 | **P** | | Toviaz (Fesoterodine) | 2 |
| Azilect (Rasagiline) | 7 | Fastin | 4 | Paliperidone (Invega) | 5 | Tracleer (Bosentan) | 2 |
| **B** | | Fesoterodine (Toviaz) | 2 | Pamelor | 7 | Trandate | 2 |
| Barnidipine | 7 | Fetzima (Levomilnacipran) | 3 | Paroxetine | 7 | Transderm-Scop | 3 |
| Bellafoline | 2 | Flavoxate | 5 | Patanol (Olopatadine) | 3 | Trazdone | 7 |
| Benadryl | 2 | Flexeril | 7 | Paxil | 7 | Trental | 1 |
| Bentyl | 2 | Flomax (Tamsulosin) | 3 | Pentoxifylline | 1 | Treprostinil (Remodulin) | 1 |
| Benylin | 2 | Florinef | 3 | Pepcid | 1 | Trihexyphenidyl | 1 |
| Betapace | 4 | Fludrocortisone | 5 | Periactin | 2 | Trimipramine (Surmontil) | 7 |
| Biperiden | 4 | Fluoxetine | 7 | Phenergen | 2 | Trinalin | 3 |
| Bisoprolol | 3 | Fluvoxamine | 5 | Phentercot | 4 | Trintellix (Vortioxetine) | 9 |
| Bosentan (Tracleer) | 2 | Focalin (Dexemthylphenidate) | 1 | Phentride | 4 | Triptone | 2 |
| Brexanolone (Zulresso) | 1 | Foradil (Formoterol) | 3 | Phentolamine | 3 | Trospium (Sanctura) | 5 |
| Brintellix | 9 | Formoterol (Foradil) | 3 | Pindolol | 2 | **U** | |
| Bromocriptine (Cycloset) | 5 | Fluvoxamine | 5 | Pirabuterol (Maxair) | 1 | Umeclidinium bromide (Ellipta) | 3 |
| Brompheniramine | 5 | **G** | | Polaramine | 4 | Urispas | 5 |
| Brovana (Arformoterol) | 6 | Geodon (Ziprasidone) | 2 | Pramipexole | 7 | **V** | |
| Buproprion | 7 | Glycopyrrolate | 2 | Phentermine | 4 | Valpin | 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Buspirone | 1 | Guanfacine | 5 | Prazosin | 1 | Valsartan | 1 |
| Bystolic (Nebivolol) | 7 | **H** | | Pressair (Aclidinium Tudorza) | 1 | Ventavis (Iloprost) | 1 |
| **C** | | Hydralazine | 2 | Pristiq (Pristiq) | 3 | Ventolin | 2 |
| Calan | 2 | Hydrochlorothiazide (HTCZ) | 3 | Proamatine (Midodrine) | 1 | Verapamil | 2 |
| Carbinoxamine maleate | 4 | Hydroxyzine | 2 | Promethazine | 2 | Venlafaxine | 5 |
| Candesartan | 2 | Hyoscyamine | 2 | Procardia | 1 | Vesicare (Solifenacin) | 7 |
| CAPLYTA (Lumateperone) | 4 | Hyperstat | 2 | Procoralan | 2 | Viibryd (Vilazodone) | 6 |
| Cardura | 5 | Hytrin | 3 | Procyclidine | 2 | Vilazodone (Viibryd) | 6 |
| Carvedilol | 2 | **I** | | Propranolol | 2 | Viloxazine (Qelbree, Vivalan) | 1 |
| Catapres | 5 | Iloperidone (Fanapt) | 5 | Propulsid | 1 | Visken | 2 |
| Celexa | 7 | Iloprost (Ventavis) | 1 | Provigil (Modafinil) | 3 | Vistaril | 2 |
| Cetirizine | 2 | Imdur | 1 | Prozac | 7 | Vortioxetine (Trintellix) | 9 |
| Chlorpheniramine (Chlor-Trimeton) | 4 | Imipramine | 5 | **Q** | | Vyvanse (Lisdexamphetamine) | 3 |
| Chlorpromazine | 7 | Imodium | 5 | Quarzan | 1 | **W** | |
| Cimetidine | 1 | Incruse (Ellipta) (umeclidinium bromide) | 3 | **R** | | Wellbutrin | 7 |
| Cisapride | 2 | Indapamide | 4 | Rapaflo (Silodosin) | 5 | **X** | |
| Citalopram | 7 | Inderal | 2 | Rasagiline (Azilect) | 7 | Xenazine (Tetrabenazine) | 2 |
| Clarinex (Desipramine) | 6 | Invega (Paliperidone) | 5 | Reboxetine (Edronax) | 3 | Xopenex hfa (Levalbuterol) | 2 |
| Claritin | 2 | Irbesartan | 3 | Regitine | 3 | Xyzal (Levocetirizine) | 2 |
| Clemastine | 4 | Isopropamide | 7 | Reglan | 3 | **Y** | |
| Clenbuterol | 6 | Isoxsuprine (Duvadilan) | 1 | Remodulin (Treprostinil) | 1 | **Y**ohimbine (Aphrodyne, Yocon) | 1 |
| Clindium | 1 | **K** | | Requip Ropinirole) | 2 | **Z** | |
| Clomipramine | 7 | Kenadrin | 2 | Reserpine | 7 | Zanaflex (Tizanidine) | 1 |
| Clonidine | 5 | **L** | | Resperidone | 5 | Zantac | 3 |
| Cogentin | 2 | Labetalol | 3 | Risperdal | 5 | Zebeta | 2 |
| Concerta | 3 | Lavatol | 2 | Ritalin | 1 | Zegerid | 5 |
| Coreg | 2 | Letairis (Ambrisentan) | 2 | Ritalin LA | 3 | Ziprasidone (Geodon) | 2 |
| Corgard | 5 | Levalbuterol (Xopenex HFA) | 2 | Robinul | 2 | Zofran | 5 |
| Corlanor (Ivabradine, Procoralan) | | Levsin | 2 | **S** | | Zoloft | 7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cyclizine | 4 | Levocetirizine (Xyzal) | 2 | Salbutamol | 1 | Zulresso (Brexanolone) | 1 |
| Cycloset (Bromocriptine) | 5 | Levomilnacipran (Fetzima) | 3 | Salmeterol (Serevent) | 2 | Zyrtec | 2 |
| Cymbalta | 7 | Lexapro | 7 | Sanctura (Trospium) | 5 | | |
| Cyproneptadine | 3 | Librax | 4 | Saphris (Asenapine) | 4 | | |
| Cystospaz | 2 | Lisdexamphetamine (Vyvanse) | 3 | Savella (Milnacipran) | 2 | | |