Lung HIV Microbiome Project

Manual of Procedures

February 20, 2015 - Public

Sponsored by
National Heart, Lung, and Blood Institute
National Institutes of Health

U.S. Department of Health and Human Services
National Institutes of Health

National Heart Lung and Blood Institute

Maintained and distributed by
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Table of Contents

Abbreviations Used

ACD	Acid Citrate Dextrose
ACTG	AIDS Clinical Trial Group
ARDS	Adult Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BioLINCC	Biologic Specimen and Data Repository Information Coordinating Center
BSA	Bovine Serum Albumin
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disease
CPT	Cell Preparation Tube
CRF	Case Report Form
DACC	Data Analysis and Coordinating Center
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic Acid
EXHALE	Examinations of HIV Associated Lung Emphysema
FBS	Fetal Bovine Serum
HIV	Human Immunodeficiency Virus
HIPAA	Health Insurance Portability and Accountability Act
HMP	NIH Roadmap Human Microbiome Project
HSV	Herpes Simplex Virus
ICF	Informed Consent Form
IRB	Institutional Review Board
KS	Kaposi Sarcoma
LHMP	Lung HIV Microbiome Project
LN2	Liquid Nitrogen
MAC	Mycobacterium Avium
MIDAS	Multimodal Integrated Data Acquisition System
MOP	Manual Of Procedures
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
NPO	Nothing by mouth
OSMB	Observational Study Monitoring Board
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCP	Pneumocystis Pneumonia
PDR	Physician’s Desk Reference
PI	Principal Investigator
PPD	Purified Protein Derivative standard (Tuberculin skin test)
RBC	Red Blood Cell
RPM	Revolutions Per Minute
SAE	Serious Adverse Event
SC	Steering Committee
SCOR	Specialized Center Of Research
SOP	Standard Operating Procedure
TB	Tuberculosis
UP	Unanticipated Problem

1. OVERVIEW OF THE LUNG HIV MICROBIOME PROJECT

1.1. Background

The entire respiratory tract is constantly exposed to the external environment and serves as the portal of entry for many microbes. It is in the airways and lungs that numerous pathogens, opportunistic organisms, and commensals first encounter the host defense system. The characteristics and mix of organisms populating the respiratory tract, coupled with the state of local respiratory defenses (e.g., mechanical, immune, and non-immune) are key factors in determining whether a person remains healthy or develops acute, chronic, or latent infection. Inflammation in the airways and lungs and how it progresses or is resolved affects not only local immune mediators and primary gas exchange functions, but may also have consequences for the whole immune system and the progression of diseases in other organs.

HIV-infected individuals are at very high risk of developing pneumonias caused by pathogenic and opportunistic microorganisms. These respiratory infections frequently cause morbidity, and they are often life threatening. They also may increase the rate of replication of HIV, accelerating the course of HIV disease. HIV-infected individuals experience a decremental of lung function following pneumonias which are not observed in normal, HIV-uninfected populations. Furthermore, lung infections and microbial colonizations are suspected in the etiology of HIV-associated emphysema and pulmonary hypertension. A new issue that is becoming increasingly more important is that lung infections may play a role in inducing the immune reconstitution syndrome seen in some HIV-infected patients following initiation of multi-drug antiretroviral regimens.

1.2. Mission Statement

The Lung HIV Microbiome Project (LHMP) is a collaborative project funded by the National Heart Lung and Blood Institute (NHLBI) to characterize and analyze changes in the lung microbiota and other sites. The LHMP is committed to addressing hypothesis driven research, conducting the highest quality research through a cooperative multi-center and multi-study network, and informing the diagnosis, prognosis and treatment of HIV infection and respiratory complications.

1.3. Goals

Determining the lung microbiome enables us to learn which microbes are present and where they are located. Starting from this basic knowledge, we will be able to study how these microbes grow, interact among themselves, relate to other microbial species co-existing in the same niche and with the cells in the lung, and how they are altered by other environmental factors such as the physical environment and cigarette smoke. This knowledge will enhance our understanding of the role of the lung microbiome in preserving health or causing disease and also in the divergent effects observed in HIV-infected versus uninfected individuals. Knowledge of the lung microbiome and that of other components of the respiratory tract in health and diseased states may lead to the identification of predictors of disease progression and therapeutic targets for translation into better preventive and treatment strategies. In the future it may also provide an opportunity to study gene-gene and gene-environment interactions under specific disease conditions and also interactions of the lung microbiome with microbial populations located elsewhere in the respiratory tract or other organs.

1.4. Lung HIV Microbiome Project (LHMP) Overview

The Lung HIV Microbiome Project (LHMP) as a cooperative network is responsible for carrying out both joint protocol(s) and integrating independent protocols that benefit from increased sample size.

1.4.1. Participating Institutions

The LHMP network is comprised of representation from the National Heart, Lung, and Blood Institute (NHLBI), six initial clinical centers and their associated sequencing centers, and a data analysis and coordinating center. Detailed contact information on all study staff at the participating institutions is provided in the LHMP directory on the study website.

1.4.1.1. National Heart, Lung, and Blood Institute (NHLBI)

As the contracting institution, staff members at the NHLBI in Bethesda, Maryland are responsible for the overall design and conduct of the LHMP. The project officer will oversee the study design, implementation, data quality, and information dissemination.

1.4.1.2. Clinical and Sequencing Centers

Each of the clinical and sequencing centers are responsible for conducting their own individual project proposed in their grant applications and for participating in the development and prioritization of common protocols for harvesting biospecimens and for genetic analysis of the lung microbiome. Each Clinical/Sequencing Center designs their own project, stores the biospecimens, transmits data electronically, and analyzes the data generated for their individual projects. The clinical/sequencing centers are required to collaborate with the Data Analysis and Coordinating Center (DACC) and the other clinical/sequencing centers that are part of the LHMP. Centers agree to share data and biospecimens. They are responsible for conducting the research (participant recruitment and implementation of all study procedures), analyzing and interpreting their own research results, and disseminating research findings.
Clinical/sequencing centers are responsible for working with the DACC to develop common definitions, setting milestones and continued submission of key data to the DACC for a collaborative database. Each clinical/sequencing center is required to participate in a cooperative and interactive manner with all other clinical/sequencing centers and the DACC in all aspects of the project.

1.4.1.3. Data Analysis and Coordinating Center (DACC)

The Data Analysis and Coordinating Center (DACC) supports the individual projects at each of the centers through the development and implementation of the agreed upon collaborative protocols at each of the centers. The DACC is responsible for the data acquisition and data management of all phenotype, sequencing and specimen characterization data which includes keeping an inventory of all biospecimens collected at the various centers. The DACC provides support for quality control, particularly for collaborative protocols. The DACC also coordinates all meetings and conference calls including preparation of printed materials for meetings/conference calls.

The DACC initiates and coordinates activities to promote standardization of lung microbiome research vocabularies and methodologies and coordinates the LHMP activities with NHLBI bioinformatics programs. These are developed to be compatible with Roadmap HMP whenever possible.

The DACC is responsible for arranging meetings and conference calls, preparing reports, implementing a web-based system to coordinate joint project activities, and facilitating the

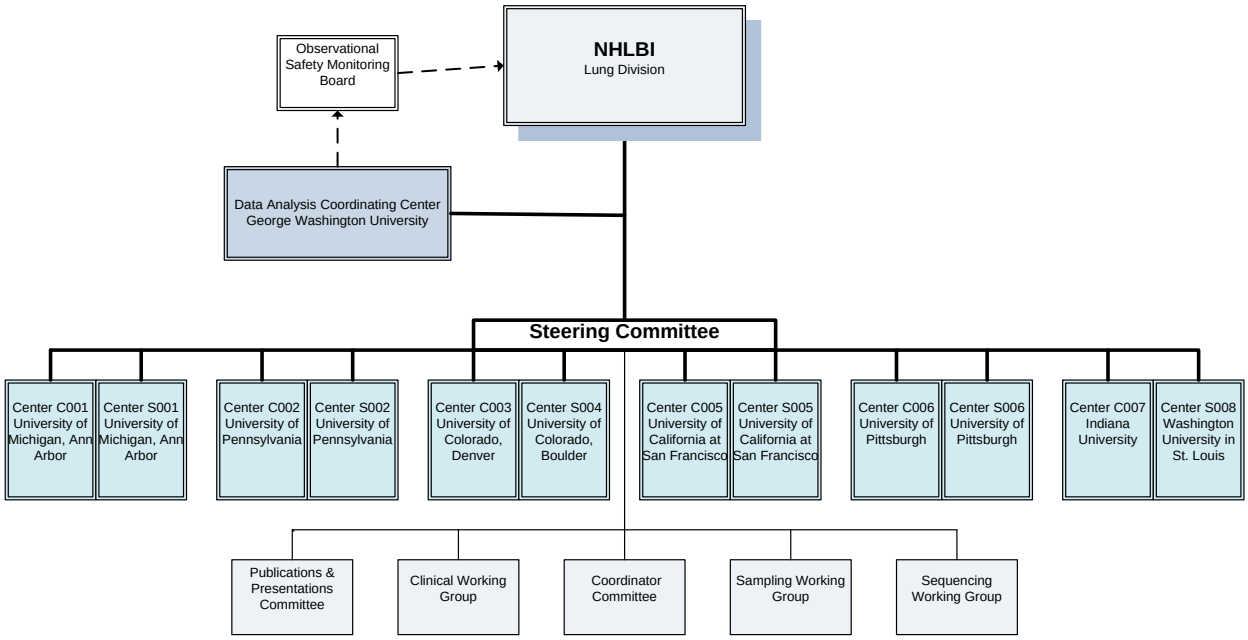
sharing of data and specimens. The DACC provides data management and quality control, develops forms, and maintains common data sets. The DACC provides statistical support in designing and conducting collaborative protocols and in analyzing data. The DACC also supports specimen banking activities, as needed, and activities associated with preparing and submitting presentations and publications. The DACC provides support to the clinical/sequencing centers in monitoring the milestones developed by the investigators.

The DACC provides editorial and meeting coordination for manuscript preparation, coordination of the activities of the Steering Committee (SC), Observational Safety Monitoring Board (OSMB), and overall study coordination and quality assurance.

1.4.2. Organizational Structure

The structure of the LHMP is designed to enhance collaboration and awareness among all participating centers. The organization structure is summarized in the following diagram.

Lung-HIV Microbiome Project Organizational Structure



1.4.2.1. Steering Committee (SC)

The Steering Committee (SC) is the overall governing body for LHMP. The committee consists of representatives from each of the clinical/sequencing centers, the DACC, and NHLBI. The committee meets monthly via conference call and bi-annually in person. The members from the clinical centers and sequencing centers will include the Principal Investigators and Co-Investigators. Steering Committee voting membership shall consist of the principal investigators (i.e., cooperative agreement awardees), the NIH Project Scientist, and the Chairperson. Each full member will have one vote, with lack of response being counted as acceptance. Members of the Steering Committee will be required to implement policies approved by the Steering Committee.

1.4.2.1.1. Overall Responsibility

The Steering Committee is primary responsible for the general organization of the Lung HIV Microbiome Project, standardizing vocabulary, methodology, forms, prioritizing proposed topics for joint investigations, finalizing protocols, facilitating the conduct and monitoring of the studies, reporting study results in a timely manner, and working with the NHLBI to promote dissemination of the findings. Topics for the joint protocols may be proposed to and prioritized by the Steering Committee. The entire Steering Committee will provide input and will be responsible for assuring development of a common protocol(s) in areas implemented by the clinical/sequencing centers. The Steering Committee has final responsibility for approving joint protocols and protocol revisions before review by the OSMB.

1.4.2.2. Publications Committee

The goal of the Publications Committee is to facilitate and coordinate collaborative dissemination of the LHMP results through high-impact publications, without delaying those groups that have already accumulated substantial data.

1.4.2.3. Other Committees and Working Groups

Other committees are established as needed during the development, implementation, and reporting phases of the project. Three working groups have been established during the development phase of the project

- The Sampling Working Group is tasked with assuring the samples are collected and stored using the same or similar processes.
- The Sequencing Working Group is tasked with assuring that samples are processed using the same methods and with the development of procedures to assess quality assurance between labs.
- The Clinical Working Group is tasked with the establishment of common definitions of phenotypes and variable definitions and with the development of questionnaires and forms to collect this data.

1.5. Confidentiality Procedures

The study leadership outlines and enforces participant and study data confidentiality policies. Study staff should be instructed in their responsibilities regarding data safeguards and cautioned against the release of data to any unauthorized individuals, unless such a release is approved by the study leadership and NHLBI and is not in violation of applicable Federal and state laws.

This section of the MOP discusses the safeguards been put in place by the LHMP to ensure participant confidentiality and data security.

Participant confidentiality safeguards includes, but is not limited to:

- ***Data flow procedures*** – data identifying participants should not be transmitted from study centers to the DACC.
- ***Electronic files*** – data identifying participants that are stored electronically should be maintained in an encrypted form or in a separate file.
- ***Forms*** - forms or pages containing personal identifying information should be separated from other pages of the data forms.

- ***Data reports*** - participant name, name code, hospital chart, record number, Social Security Number, or other unique identifiers should not be included in any published data.
- ***Data distribution*** - data that contain participant name, name code, or other identifiers easily associated with a specific participant should not be distributed.
- ***Data disposal*** - computer listings that contain participant-identifying information should be disposed of in an appropriate manner.
- ***Access*** - participant records should not be accessible to persons outside the study without the express written consent of the participant.
- ***Storage*** - study forms and related documents retained both during and after study completion should be stored in a secure location. If computers are used to store and/or analyze clinical data, the DACC and or the study center investigators should address the following elements of computer security to ensure that the data remain confidential:
    - Passwords - Passwords limit access to data and must be changed on a regular basis.
    - User Training - Study staff are to be trained on accessing the system and on the importance of the system security.
    - System Backups - Backup copies of electronic data are made at specified intervals. Backups are stored securely.

1.6. Observational Safety Monitoring Board (OSMB)

The LHMP Observational Safety Monitoring Board (OSMB) is appointed by the NHLBI to help assure the integrity of the project through monitoring data acquisition for comprehensiveness, accuracy, and timeliness, and monitoring other concerns such as participant confidentiality. An NHLBI staff member, who is not the primary program official, serves as the Board's Executive Secretary. The OSMB makes its recommendations to the Institute's Office of the Director. The Board members are to reflect the necessary expertise for the LHMP. The OSMB membership will consist of a minimum of three members who will collectively provide representation in the relevant disciplines, e.g., pulmonary medicine, immunology, microbiology, biostatistics, and ethics. The OSMB will assure the NHLBI that every effort is made to protect the participants. NHLBI Guidelines: http://public.nhlbi.nih.gov/ocr/home/GetPolicy.aspx?id=8

The Observational Safety Monitoring Board will meet approximately semi-annually either by telephone conference or in-person meetings in Bethesda, Maryland.

1.7. LHMP Timeline

<table>
<tr><td>Year 1</td><td></td><td>Year 2</td><td></td><td>Year 3</td><td></td><td>Year 4</td><td>Year 5</td></tr>
<tr><td>Design & develop single site studies
Hire & train study staff
IRB submit</td><td>Single site protocols begin……</td><td>Single site protocols continue recruitment, data collection, specimen banking.....</td><td>Analysis & report initial single site protocols

……..</td><td colspan="2">Second generation of single site protocols begin recruitment, data collection, specimen banking.....</td><td colspan="2">Analysis & reporting continues

……</td></tr>
<tr><td colspan="8">Web development and maintenance throughout ……..</td></tr>
<tr><td colspan="8">Monthly Steering Committee throughout ……..</td></tr>
<tr><td colspan="2"></td><td>OSMB</td><td colspan="2">OSMB call</td><td>OSMB</td><td>OSMB call</td><td>OSMB</td></tr>
<tr><td colspan="3"></td><td>Clinical/seq ctr site visits</td><td>Clinical/seq ctr site visits</td><td colspan="2">Clinical/seq ctr site visits</td><td></td></tr>
<tr><td></td><td>Design collaborative pilot study, DSMB review
IRB submit</td><td colspan="4">Collaborative pilot protocol begins staff training, participant recruitment, data collection, specimen banking.....</td><td>Analysis & reporting</td><td></td></tr>
<tr><td colspan="4"></td><td>Design collaborative pilot study, DSMB review, IRB submit</td><td colspan="2">Collaborative pilot protocol begins recruitment, data collection, specimen banking.....</td><td>Analysis & reporting</td></tr>
</table>

2. COLLABORATIVE PROTOCOL DEVELOPMENT AND PUBLICATION

The protocols for the collaborative studies are designed to extend research on the lung microbiome in HIV beyond what can be accomplished at one Clinical/Sequencing site. The collaborative studies explore an area of research related to the lung microbiome that requires the population and other resources from one or more collaborating clinical/sequencing center. Center specific projects are those unique to each site/center and generally require smaller sample sizes. The procedures outlined below are designed to encourage contact and collaboration across the LHMP, and to ensure transparency of the process leading to publications.

2.1. Publication Responsibility

Investigators have a responsibility to the public to make study results available as soon as possible. The Manual of Procedures (MOP) details the publication policy so that data are released appropriately, and manuscripts are communicated to other members of the project.

There is great potential for the cooperative approach with collaboration in the LHMP, particularly since all the participating centers are currently studying a variety of aspects of the lung microbiome. The LHMP and its Publications Committee collaborates on important group-level papers and also work individually or in smaller "thematic" groups. The purpose of the 4 tiers described below is to provide a framework for LHMP starting with group-level projects intended to result in one or more high-impact publications, while not delaying those groups that have already accumulated substantial data in a particular thematic area. **Regardless of what tier a project is determined to be, these guidelines apply if LHMP project funds are used on the project or listed as a funding source on the manuscript.**

2.1.1. Tier 1: Group-Level Projects

Ideally, these LHMP group-level projects:

1. Address an important scientific question of group interest;
2. Invite all pulmonologists as appropriate (to maximize scientific questions, i.e., geographic differences in the lung microbiome and maximize sample size/power) to participate;
3. Invite all lab Co-PIs as appropriate (depending on their interest and assuming that everyone has a defined role/assay) to participate; and
4. Involve the DACC and NHLBI.

Authorship will be decided by the study group.

2.1.2. Tier 2: Thematic Group Collaborations

Thematic group collaborations are ad hoc and involve individual groups working together according to a theme (i.e., studying similar cohorts, acute HIV and chronic HIV are two common cohorts – in which specimens can be shared) and/or according to capacity (e.g., sending specimens to specific lab that has unique capabilities/assays). Other areas of potential collaboration, based on similar interests and specific aims from different sites, include the effect of smoking and COPD on the lung microbiome and an interest in international studies.

These collaborations add value to participating groups and to the LHMP.

2.1.3. Tier 3: Individual Projects

<Not included>

2.1.4. Tier 4: Peripheral Projects

<Not included>

2.2. Concept Proposal (Applies to Tier 1 & Tier 2 Projects)

Preliminary concepts for collaborative protocols are formulated by an investigator from either clinical centers or sequencing centers and presented to the SC. The proposing investigator identifies the Lead Investigator. Leaders and responsibilities of the group members will be determined at the initiation of the proposal, final manuscript authorship will be determined based on merit.

2.3. Study Protocol Development (Applies to Tier 1 & Tier 2 Projects)

The Lead Investigator and writing group are responsible for concepts approved by the SC. The Lead Investigator and writing group works with the DACC on the development of a detailed protocol which includes an analysis plan. The protocol will be made available through a wiki on the LHMP web site and could include:

- a list of writing group members and authors,
- a rationale for the study including study hypothesis(es) and specific aim(s),
- estimates of the numbers of participants needed and the inclusion/exclusion criteria, with the rationale based on expected effect size and statistical power,
- a list of key clinical variables from LHMP forms and justification for any requested new variables not contained in the LHMP forms (note: variables not contained in LHMP forms are discouraged and will not be approved unless there are compelling reasons to collect them),
- details of specimen collection, processing, and storage requirements if different from LHMP SOPs,
- the expected project duration,
- the proposed end points,
- the required clinical center, sequencing center and DACC staff time, and other needed materials and resources, and
- analysis methods.

2.4. Collaborative Protocol Implementation

Once a protocol is finalized, the Writing Group works with the DACC, as needed, to:

- complete the study forms, including the informed consent form (ICF), (see section on Form Change Procedures),
- generate generic IRB materials, and
- modify applicable sections to the Manual of Operations.

If needed, the DACC sets up the collaborative protocol forms in the Multimodal Integrated Data Acquisition System (MIDAS) for web data entry and or electronic data transfer. The DACC trains and certifies designated staff at LHMP Centers.

If necessary or requested, the DACC holds a training session for coordinators and certifies that the participating clinical centers meet all requirements for beginning the study. Individual clinical centers begin recruitment when these and any other NHLBI requirements are met, and they have local IRB approval.

2.5. Analysis/Manuscript Drafting/Review/Submission (Tier 1 & Tier 2 Projects)

There are various approaches for collaborative manuscript development; the approach taken for each project will be at the discretion of the Writing Group Chair.

The Writing Group prepares and revises the manuscript draft. The Lead Investigator/Writing Group Chair provides the publications committee with a detailed outline of a manuscript prior to writing the paper. The Lead Investigator must submit the final, pre-submission version of a manuscript to the Publications Committee for comments. Members of the committee have 5 working days to respond. If there are issues that cannot be readily resolved, further discussion will be brought to the Steering Committee. The authors must also send a final version of a manuscript to NHLBI for their information upon submitting for publication. The DACC maintains tracking of these submissions.
The Writing Group determines the corresponding author who submits the final, reviewed manuscript to the selected journal.

2.6. Individual Center Projects (Tier 3 & 4): Manuscript sharing

2.6.1. Tier 3.

<Not included>

2.6.2. Tier 4.

<Not included>

2.7. Review by Publication Committee (Tiers 1-3)

- The paper to be reviewed is sent to the DACC, in PDF format, watermarked "Confidential – Unpublished – Please do not share" or "For Review only"
- The DACC will send the paper to the Publication Committee, requesting that comments be sent to the DACC within 5 days.
- The DACC will compile all of the responses and send them to the chair of the Publication Committee.
- The chair of the Publication Committee will then provide the feedback to the lead author.

2.8. Adjudication in the event of conflict

<Not included>

2.9. Abstracts

<Not included>

3. ELIGIBILITY CRITERIA

3.1. Screening

Participant eligibility for the LHMP is confirmed by each center according to the parameters of their individual protocols. The collaborative protocols may also define specific inclusion/exclusion criteria or eligibility as agreed to by the Steering Committee. The LHMP Participant ID number is assigned after informed consent is obtained. The LHMP Participant ID is assigned by one of the following methods, depending on how the participant's data is transferred to the DACC:

- For centers using the DACC's data entry system (MIDAS) to enter directly into the LHMP database, the LHMP Participant ID is assigned upon creation of the Base record when data is first entered for that participant.
- For centers transferring electronic data files to the DACC via secure File Transfer Protocol (FTP), the LHMP Participant ID is assigned when the data files are processed into the LHMP database by the DACC's Extract, Transform, and Load (ETL) system.

3.2. Prior Review of Participant Records

Record review and participant recruitment efforts are unique to each center and are conducted according to each center's independent protocol(s).

4. INFORMED CONSENT

Obtaining informed consent is essential prior to involvement in human subject research. The purpose of the informed consent process is to protect an individual's rights. The principles guiding this process are those of respected persons, beneficence and justice. Each center will document the consent process by use of consent forms that have been approved by their institution according to the individual protocol.

Each participating center follows all guidelines of its own governing Institutional Review Board (IRB) in obtaining informed consent. All study participants must sign an Informed Consent Form (ICF), provided by the participating center, before being screened. Each site prepares its own ICF and then submits the ICF to the DACC, which will review the ICF with the NHLBI for the required elements. After this review is complete, then each center submits the ICF to their IRB for approval. After obtaining IRB approval, the center sends copies of the IRB approval letter and stamped ICF to the DACC.

Each institution's IRB reviews the ICF annually. Unless the IRB requests a change, or the study changes in a way that directly affects the participant (e.g., procedure change or addition), the original signed ICF is valid for the entirety of the study. If a change is necessary, active participants may need to sign an addendum to the ICF. If recruitment is still in process, then new participants will sign an ICF that incorporates any changes or additions.

Each center is responsible for maintaining up to date copies of the IRB letter and stamped ICF on file with the DACC.

The DACC will maintain a record of the level and or type of consent the participant has consented to, using the Consent Record Form.

4.1. Obtaining Consent

Written consent is obtained before any procedures, tests, or examinations are performed. The Principal Investigator (PI), or designee, explains the study's purpose, methods, risks, and benefits to the potential participant. The first step of the process is the assessment on the part of the PI, or designee, of whether the individuals providing consent have the capacity to make this decision. If decisional capacity is impaired, then consent cannot be obtained.

The PI, or designee, reviews the ICF with participant and answers any questions they may have. As a part of the process the participant is told about the study procedures, what is expected in terms of time commitment, and the risks and benefits of participating in the study. The ICF contains the required information that is to be disclosed. Each portion of the ICF should be explained in detail. When obtaining consent, the PI and/or designee should explain the following:

- Purpose of the Lung HIV Microbiome Project,
- Purpose of the individual study being conducted at that center,
- Procedures – A clear description of the study in terms of the types of measurements taken, the time commitment to perform the measurements, and the overall length of commitment,
- Benefits associated with participation,
- Risks, discomforts, and precautions,
- Alternatives to participation,
- Confidentiality of information,

- Availability of information – Participants should be told who to contact if they have questions about study procedures (the PI) or questions about their rights as a research subject (the chair of the local IRB),
- The right to withdraw from the study at any time,
- Tiered consent: regarding information about specimens sent to repositories,
- Compensation of participants for time involved/ study visits.

After providing information regarding the procedures, risks, and benefits, it is important to give the participant an opportunity to ask questions. Sufficient time must be provided to answer all participant questions prior to signing the consent. It is important to ascertain whether the participant understands and appreciates what they are agreeing to do. Not only does this protect their rights, but it also minimizes the risk of the participant dropping out of the study.

Care is used throughout the recruitment and informed consent process so a participant does not feel coerced into participation. They should be reassured that their future care at the institution will not be affected should they decide not to participate.

The participant must sign the consent form in the presence of a qualified staff member Lung HIV Microbiome Project study staff indicating their consent to participate in the study. At least one LHMP Coordinator or investigator witnesses the signature. Additional witnesses may be required by the local IRB. All signatures on the consent form are in a non-erasable ink. The participant's LHMP Participant ID number DOES NOT appear on the consent form. A copy of the signed consent form is given to the participant to keep.

4.2. Time Constraints for Obtaining Consent

While a participant may be screened for the LHMP, no other LHMP study interviews or procedures are performed until written consent has been obtained. Consent is obtained far enough in advance of procedures to allow for the completion of other LHMP interviews or tests without interfering with preparations for the procedures or unduly stressing the participant physically or mentally.

4.3. Lung HIV Microbiome Project Consent Form Handling

The LHMP Clinical Centers may have an informed consent form for each contributing parent study. Additionally there may also be the following:

- Separate consent forms for cohorts within a parent study,
- Separate consent forms for centers participating in the same parent study,
- Addendums to the above consent forms for supplementary procedures, such as bronchoscopy.

Consent forms and/or addendums may allow for partial exclusions. Participants have the opportunity to select specific elements which they consent to:

- Procedures performed,
- Data sharing - authorized institutions (including the DACC,)
- Specimen storage and future use.

Signed Informed Consent Forms are important legal documents. These original forms should be kept in the participant's LHMP file together with their other LHMP study forms and documents, or in a separate Informed Consent Binder that contains all Informed Consent Forms for participants at that center. These forms are not part of the participant's institutional medical records but are a part of their participation in the LHMP. The LHMP Participant ID should not be

written directly on any Informed Consent Form. Informed Consent forms and study forms are examined during site visits by DACC staff, and must be made available.

4.4. Essential Elements of Informed Consent

The following list is derived from Informed Consent in Research Involving Human Participants, NIH Guide, Volume 25, Number 32, September 27, 1996 (http://grants.nih.gov/grants/guide/rfa-files/RFA-OD-97-001.html):

- a statement that the study involves research, an explanation of the purposes of the research and the expected duration of the participant's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;
- a description of any reasonably foreseeable risks or discomforts to the participant ;
- a description of any benefits to the participant or to others that may reasonably be expected from the research;
- a disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant ;
- a statement describing the extent, if any, to which confidentiality of records identifying the participant will be maintained;
- for research involving more than minimal risk, an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they consist of or where further information may be obtained;
- an explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the participant ;
- a statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled, and that the participant may discontinue participation at any time without penalty or loss of benefits to which the participant is otherwise entitled.

In addition, when appropriate, one or more of the following elements of information will be provided to each participant:

- a statement that the particular treatment or procedure may involve potential risks, discomfort, or adverse effects, a description of any reasonably foreseeable risks or discomforts to the participant and risks that are currently unforeseeable;
- anticipated circumstances under which the participant's participation may be terminated by the investigator without regard to the participant's consent;
- the consequences of a participant's decision to withdraw from the research and procedures for orderly termination of participation by the participant.

4.5. HIPAA Authorization

All participants must sign a Health Insurance Portability and Accountability Act (HIPAA) authorization form. Without it, participating clinical Centers cannot share participant data with the LHMP Study Group. Each participating Center follows all guidelines of its own governing IRB regarding this document. It must include the text that specifically mentions that data from the participant's medical records will be shared with a data coordinating center or agents for the NIH or NHLBI.

The original form is kept in the participant's study file and a copy is given to the participant. The date that the participant signed the ICF and HIPAA authorization form is recorded on the Consent Record Form, and the information on this form will be entered into the LHMP study database either through MIDAS or electronic transfer.

4.6. Revoking Consent and Reinstatement

A participant may revoke their consent to collect new study data and/or HIPAA authorization to share newly collected data and specimens with the LHMP Study Group. They may revoke verbally or in writing. If the participant revokes verbally, then the clinical center must mail a letter of confirmation to the participant. The center updates the participant's Consent Record Form by recording the date that consent and/or authorization was revoked. The information on this form is then updated in the LHMP study database either through MIDAS or electronic transfer. Data and specimens collected up until the time of consent revocation will remain the property of the clinical center; however all attempts will be made to trace and discard data and specimens previously collected.

If a participant requests reinstatement, then they must sign a new ICF and a new HIPAA authorization form. The center updates the participant's Consent Record Form by recording the date that the new documents were signed and the information on this form is then updated in the LHMP study database either through MIDAS or electronic transfer.

5. Definition of Events and Procedures for Reporting

5.1. Unanticipated Problem (UP)

"Any incident, experience, or outcome that meets all of the following criteria: 1) unexpected 2) related or possibly related to participation in the research; and 3) suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized."
http://www.nhlbi.nih.gov/crg/glossary.php#unanticipated

5.2. Adverse Event (AE)

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.
http://www.nhlbi.nih.gov/crg/glossary.php#adverseevent

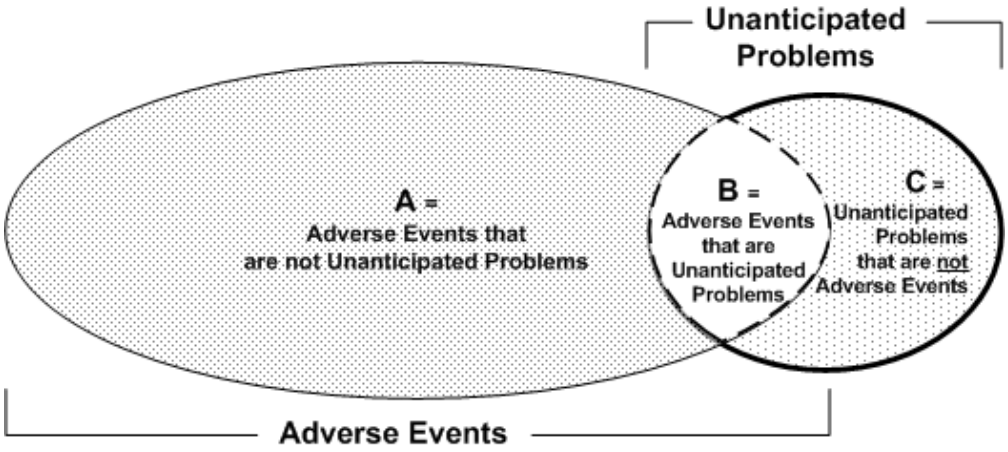
5.3. Serious Adverse Event (SAE)

Any adverse event that is fatal or life threatening, resulting in significant or persistent disability, requiring (prolonged) hospitalization, resulting in a congenital anomaly/birth defect, or that which investigators judge to represent significant hazards or harm to research subjects.
http://www.nhlbi.nih.gov/crg/glossary.php#unanticipated

5.4. Policy

The LHMP will follow the NHLBI policy which includes a requirement for reporting adverse events (AE) and unanticipated problems (UP) for the clinical research studies funded in whole or in part by National Heart, Lung, and Blood Institution (NHLBI) extramural programs.
As the LHMP consists of a cooperative group of individual parent studies, which should already be reporting these events through established procedures the DACC is requesting that each center provide a copy of these reports to the DACC at the same time the reports are provided to the primary governing body. All identifying information is to be removed prior to sending to the DACC. LHMP Centers that have approved reporting forms available will send a copy of that report by fax or email to the DACC. If no such form is available there are LHMP templates available in Appendix 7.

The following diagram summarizes the general relationship between adverse events and unanticipated problems:



Under 45 CFR part 46: Do not report A, Do report (B+C)

- Studies involving human subject research must include procedures for identifying, monitoring, and reporting adverse events (AE) and unanticipated problems (UP). For clinical trials and studies with greater than minimal risk, these procedures should be described in the study's Institutional Review Board (IRB)-approved data and safety monitoring (DSM) plan which is sent to the NHLBI. (See NHLBI Data and Safety Monitoring Policy)
- Expedited reporting to the NHLBI Program Official or Project Officer (PO) is required for unanticipated problems(UP) or unexpected serious adverse events (SAE) that may be related to the study protocol as follows:

  Any event or problem that is

  - **Unexpected;**

  AND

  - **Possibly, probably, or definitely related to study participation;**

  AND one of the following:

Is fatal, life-threatening, or serious (SAE + UP)	**Within 7 calendar days**
Suggests greater risk of harm to study participant(s) than was previously known or recognized(UP)	**Within 30 calendar days**

  http://www.nhlbi.nih.gov/funding/policies/adverse.htm

- Expedited SAE/UP reports to NHLBI should include the following elements:
  - Study title, grant/contract number, PI name
  - Description and date of the event or problem, including why it merits expedited reporting
  - When available, date(s) when the event was reported to applicable governing bodies (e.g., IRB, Food and Drug Administration)
  - Any corrective action planned or taken in response to the event or problem (e.g., study suspension, consent or protocol changes, additional training or security measures)

- Investigators are responsible for reporting to and following the guidance of any other applicable oversight bodies, including (but not limits to) the following:
    - Institutional Review Broad (IRB) or Ethics Board
    - Office for Human Research Protections (OHRP)
    - Food and Drug Administration (FDA)
    - Monitoring entity ---- e.g., independent monitoring group, data and safety monitoring board (DSMB) or observational study monitoring board(OSMB)
    - NIH Office of Biotechnology Activities (OBA)

  NOTE: Communications from the above oversight bodies regarding any applicable SAE/UP must be reported to NHLBI according to the (see NHLBI Data and Safety Monitoring Policy)

6. PARTICIPANT ASSESSMENT PROCEDURES

This chapter provides information related to the procedures to assess participants in LHMP.

6.1. Interviews

Advance work by the clinical center staff facilitates completion of interviews and reduces waiting time. It is suggested that clinical center staff follow the procedures described below to properly prepare for the participant's visit.

The Coordinator, or center assigned staff in each clinical center assembles packets so that forms and other materials are readily available. The packets include a consent form for the potential participant to sign and the appropriate LHMP forms for that visit. The Site Participant ID number assigned to the potential participant is written on the forms in the header spaces provided.

At the beginning of the interview, the interviewer determines the potential participant's willingness to participate in the study and, if willing, obtains informed consent. This is estimated to take about .5 to 1 hour to complete.

The interviewer proceeds to the demographic and history questionnaires. This is estimated to take about 1 to 2 hour(s) to complete. All interviews are conducted in a private, quiet office environment that facilitates the participant's cooperation and puts them at ease.

During an interview, if the participant expresses doubt as to the meaning of a question, repeat it exactly. Emphasizing individual words or phrases often makes the meaning clear. Further explanation may be needed, but do not cross-examine the respondent. When, after a brief explanation, doubt remains as to whether the answer should be 'yes' or 'no', the answer is recorded as "unknown or don't know".

6.2. Pulmonary Function Testing

The LHMP Centers that are performing the pulmonary function testing will conduct them in a manner which is in accordance to their institutional protocols.

7. SPECIMEN COLLECTION PROCEDURES

7.1. Purpose

An important objective of the Lung HIV Microbiome Project is the collection and sequencing of biological specimens to provide an understanding of the microbes present in the pulmonary system of individuals with and without positive HIV status, with or without chronic obstructive pulmonary disease (COPD). Through the collection of data regarding the interrelationship among lower and upper respiratory specimens which are obtained by bronchoscopy, it is proposed that it will be possible to distinguish upper respiratory tract contamination from true lower respiratory tract microbiota in the LHMP

7.2. Types of Specimen Collections

All participants agreeing to participate in the LHMP have provided informed consent for specimen collection. The following will be collected:

1. Oral wash sample,
2. Bronchial sample,
3. Blood specimens.

7.2.1. Oral Wash Collection and Processing

7.2.1.1. Biorepository Submission- Oral Wash

The Biorepository submission goal for Oral Wash is one to three 1 ml aliquots of neat Oral Wash, this is a required goal. Three Biorepository labels are provided as part of the Respiratory Label Set.

7.2.1.2. Procedure

- Prior to the oral wash: instruct participant to rinse their mouth with tap water and spit into sink.
- Oral Wash Collection: Ask the participant to gargle with 5-10 mls of a saltwater solution (1% saline) for 1 minute. Have them swish the saline around the oral cavity for 30 seconds, follow with a throat gargle. Continue swishing the saline for the remainder of the 60 seconds and then have the participant spit this wash into a sterile container. Keep on ice until processed or at -20°C until shipped.
- Oral Wash Control: 5mls of the saline solution is set aside in a sterile container. (Additionally some sites may open a tongue scraper and expose it to the air and then swish it in the saline.) This sample is processed in the same manner as the Oral Wash.

7.2.1.3. Notes:

- Oral washes should be processed immediately after collection. Alternately, they can be stored “neat” at -80°C, then thawed and processed immediately. (‘Immediately’ in this case generally means within 30 minutes would be reasonable, whereas anything over 2 hours would be considered a delay.)
- All steps should be performed in a biohazard hood and follow general lab safety procedures.

7.2.1.4. Reagents:

- .1% DTT/PBS: Add 1 gram of DTT (Dithiotritol) per 1000 ml of PBS (Phosphate Buffered Saline). Sterile filter, aliquot and store in -20°C.

- PBS/EDTA: Add 2.0 ml of 0.5M EDTA to 1 Litre of PBS. Sterile filter and store at 4°C

7.2.1.5. Processing:

- Collect one to three ml aliquots of neat OW. Label with Biorepository labels, store -80C.
- Divide in half the total remaining volume of the collected oral wash into two 15 ml conical.
- To one add an equal volume of .1% DTT/PBS. Place the cap back on, swirl and let sit at room temperature for 2 minutes.
- To the other OW sample add an equal volume of sterile PBS swirl and let sit at room temperature for 2 minutes.
- Centrifuge both samples at approx. 10,000g for 10 minutes.
- Pipette off supernatant and discard, or collect 1 ml aliquots of OW-supernatant, store -80C.
- Flick the pellet to resuspend and add 2.0 ml of PBS/EDTA to the tube.
- Pipette up and down to resuspend the pellet and transfer to two 1.5 ml microfuge tubes.
- Spin at 15,000g (13,000rpm, high speed) in a tabletop microfuge for 3 minutes.
- Pipette off supernatant and discard.
- Store dry cell pellets at -80°C or continue with DNA extraction. (Both the method of extraction and parent/child relationship will be recorded LHMP Tracking, Receiving and DNA Extraction forms and entered into MIDAS.)
- All centers will provide neat oral wash specimens to the repository, and will use the Biorepository provided labels.
-

7.2.1.6. Labeling

Each center, as appropriate, will follow ISBER 2008 Best Practices for Repositories (pg. 34).

7.2.1.6.1. Barcoding

Whenever possible, labels should be printed with a linear (one-dimensional or 1D) barcode that uniquely identifies the specimen. Under some circumstances, two-dimensional (2D) barcodes may be utilized. 2D barcodes have the advantage that scanning error rates may be lower and more information can be included on the label and may be optimal for use on smaller vials. Cost considerations may influence the systems selected for creating and reading barcodes.

7.2.1.6.2. Labels for Human Specimens

Human specimens should be labeled in such a way that protects privacy and confidentiality and is in compliance with applicable laws and institutional policies. The unique identifier for each specimen should be printed on the label in both barcode format and human readable form. Specimens should be labeled with a unique code not derived from information about the subject or related to its storage location in the repository. No other study or personal health information should be encoded in the specimen ID. In addition, the specimen ID should not be tied to the storage location.

7.2.1.6.3. Repository Labels

See Appendix 12 for instructions.

7.2.2. Bronchoalveolar Lavage Procedure and Processing

The following protocol contains guidelines and operating procedures to standardize the performance of bronchoscopy with airway washing and bronchoalveolar lavage for the Lung HIV

Microbiome Project. The protocol was adapted from a composite of protocols submitted by the Examinations of HIV Associated Lung Emphysema (EXHALE) project developed by Kristina Crothers, MD; the UCSF Airway Clinical Research Center Protocol developed by Prescott Woodruff, MD, MPH; and from protocols for processing of bronchoalveolar lavage specimens from the Seattle ARDS SCOR Core Laboratory by Lynn Schnapp, MD and from ACTG 723 by Homer Twigg, MD at Indiana University.

Patient care, including assessment and monitoring, topical anesthesia, and conscious sedation are performed at the discretion of the bronchosopy team and in accordance with local institutional protocols. All equipment for bronchoscopy is prepared according to institutional protocols, with the exception of specific procedures related to preparing, obtaining, and handling of the airway wash and bronchoalveolar lavage (BAL) specimen as outlined below.

7.2.2.1. Supplies and Equipment

1. Sterile polypropylene conical centrifuge tubes (50 ml and 15 ml)
2. Frosted Plus slides and coverslips
3. Shandon Diff Quick stain kit (# 9990700)
4. Hemocytometer
5. Cell counter
6. Cytoseal 60 (VWR cat # 48212-154)
7. Sterile normal saline
8. Microscope
9. Centrifuge (with appropriate rotors) for 50 ml and 15 ml tubes of BAL
10. 100 µm cell strainer (BD Falcon cat# 352360 Bedford, MA)
11. Shandon cytocentrifuge, metal slide holders, cytofunnels and filter cards [Shandon or similar (800)245-6212].
12. Common laboratory supplies: Pipette aid, graduated pipets, Pipetmen and pipette tips etc
13. Sterile beaker for mixing/pooling lavage fluid
14. Sterile RNase-free PBS
15. TRIzol or RNALater isolation buffer
16. RBC Lysing Solution: [1L sterile water, 1.09 g $KHCO_3$ (Sigma #P-9144), 8.12g NH_4Cl (Sigma # A-4514)]

7.2.2.2. Biorepository Submission – BAL

The Biorepository submission goal for BAL is five1 ml aliquots of accelular BAL, this is a required goal. Five Biorepository labels are provided as part of the Respiratory Label Set. Additionally one1 million cell aliquot of BAL dry pellet and one 1million cell aliquot of BAL in RNALater are optional submission calls, the Biorepository labels are provided as part of the Respiratory Label Set.

7.2.2.3. Procedures

7.2.2.3.1. General Patient Safety Considerations

- Written reports pertaining to pre- and post-bronchoscopy care, and operative reports must be available for review by the LHMP Sampling Committee team in order to document patient safety and adherence to protocols.
- Information recorded must conform to local institutional requirements, and must include total dose of lidocaine and medications used for conscious sedation.

- For participant safety, an upper limit of 600 mg or 9 mg/kg of lidocaine, whichever is less, is suggested for the total dose administered. This is based on a publication (Langmack EL, et al. Chest 2000; 117: 1055-60) that demonstrates levels below potentially toxic range after administration of up to 9 mg/kg of lidocaine in asthmatic subjects undergoing research bronchoscopy. Calculate total lidocaine amount administered by subtracting remaining lidocaine volume from your starting volume and calculate mg lidocaine delivered. The total lidocaine dose includes any aerosol mixtures used for initial airway anesthesia as well as that administered during the brochoscopic procedure.
- Sedatives and/or anxiolytics may be administered under appropriate monitored settings. All medications are given in small incremental doses as needed (local institutional guidelines for conscious sedation must be followed).
- If sedation with narcotics and/or benzodiazepines is administered, have narcan and flumazenil available, respectively.
- Bronchoscopy team should know the location of emergency equipment (crash carts). The procedure is aborted if an adverse event occurs.
- All Adverse Events and Serious Adverse Events are reported as required at each center and the centers are responsible for sending a copy of all Serious Adverse Event forms to the DACC.

7.2.2.3.2. Patient instructions

The Coordinator and/or medical team performing the bronchoscopy should confirm instructions, follow-up and potential complications with the participant.

<u>Pre-Bronchoscopy</u>

- Participants should be advised to dress comfortably.
- No food or drink (including water) for at least 6 hours prior to the procedure. Important scheduled medications (i.e. antiretroviral medications) can be taken with a sip of water.
- No smoking (of any substance) for at least 6 hours prior to the procedure.
- An adult should be available to drive and accompany participant home after the procedure.
- Routine laboratory testing and medication review is performed according to institutional protocols and the discretion of the bronchoscopist.

<u>Post-Bronchoscopy</u>

- Review post bronchoscopy instructions with the participant, including provision of instructions to contact the designated study physician on call at any time the participant thinks he or she may be suffering from a complication.
- The participant should be informed of the possibility of a low-grade fever and a sore throat the night after bronchoscopy, but should also be instructed to call for rigors, production of purulent sputum, hemoptysis, or shortness of breath not promptly relieved by albuterol inhalation.
- If the participant has received any sedation, he/she should be instructed not to drive for 8 hours, and should be accompanied by a friend on discharge home.

7.2.2.3.3. Performance of Bronchoscopy, Proximal Airway Wash, and Bronchoalveolar Lavage

General Preparation and Instructions

- Before local anesthesia is administered for broncholavage, have the participant rinse mouth for 60 seconds with 5 ml of Listerine and discard.
- Use room temperature 0.9% saline
- BAL Pre-wash Control – saline is drawn into a syringe and flushed through the clean bronchoscope. This is collected in a sterile container prior to the brochoalveolar lavage and processed with the BAL samples.
- Use 60 ml syringe to draw up and to instill saline
- Place specimen trap(s) on ice.
- Use minimal to no suction prior to collection of airway wash and lavage sample in order to decrease contamination of the bronchoscope with upper airway microbes.
- No air is introduced behind the wash or lavage sample and gentle suction is applied. This can be accomplished by placing the wall suction at the highest setting and controlling suction by bronchoscope, placing the wall suction to low suction, or using hand suction to withdraw fluid back into the syringe used to instill the saline.
- The samples are collected into specifically designated specimen traps (lidocaine free) on ice.
- The bronchoalveolar lavage is collected from the right middle lobe (first choice) or lingula.
- Total volume of saline instilled is dependent on patient tolerance. In patients without underlying lung disease 300 cc lavages (150 cc in two separate segments) are well tolerated. In patients with obstructive lung disease we recommend a 180 cc lavage, all into one segment. Expected return is around 50% of total instilled.

Bronchoalveolar Lavage (BAL)

- Wedge the bronchoscope in a subsegment of the right middle lobe or lingual
- Instil room temp sterile saline in 50 cc aliquots and apply gentle suction until the return slows. This is repeated twice more for an instillation of 150 cc.
- The bronchoscope is then wedged into another subsegment of the same lobe (for example, first in the medial and then in the lateral segment of the right middle lobe), and a second lavage with 50 cc x 3 is repeated. The total volume for lavage is thus 300 cc. If performing in patients with obstructive lung disease should use three 60 cc aliquots for a total volume of 180 cc.
- Return specimen traps are switched if full, and are collected on ice.
- If participant has difficulty with coughing, the BAL may need to be aborted.

Proximal Airway Wash

- Obtained after bronchoalveolar lavage.
- Performed in a segmental (not subsegmental) bronchus separate from where the lavage was performed. For example, can be done in an upper or lower lobe segmental bronchus.
- One 20 cc aliquot of sterile room temp saline is instilled and then removed immediately using high suction. A good return is about 5 ml.

7.2.2.4. BAL Handling and Processing

7.2.2.4.1. General instructions

- Store BAL fluid on ice after it is obtained and throughout processing.
- BAL must be processed within 60 minutes of being obtained (within 30 minutes if oxidant analyses planned).
- All work should be performed aseptically in bio-safety hood.
- All specimens highlighted in red represent the basic samples all LHMP sites should attempt to acquire for joint studies. For BAL fluid this represents a total of 35 ml.

7.2.2.4.2. Processing of BAL Fluid

1) **Bold red text refers to preparations specific for Biorepository and/or joint projects throughout.**
2) Transfer the specimen from the collection vials to a sterile container capable of holding all of the collected fluid. Mix gently.
3) Record total volume of return fluid.
4) **Remove 10 ml of fluid and store in one 15 ml polypropylene conical tube as "neat" or "whole" BAL. This sample is reserved for joint projects. Fluid should be stored at -80°C.s**
5) Use a 10 ml pipet to transfer the specimen thru a 100 µm cell strainer (cell strainer BD#352360 or Fisher # 877119, case of 50, individually wrapped) into a 50 ml conical tubes. If the total volume is greater than 50 ml you will need to use additional 50 ml conical tubes, splitting the specimen into multiple tubes.
6) Discard cell strainer.
7) Centrifuge all of the BAL fluid at 300 x g for 7 min.
8) **Decant the BAL supernatant from the 50 ml tube(s). Separate the BAL into 5 aliquots of 1 ml each. This is "acellular", filtered BAL fluid.** Use the Biorepository provided labels. The remainder of the fluid may be processed and used for site specific purposes.
9) Using sterile 1x PBS or protein free media of choice (i.e. RPMI), resuspend and combine cell pellets from all 50 ml conical tubes into one 50 ml conical.
10) If the pellet contains a large amount of red blood cells (small amounts are normal) it can be lysed by adding 6 ml of the RBC lysing solution, resuspending the cells and filling the tube to the top with lysing solution. Spin and wash 2X. Resuspend as described above.
11) Count and record total number of cells using a hemocytometer.
12) Adjust cell suspension to 1 million cell/ml final concentration by adding the necessary amount of 1X PBS or protein free media of choice. Most of the time your combined cell pellet suspension from step 8 will contain cells at greater that 1 million cells/ml. If your cell suspension contains less than 1 million cells/ml you will have to spin down the cell suspension at 300 x g for 7 min and resuspend appropriately. (***Example***: if you have 10.5 million total cells, you will need to add 10.5 ml 1X PBS or protein free RPMI.)

7.2.2.4.3. BAL Cell Cytospins

1) Make 8 cytospin slides
2) Cytospins are best using 25,000 to 50,000 cells per slide.
3) Liquid volume per cytospin slide is 350 ul
4) Thus, based on total cell count in pooled BAL cell preparation, remove 200,000 to 400,000 cells (200 – 400 ul of the 1 million cell/ml mixture) and add additional 1X PBS or protein free media to bring the total volume to 2.8 ml.
5) Load 350 ul sample into each cytospin funnel.

6) Spin for 5 minutes at 1350 rpm.
7) Perform diff-quick stain on two of the slides.
    a. Staining Cytospin Slides
        i. Stain and coverslip 2 slides fully. For remaining slides perform fixation step only (Step 1– do not coverslip, or stain). Allow fixed slides to air-dry before inserting into MDS supplied slide shippers.
        ii. Use Shandon Diff-quick kit:
        iii. Dip in fixative solution for about 10 seconds. Drain/blot excess fixative from end of slide.
        iv. Dip in Solution I for 5 seconds. Drain/blot excess solution.
        v. Dip in Solution II for 15 to 20 seconds. Drain excess solution. (Dipping in Solution II longer will heighten the contrast between blue cytoplasm and purple nucleus.)
    b. Rinse with distilled water. Allow water to gently run down slide, avoid disturbing cell layer.
    c. Check slide for staining quality under microscope. Re-stain if necessary, repeating steps ii – iv.
    d. Allow to air dry. Apply 2 - 3 drops of Cytoseal 60 adjacent to cell layer. Place coverslip on the slide at a 90^0 angle and gently lower to slide surface. Squeeze out bubbles. Allow to dry before placing inside mailers.
    e. Perform cell differential count on 300 cells. Record percentage of alveolar macrophages, neutrophils, lymphocytes, and eosinophils. Also record the percentage of ciliated airway epithelial cells and oral buccal mucosa squamous epithelial cells. If the combination of epithelial cells is > 15% upper airway contamination is present.

7.2.2.5. BAL Cell Handling

General comments

- The steps below highlighted in red represent the basic samples all LHMP sites should attempt to acquire for joint studies. This represents 3 million BAL cells.
- If enough cells are obtained from the bronchoscopy, sites are encouraged to also save cells in RNA preservation media and to cryopreserve cells. This would require an additional 13 million BAL cells.

7.2.2.5.1. Dry cell pellets and cells for future DNA and RNA analysis:

1) Place six 1 ml aliquots of the cells suspended in 1X PBS or protein free media (1 million cells/ml) into Sarstedt 1.5 ml microtubes with screw O-ring caps (Fisher catalog number NC9145227; Sarstedt cat# 72.730.005).
2) Centrifuge 500 g for 5 minutes.
3) Remove the supernatant carefully without disturbing the pellet
4) **Immediately freeze three of the aliquots as a dry cell pellet. These samples represent "BAL cells".** This can be done in a dry-ice-ethanol bath or liquid nitrogen.
5) Add 700ul RNALater or TRIzol to each of the other three aliquots and vortex for 1 min on highest setting.
6) The dry cell pellets and the RNALater aliquots are an optional contribution to the repository; all centers that will contribute cells for RNA analysis will use RNALater. If contributing to Biorepository, use the Biorepository provided labels. Store at -80C.

7.2.2.5.2. Cryopreserved BAL cells:

1) Place 10 ml of the 1 million cells/cc mixture into a 15 ml conical.
2) Centrifuge at 300 x g for 7 min and decant the supernatant.

3) Resuspend the cell pellet in two ml of freezing media. Freezing media consists of chilled 90% fetal bovine serum and DMSO at a 9 to 1 ratio (i.e. 10% DMSO, freshly made). Freezing medium may be stored up to one week.
4) Place 1 ml aliquots (5 million cells) into each of two cryovials.
5) Place the vials in “Mr. Frosty” (Nalgene #5100-0001) then into the –80°C overnight.
6) Remove vials from the Mr. Frosty cryocontainer and store in vapor phase of liquid nitrogen.

7.2.2.6. Labeling

Each center, as appropriate, will follow ISBER 2008 Best Practices for Repositories (pg. 34)::

7.2.2.6.1. Barcoding

Whenever possible, labels should be printed with a linear (one-dimensional or 1D) barcode that uniquely identifies the specimen. Under some circumstances, two-dimensional (2D) barcodes may be utilized. 2D barcodes have the advantage that scanning error rates may be lower and more information can be included on the label and may be optimal for use on smaller vials. Cost considerations may influence the systems selected for creating and reading barcodes.

7.2.2.6.2. Labels for Human Specimens

Human specimens should be labeled in such a way that protects privacy and confidentiality and is in compliance with applicable laws and institutional policies. The unique identifier for each specimen should be printed on the label in both barcode format and human readable form. Specimens should be labeled with a unique code not derived from information about the subject or related to its storage location in the repository. No other study or personal health information should be encoded in the specimen ID. In addition, the specimen ID should not be tied to the storage location.

7.2.2.6.1. Repository Labels

See Appendix 12 for instructions.

7.2.3. Blood Specimens

7.2.3.1. Solutions and Materials

- 50 ml Falcon tube (BD Falcon Ref# 352073)
- PBS: 1x Dulbecco's Phosphate-Buffered Saline, Invitrogen Cat.# 14190-144 Ficoll
- Hypaque: Histopaque®-1077 (Sigma Cat# 10771)
- Staining Buffer: PBS with 1% BSA

7.2.3.2. Biorepository Submission - Blood

The Biorepository submission goal for Blood is five 200 ul aliquots of plasma, this is a required goal. Five Biorepository labels are provided as part of the Blood Label Set. Additionally three 1 million cell aliquots of Blood PBMC dry pellet and three 1million cell aliquot of Blood in RNALater are optional submission goals, the Biorepository labels are provided as part of the Blood Label Set.

7.2.3.3. Collection Procedures

Collect approx. 10 ml blood by venipuncture into a CPT (marble top) tube.
Collect approx.10 ml blood by venipuncture into a ACD (yellow top) tube or a EDTA (purple top) tube.

7.2.3.4. Cell Preservation Procedures

7.2.3.4.1. Processing of PBMC's from CPT tubes

- Collect approx. 10 ml blood by venipuncture into a CPT (marble top) tube. Mix by inversion 8-10 times and store upright at room temp for transport to lab. (Alternately blood collected in an EDTA tube (purple top) or an ACD citrate tube (yellow top) can be used for manual ficoll separation. See section 7.2.3.5.2 for method)
- Centrifuge sample 1500g/25C/15 min.
- Transfer the entire buffy coat (PBMCs) to a 15 ml conical. Bring volume to 10 ml with cold PBS. Mix by inversion.
- Centrifuge sample 400g/4C/10min. Discard supernatant.
- Resuspend PBMC pellet in cold 1X PBS or protein free media at a concentration of 5 million cells/ml and proceed to section 7.2.3.5.3 for directions on storage.

7.2.3.4.2. Ficoll PBMC Isolation Protocol

- Collect approx.10 ml blood by venipuncture into a ACD (yellow top) tube or a EDTA (purple top) tube. Mix by inversion and store upright on ice for transport to lab.
- Centrifuge sample 800g/4C/10 min.
- Remove and save plasma if desired in 200ul aliquots.
- Transfer remaining whole blood to a 50 ml conical tube. Dilute to 30 ml in PBS, & mix gently with pipette.
- Add 10 ml room temp 1.077g/ml Ficoll Hypaque solution to a 50 ml Falcon tube.
- Gently underlay Ficoll below diluted blood.
- Spin tubes 400g, 20 min., RT (acc=1, dcl=1, no brake).
- Collect PBMCs from interphase of PBS/ Ficoll with a transfer pipette and transfer to a clean 50 ml conical tube, add PBS to 40 ml.
- Spin cells down 800g/RT/8 min.
    - Dilute cells to 10 ml with PBS and spin down cells at 240g/RT/ 8 min.
    - Resuspend PBMC pellet in cold 1X PBS or protein free media at a concentration of at least 5 million cells/ml and proceed to section 7.2.3.5.3 for directions on storage.

7.2.3.4.3. Storage of PBMC

Note that even though directions are being provided for storing live cells, dry cell pellets, and cells in RNALater, only the latter two are being requested by the repository.

- Aliquot the PBMCs (at 5 x 10^6 cells/ml) into Sarstedt 1.5 ml microtubes with screw O-ring caps (Fisher catalog number NC9145227; Sarstedt cat# 72.730.005) as follows:
    - Place 1 ml (5 x 10^6 cells) into tubes to be stored in freezing media
    - Place 200 ul (1 x 10^6 cells) into tubes to be stored in RNALater. Add 800 ul 1X PBS or protein free media to bring total volume to 1 ml.
    - Place 200 ul (1 x 10^6 cells) into tubes to be stored as a dry pellet. Add 800 ul 1X PBS or protein free media to bring total volume to 1 ml.
- Spin down cells in Centrifuge at 500 g for 5 minutes
- Remove the supernatant carefully without disturbing the pellet

- Storage of live cells in DMSO
  - Resuspend 5 x 10^6 PBMC cell pellet in 1 ml freezing media (90%FBS, 10%DMSO).
  - Transfer to a cryotube. Hold cells at -80°C for 24-48hrs.
  - Transfer the cryotube to the gaseous phase (top) of liquid Nitrogen. (Cells should not be stored in the LN2 at the bottom of the tanks).

- Storage of cells in RNA preservation media
  - Resuspend 1 x 10^6 PBMC cell pellet in 700 ul RNALater and vortex for 1 minute on highest setting.
  - Store -80°C.

- Storage of cells as dry pellet
  - Do not resuspend 1 x 10^6 PBMC cell pellet. Instead freeze immediately as a dry cell pellet.
  - Store -80°C.

  The dry cell pellets and the RNALater aliquots are an optional contribution to the repository; all centers that will contribute cells for RNA analysis will use RNALater. If contributing to Biorepository, use the Biorepository provided labels.

7.2.3.5. Labeling

Each center, as appropriate, will follow ISBER 2008 Best Practices for Repositories (pg. 34).

7.2.3.5.1. Barcoding

Whenever possible, labels should be printed with a linear (one-dimensional or 1D) barcode that uniquely identifies the specimen. Under some circumstances, two-dimensional (2D) barcodes may be utilized. 2D barcodes have the advantage that scanning error rates may be lower and more information can be included on the label and may be optimal for use on smaller vials. Cost considerations may influence the systems selected for creating and reading barcodes.

7.2.3.5.2. Labels for Human Specimens

Human specimens should be labeled in such a way that protects privacy and confidentiality and is in compliance with applicable laws and institutional policies. The unique identifier for each specimen should be printed on the label in both barcode format and human readable form. Specimens should be labeled with a unique code not derived from information about the subject or related to its storage location in the repository. No other study or personal health information should be encoded in the specimen ID. In addition, the specimen ID should not be tied to the storage location.

7.2.3.5.3. Repository Labels

See Appendix 12 for instructions.

7.3. Center C002 University of Pennsylvania - Bronchoscopy Protocol

v.3.7-RC 6/18/10

7.3.1. Introduction & Overview

7.3.2. Rationale:

1. Lower respiratory tract microbiota may be present at low density, and there is potential for significant skewing by even very low level cross-contamination by upper respiratory tract microbiota, since they are likely present at high density.
2. The upper respiratory tract likely represents multiple distinct microbiomes, and sampling of specific areas distinct from those through which the bronchoscope directly passes may not accurately represent upper tract contaminants.
3. The literature suggests that contamination can be minimized by using 2-scope method (1,2), and by discarding first aliquot of BAL return (3,4).
4. Upper respiratory sampling: Nasopharyngeal (NP), oropharyngeal (OP) and oral wash (OW) will be complemented by bronchoscope #1 that will assess upper resp tract as close to cords as possible.

references

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(2) Pang JA, Cheng AF, Chan HS, French GL: Special precautions reduce oropharyngeal contamination in bronchoalveolar lavage for bacteriologic studies, Lung 1989, 167:261-267

(3) Qvarfordt I, Riise GC, Andersson BA, Larsson S: Lower airway bacterial colonization in asymptomatic smokers and smokers with chronic bronchitis and recurrent exacerbations, Respir Med 2000, 94:881-887

(4) Soler N, Ewig S, Torres A, Filella X, Gonzalez J, Zaubet A: Airway inflammation and bronchial microbial patterns in patients with stable chronic obstructive pulmonary disease, Eur Respir J 1999, 14:1015-1022

7.3.3. Bronchoscopy Procedure Overview

1. Two bronchoscopes – scope #1 for anesthesia and sampling above the cords; scope #2 for sampling below the cords
2. Pre-bronch flush & aspiration through channel of each scope as control for nucleic acid carriage within instrument as well as contamination during specimen handling & processing.
3. Compare the 'BAL first return' with remainder of fluid return, and with a second BAL in an adjacent area of lung. Upper airway contaminants should decrease serially in the 3 samples, while true lower airway contents will be present in all three.
4. We will give future consideration of an oral airway (Williams Airway) to minimize "tongue scraping" by tip of scope but will not include at present.
5. Both bronchoscopes will have a channel flush (pre-wash) prior to insertion into subject as negative control

7.3.4. Specimen collection overview:

1. NPO and no gum, mints, etc
2. RN evaluation, consent, forms
3. NP/OP swab collection (NP & OP) – for DNA Microbiome and Micro Culture
4. Oral Wash collection (OW) – for DNA Microbiome
5. Listerine rinse & gargle
6. Local spray anesthesia & conscious sedation
7. Bronchoscope #1 for local anesthesia & upper tract contamination samples (2 samples – tip & channel rinse-out) – for DNA Microbiome

8. Bronchoscope #2 for lower tract BAL (3 BAL samples) – for DNA Microbiome, Micro Culture and BAL cells & fluid
9. Lower tract protected specimen brush (3 brushes) - for DNA Microbiome, Micro Culture & epithelial cell RNA

Materials needed for Bronch Suite Sample Collection (in addition to standard bronchoscopy supplies)

1. Sterile 1% saline for BAL (and Oral Wash, if being carried out)
2. Listerine
3. paper cups
4. tongue depressors (for NP/OP sampling, if being carried out)
5. ice bucket with wet ice
6. bead-beater tube (1) for PSB (OP, NP and "scope #1 tip swab" will be put in beadbeater tubes once back in the lab)

7. specimen containers (6) for: oral wash sample
   Scope #1 channel pre-wash & post-wash
   Scope #2 channel pre-wash
   BAL-A, BAL-B

8. 50 ml conical tube for 'BAL First return'
9. 15 ml tube (for BAL-B to go to Micro Lab for culture)
10. 15 ml tube with 2 ml saline (for PSB to go to Micro Lab for culture)
11. 2 ml cryotube with 1 ml RNALater for PSB
12. OP swabs (3): one for "scope #1 tip swab"
    two if OP/NP sampling being done:
    - one for OP DNA
    - one for OP Micro culture (with micro transport media)
13. NP swabs (2): two if OP/NP sampling being done:
    - one for NP DNA
    - one for NP Micro culture (with micro transport media)
14. Protected specimen brushes (2 – for DNA and for Micro) – Conmed #130 Microbiology Brush)
15. Cytology brush (1 – for Epithelial Cell sample) – Conmed #129 sheathed
16. Sterile (flamed) scissors or wire-cutters (to cut PSB in bronch suite)
17. sterile 5 or 10 ml pipette (in bronch suite / ABI lab)
18. 10 ml, 20 ml and 50 ml syringes
19. Lukens traps (should have up to 10 available though likely fewer necessary):
    scope #1 channel pre-wash
    scope #1 channel post-wash
    scope #2 channel pre-wash
    BAL first return
    BAL-A (may require ≥ 2 traps)
    BAL-B (may require ≥ 2 traps)

7.3.5. Bronchoscopy protocol:

7.3.5.1. Pre-Procedure

1. Subject will be NPO p-midnight (AM meds ok with small sips water).
2. RN evaluation: interim history, physical exam, consent reviewed, confirm companion to accompany home
3. URT sampling will be done in the prep area, prior to any inhalational local anesthesia
   - Upper **respiratory tract sampling (OP & NP**) will be done for DNA extraction and Micro culture if appropriate and samples placed on ice (DNA) or at RT (Micro culture) - see NP/OP sampling protocol.
   - **Oral Wash (OW)** will be carried done and sample placed on ice (10 ml, swish and gargle) - see Oral Wash sampling protocol.
   - Subject will carry out oral rinse with water followed by gargle with 10 ml Listerine (30 sec each) as per LHMP protocol. Discard.
4. In the bronch suite, standard oropharyngeal local anesthesia and conscious sedation will be done as per routine.

7.3.5.2. Anesthesia – bronchoscope #1 (three samples)

5. Prepare 3 specimen containers with 10 ml sterile saline in each, to be used for channel washes
6. Prior to insertion into patient, tip of scope #1 is placed into saline-containing sterile specimen container. The 10 cc of sterile saline is drawn up through scope in using a syringe, then flushed back down into the specimen container (**"scope #1 channel pre-wash"**). The specimen container is labeled and placed on ice (this serves as a control for DNA in saline, bronchoscope channel or any of the subsequent handling and processing steps).
7. Bronchoscope is inserted trans-orally and local lidocane anesthesia administered to the epiglottis and vocal cords as per routine. Following final lidocane administration, residual secretions around glottis should be suctioned into the scope.
8. Scope is removed and tip is swabbed with an Oropharyngeal swab. Swab is placed into hard plastic case, and placed on ice for transport back to lab (**"scope #1 tip swab"**)
9. Step #6 is repeated using a new specimen container with saline, in order to sample the channel contents (**"scope #1 channel post-wash"**). The specimen container is labeled and placed on ice.

7.3.5.3. Bronchoscopy & BAL – bronchoscope #2 (4 samples)

10. Prior to insertion into patient, step #6 is repeated using a new specimen container with saline (**scope #2 channel pre-wash**). The specimen container is labeled and placed on ice.
11. Bronchoscope will be inserted trans-orally and passed through the vocal cords as per routine, without suctioning of the upper airway. Anesthesia below the cords to the large airways should be administered as per routine (special attention to RML where BAL will be done and LLL bronchus where brushing will be taken). There should be no further suction following administration of final lidocane bolus prior to BAL.
12. Scope is advanced into the RML and wedged into position in a segmental/subsegmental bronchus. 50 ml saline is administered with a syringe, and aspirated back into the syringe. This is then ejected from the syringe into a 50 ml conical tube (**BAL First Return**), which is labeled and placed on ice.
13. Without losing the wedge position, an additional 100 ml saline is administered to same area using a new syringe. Fluid is then aspirated back into the syringe (**BAL-A**), then ejected into a new specimen container, which is labeled and placed on ice

14. Scope tip is repositioned into an adjacent segmental/subsegmental bronchus. 150 ml saline is administered using a new syringe, then aspirated back into the syringe. Approximately 1 ml of this fluid is ejected into a 15 ml conical tube to be sent to Micro lab. The remainder is then ejected from the syringe into a new specimen container, combining multiple syringe-fulls if needed (**BAL-B**). Specimen container and 15 ml conical tube are labeled and placed on ice.
15. If more than one syringe-full of fluid is aspirated from any one step, they may be combined (being sure to keep the 3 samples separate: BAL-first return; BAL-A; BAL-B).

7.3.5.4. Bronchial Brushings–protected specimen brush(PSBX3 to be carried out after BAL completed)

16. Bronchoscope is positioned in the LLL and PSB #1 inserted under direct visualization. When PSB has cleared tip of scope, plug is ejected, inner sheath extended, and brush extended from sheath. Gentle brushings of bronchial mucosa (4-8x) are done. If visible mucous or airway secretions are visible they should be included in the brushing. Brush is pulled back into sheath and removed from bronchoscope.
17. The outside of the sheath is gently wiped (top to bottom) using sterile gauze. One person should hold the bead-beater tube and scissors while a second person holds the brush, extends it out from the sheath and places the tip inside the Beadbeater tube. Be careful that sheath does not touch the bead-beater tube. Using sterile scissors the brush is cut off into Beadbeater DNA Extraction tube.
18. Second PSB brush is inserted and brushings taken in a similar manner (including mucous or secretions, if visible) in an adjacent undisturbed region of mucosa. Brush and sheath are removed and brush is clipped off into 15 ml tube containing 2 ml of saline for micro culture (same pair of scissors may be used, as long as kept aeseptic).
19. Third brush (sheathed cytology brush) is inserted and brushings taken in a similar manner (except that *secretions and mucuous should be avoided*) in an adjacent undisturbed region. Brush and sheath are removed and brush is clipped off into 2 ml tube containing 1 ml of RNALater for epithelial cell RNA analysis.

7.3.5.5. Post-procedure

20. Micro cultures (BAL-B and NP/OP swabs, if applicable) are delivered to HUP Micro lab (4 Gates)
21. On URT and Bronch CRFs note time of BAL specimen collection, time placed on ice, time returned to lab, and time processed.
22. Post-bronchoscopy monitoring & discharge as per routine
23. Micro specimens are delivered to Micro Lab, 4 Gates
24. Other samples are brought to lab for processing as per SOP

Specimens & containers

	DNA / microbiome	Micro culture	RNA
NP swab	Hard case w/ swab	Culture swab	
OP swab	Hard case w/ swab	Culture swab	
Oral Wash	Specimen container		
scope #1 channel pre-wash *	Specimen container		
scope #1 tip swab **	Hard case w/ swab		
scope #1 channel post-wash **	Specimen container		
scope #2 channel pre-wash *	Specimen container		
BAL first return **	Specimen container		†
BAL-A	Specimen container		†
BAL-B	Specimen container	15 ml tube w/ 1 ml BAL-B	†
Endobronchial mucosal brush	PSB #1- Beadbeater tube w/ brush tip	PSB #2- 15 ml tube w/ 2 ml saline & brush tip	Cyto brush - Cryovial w/ 1 ml RNALater

* negative control
** upper airway glottic sample

† BAL cells for RNA extraction will be prepared in the lab

In addition, BAL cells for immunological analysis and cell-free fluid for soluble factor analysis will be prepared in the lab

7.3.5.6. Analysis & Interpretation

A. Pre-channel wash samples serve as controls for saline, scope channel & processing contaminants (environmental source admixture).

B. Scope #1 tip swab & scope #1 post-channel wash will reflect upper airway microbiota most likely to contaminate lower airway samples by bronchoscope, and composition should be compared with BAL samples (carryover comparison).

Caveat: normal lower respiratory tract microbiome may actually be related to upper respiratory microbiota, which cannot be distinguished in analysis B above. If so it should be reflected in similar quantities in BAL-A and BAL-B, below, so both qualitative and quantitative analysis necessary.

C. BAL-A first return vs BAL-A vs BAL-B – serially decreasing levels of microbial DNA suggests source was upper respiratory tract, not lower tract.

7.3.5.7. Data Collection for the LHMP Bronchoalveolar Lavage Form

Data for the LHMP is collected on a number of different Penn forms and is imported into the LHMP MIDAS database. All specimen characterization data for the Bronchoalveolar Lavage Form for the 'BAL-A' and 'BAL-A first return' is entered in the LHMP BAL Form section for 'BAL Site-A' and 'BAL-B' is entered in the section LHMP BAL Form for 'BAL Site-B'. For sequencing purposes the 'BAL-A first return' will be used as most similar to the LHMP study group's 'BAL Site-A'.

7.4. Specimens for the Biorepository Preparation

7.4.1. Repository Submission Goals

From the LHMP, the paired lung/blood samples are the unique contribution. Very small amounts of extracted DNA are included under the assumption that these will be used for amplification.

Sample	Total Volume	Volume/ Number	Quantity of Aliquots	Storage Temperature	
Bronchoalveolar Lavage:					
Acellular BAL	5 ml	1 ml	5	-80°C	Required
BAL cells: Dry pellet	2 million	1 million	1	-80°C	Optional
In RNALater		1 million	1	-80°C	Optional
Oral Wash:					
Oral Wash neat	1-3 ml	1 ml	1-3	-80°C	Required
Blood:					
Plasma	1 ml	200 ul	5	-80°C	Required
PBMC : Dry pellet	6 million	1 million	3	-80°C	Optional
In RNALater		1 million	3	-80°C	Optional
Extracted DNA from:					
Neat BAL			1	-80°C	Optional
Oral Wash (in .1% DTT)			1	-80°C	Optional

7.4.2. Possible uses:

- The lung & blood cell pellets could be used for DNA for host genetics, viral genetics (comparing lung/blood), protein analysis (albeit volume is limited for that).
- Acellular BAL could be useful for soluble inflammatory markers, viral genetics, proteomics.
- The lung & blood cell RNA could be useful for microarray, transcriptomics, viral RNA expression.
- From the LHMP, the paired lung/blood samples are the unique contribution. Very small amounts of extracted DNA are included under the assumption that these will be used for amplification.

7.5. Transfer and Receipt Procedures for Specimens

It is essential that the LHMP ID and Specimen ID remain linked throughout any transfers occurring either when moving the specimens to a different lab within the institution or when participating in collaborative LHMP procedures. To avoid undue burden on the centers the tracking of inter-institutional transfers are the responsibility of each center. This includes specimens sent to the Sequencing Lab and or returned to the Clinical Lab for storage.

Figure 7.1 shows the flow specimens and the documentation in two Scenarios. In Scenario 1 the specimens are collected at on Center and transferred to the primary center prior to the entry into MIDAS and creation of an LHMP IDs. In this case the collection center will complete the paper forms, including a special section on the BAL, Oral Wash and Blood forms which collect the specimen characterization data. The LHMP ID is created and all data is entered into MIDAS at the receiving center.

Scenario 2 shows the flow of the specimens within an institution, and this tracking is the responsibility of the institution.

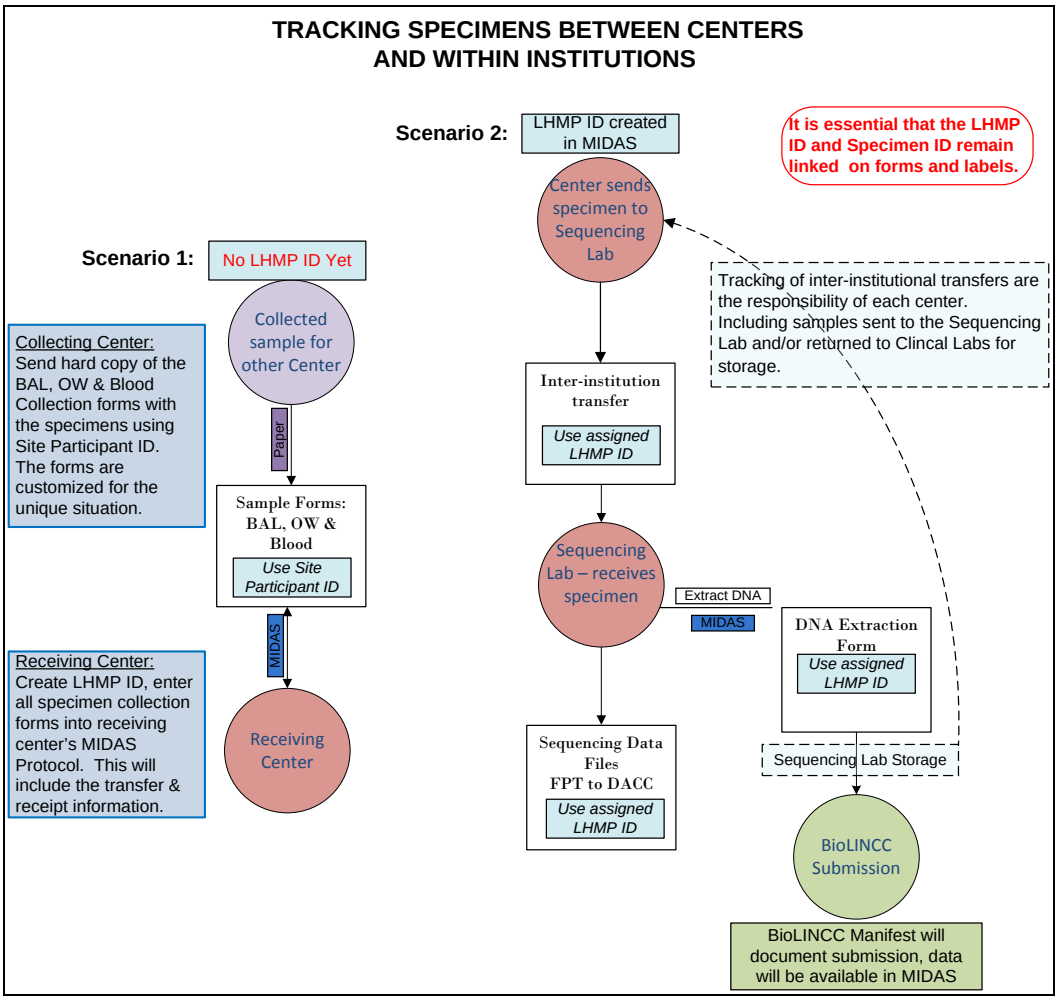


Figure 7.1

Figure 7.2 shows the flow of a specimen and the documentation, using the LHMP Transfer and Receipt Forms. These forms are available on the website, and are to be entered into MIDAS.

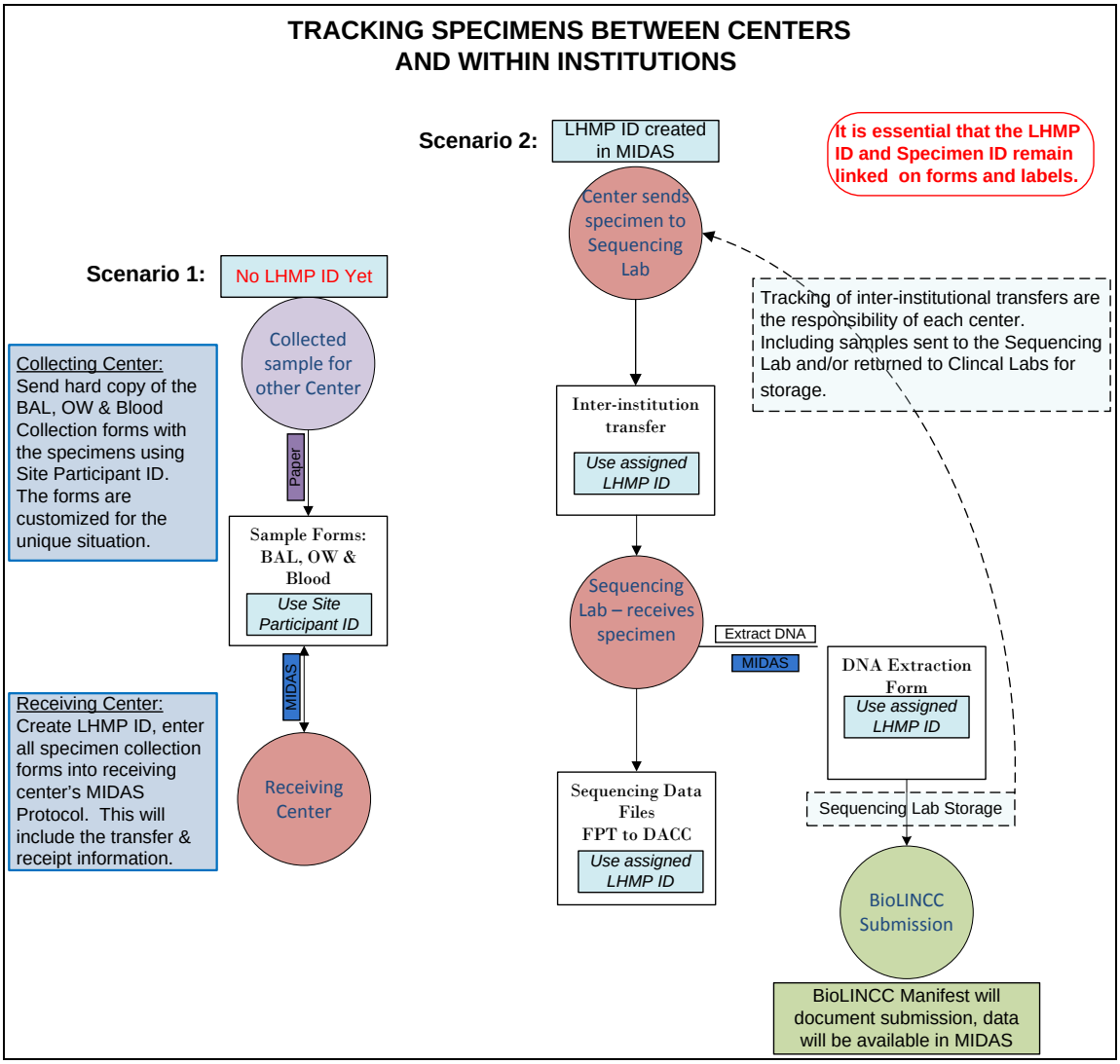


Figure 7.2

The DACC will also create collaborative study specific spreadsheets to collect this data and import it into MIDAS when there is a bulk transfer for a specific collaborative study.

8. SEQUENCING SPECIMEN PROCESSING AND ANALYSIS

8.1. Nucleic acid extraction from clinical samples

8.1.1. S001 – University of Michigan

1) Transfer freshly collected BAL samples (kept on ice) to Dry Bead Tubes (Mo Bio, Ultra Clean Fecal DNA Isolation Kit, Cat. #: 12811-100-DBT):
    a. Add 1 ml of each BAL sample to a Dry Bead Tube, centrifuge at maximum speed in a microcentrifuge (~16,000 g) for 2 min.
    b. Remove and discard the supernatant.
    c. Repeat until 5 ml of BAL has been added to each Dry Bead Tube.
    d. Store at -80° C.
2) Add 700 μl PowerSoil DNA Kit Bead Solution (Mo Bio, Cat. #:12855-50-BS) to each Dry Bead Tube (containing pellet from 5 ml of BAL). Vortex.
3) Add 60 μl Solution C1. Vortex.
4) Bead beat for 2 min (instead of 10 min vortex).
5) Continue with PowerSoil DNA Isolation Kit Protocol starting with step 6 (centrifuge for 30 seconds at 10,000 g).

8.1.2. S002 – University of Pennsylvania

We use the Powersoil kit with upstream modifications

1) Location of extraction: BSL 2+ 4th floor JP
   ***all kit reagent bottles, racks, pipettes, tips, collection tubes, DNA spin columns were cleaned with 70% ethanol and 10% bleach before placed in hood then UV irradiated for 30minutes prior to use.
   ***gloves were repeatedly changed, finger tips dipped in 10% bleach and UV light switched on during incubations to cut back on potential sources of contamination

    a. Swabs in Powersoil beadbeater tubes and liquid samples in falcon tubes were sterilized with bleach wipes and placed in the hood
    b. Sample processing:
        i. No further processing of swabs- 60ul of solution C1 was added to tubes
            1. Liquid samples (water, saline, OW, BAL) were aliquoted into 1.8mL volumes into 2mL sterile Eppendorf collection tubes provided by the Powersoil kit and centrifuged at 10,000 x g for 10min.
            2. Supernatant was discarded, pellet resuspended in 60uL solution C1 and transferred to the powersoil beadbeater tubes, vortexed 15 seconds.
    c. Beadbeater tubes incubated at 65C for 10min in a heating block then beadbeat for 2min. Beadbeater tubes centrifuged at 10,000 x g for 1min at room temp.
    d. Supernatants were transferred to 2mL collection tubes provided by the kit and protocol was followed as per manufacturer's instructions starting at step #8 of the "Experienced User Protocol".

8.1.3. C008 – Indiana University

Extraction is done at IU lab (We use the DNeasy Blood and Tissue kit "Purification of Total DNA from Animal Tissues outlined on page 30 of the handbook, including the pretreatment for Gram positive bacteria outlined on page 45-46 of the manual)

1) Thoroughly clean a cell culture hood by spraying and wiping with 70% ethanol twice. Load the hood with consumables and UV irradiate for 30 minutes.
2) Thaw samples at RT in the hood and invert to mix contents. Place tubes on ice.
3) Centrifuge each sample in a table top centrifuge at 3,000 rpm for 15 minutes.
4) Resuspend pellet in 180 ul enzymatic lysis buffer (20 mM Tris pH 8.0, 2 mM sodium EDTA, 1.2% Triton X-100 and add lysozyme to 20 mg/ml).
5) Incubate at 37 ^{o}C for 30 minutes.
6) Add 25 ul Proteinase K and 200 ul of Buffer AL, and mix by pulse vortexing.
7) Incubate at 56 ^{o}C for 30 minutes.
8) Add 200 ul 100% ethanol to the sample and mix by vortexing.
9) Pipet the entire mixture from step 8 into a DNeasy mini spin column and centrifuge at 8,000 rpm for 1 minute (step 4).
10) Follow from step 4 onwards on page 30 in DNeasy Blood and Tissue Handbook, with this exception in the final step: elute in 50 ul of Buffer AE once only.

After extraction specimens are sent to S003 Washington University at St. Louis, Genomic Institution for sequencing.

8.1.4. S004 – University of Colorado, Boulder

1. Add 25 µl of DNA free water to a PowerBead Tube provided in the kit (Mobio Powersoil Kit). Gently vortex to mix (this will be a mock extraction).
2. Resuspend and pool the four 1.25 ml BALF pellets from one patient (5 ml total) in 25 µl of DNA free water and transfer to a PowerBead Tube provided in the kit. Gently vortex to mix. Do this for each patient.
3. Add 60 µl of Solution C1 to each sample and invert several times or vortex briefly to mix.
4. Secure all PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex. Vortex at maximum speed for 10 minutes.
5. Transfer 600 ul supernatant to clean 2 ml Collection Tubes (provided in the kit).
6. Add 250 µl of Solution C2 to each sample and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
7. Centrifuge at room temperature for 1 minute at 10,000xg.
8. Avoiding the pellet, transfer up to, but no more than, 600 µl of the supernatant to a clean 2 ml Collection Tube (provided in the kit). Discard the pellet. Repeat for each sample.
9. Add 200 µl of Solution C3 to each sample and vortex briefly. Incubate at 4°C for 5 minutes. Centrifuge the tubes at room temperature for 1 minute at 10,000xg.
10. Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean 2 ml Collection Tube (provided in the kit). Discard the pellet. Repeat for each sample.
11. Shake to mix Solution C4 before use. Add 1200 µl of Solution C4 to each sample supernatant and vortex for 5 seconds.
12. Load approximately 600 µl of the supernatants from step 13 onto Spin Filters and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through.
13. Add an additional 600 µl of the supernatants to the Spin Filters and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through.
14. Load the remaining supernatants from the samples onto the Spin Filters and centrifuge
15. at 10,000 x g for 1 minute at room temperature. Discard the flow through. A total of three loads onto the same spin filter for each sample processed are required (steps 14-16).
16. Add 500 µl of Solution C5 to each sample and centrifuge at room temperature for 30 seconds at 10,000xg. Discard the flow through.

17. Centrifuge again at room temperature for 1 minute at 10,000xg.
18. Carefully place Spin Filters in clean, 2 ml Collection Tubes (provided in the kit). Avoid splashing any Solution C5 onto the Spin Filter.
19. Add 100 µl of Solution C6 to the center of the white filter membrane. Do this for each sample. C6 is a salt-free, 10 mM Tris solution that will release the DNA from the silica membrane.
20. Centrifuge at room temperature for 30 seconds at 10,000 x g.
21. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application without any further steps.

8.1.4.1. University of Colorado, Denver – Sonia Flores Lab (M014)

This lab receives samples for the longitudinal HIV project and does not collect the neat BAL. Instead, the lab does a low-speed centrifugation (at 500xg for 10 minutes) of the BAL which pellets the mammalian cells and the supernatant contains the fluid portion of the BAL plus any cell-free microbes. The lab receives from UCSF the low-speed mammalian cell pellet separately from the cell-free fluid. These are labeled these BALc and BALf. To extract gDNA from the mammalian cell pellets and for the BALf, the lab first performs a high-speed centrifugation (8000xg for 30 minutes at 4^{o}C) which pellets the microbial components and extracts gDNA from that high-speed pellet. Both BALc and BALf are sent to Wash U for sequencing.

8.1.5. S005 – University of California at San Francisco

1) Aliquot 10 ml of RNALater into multiple sterile DNAse/RNAse-free vessel in a biosafety cabinet
2) Add BAL (5 ml) to pre-aliquotted RNALater in the bronchoscopy suite immediately after collection (2:1 ratio of RNALater to BAL).
3) Tightly cap collection vessels and gently invert several times to mix contents
4) Samples are stored at 4°C for 24 hours (to permit preservative to penetrate cells) prior to freezing at –80°C until processing
5) Samples are thawed on ice and inverted to mix contents
6) 3 ml of sample is harvested by centrifugation in 1.5 ml microfuge tubes in a refrigerated bench top centrifuge (4°C) at top speed for 10 minutes
7) Supernatant is discarded and the pelleted material is resuspended in 600 ul of buffer RLT (Qiagen AllPrep kit) with 6 µl beta-mercaptoethanol
8) Transfer suspension to a lysing matrix B tube
9) Bead beat in a FastPrep-24 (MP biomedicals) for 30 sec at 5.5. m sec^{-1}
10) Centrifuge tube @ 2000 rpm for 1min.
11) Remove supernatant and pipet into the DNA Spin Column in Step 4 of AllPrep manual (Qiagen).
12) Follow Allprep manual according to manufacturer's instructions from Step 4 to isolate RNA and DNA.

8.1.6. S007 – University of Pittsburgh

1) Thoroughly clean a cell culture hood by spraying with 10% bleach solution followed by 2 washes with 70% ethanol. Load the hood with the disposables and equipment and UV irradiate for at least 15 minutes.
2) Thaw samples at RT in the hood and invert to mix contents. Place tubes on ice.
3) Harvest 3 ml of each sample by centrifugation in a 2.0 ml microfuge tube (1.5ml sample x 2) in a benchtop centrifuge (RT) at top speed for 10 minutes
4) Discard supernatant and resuspend pelleted material in 60ul C1 solution (Powersoil Kit).
5) Transfer suspended supernatant to a Powersoil beadbeating tube and incubate at 65C for 10 minutes.
6) Bead beat in a FastPrep-24 (MP biomedicals) for 60 seconds at 6.0m/s.[1]

7) Centrifuge tube at 10000 x *g* for 1 min.
8) Transfer supernatant to a new 2.0 ml microcentrifuge tube.
9) Add 250 ul C2 solution, vortex for 5 seconds, incubate at 4C for 5 min.
10) Centrifuge tube at 10000 x *g* for 1 min.
11) Transfer up to 600 ul of supernatant to a new 2.0 ml tube.
12) Add 200 ul C3 solution, vortex 5 seconds, incubate at 4C for 5 minutes.
13) Centrifuge tube at 10000 x *g* for 1 min.
14) Transfer 750 ul of supernatant to a new tube. Add 1200 ul solution C4 (shaken well before use) and vortex for 5 seconds.
15) Add 675 ul of the mixture to a spin filter and centrifuge at 10000 x *g* for 30 seconds. Discard flowthrough. Repeat with remaining mixture until all is spun through.
16) Add 500 ul solution C5 and centrifuge at 10000 x *g* for 30 seconds. Discard flowthrough.
17) Re-spin column at 10000 x *g* for 3 minutes.
18) Place column in new 2.0 ml tube.
19) Add 100 ul of solution C6 and incubate at RT for 5 minutes.
20) Centrifuge at 10,000 x *g* for 30 seconds to elute DNA.

8.2. Sequencing

8.2.1. S001 – University of Michigan

<Not included>

8.2.2. S002 – University of Pennsylvania

<Not included>

8.2.3. S003 – Washington University in St. Louis

Note: all sequencing for the LHMP was done at Washington University in St. Louis.

8.2.3.1. Purpose

16S Targeted Amplification Protocol for Lung HMP Validation Study
(based on HMP_MDG_454DefaultProtocol.V4.1) This protocol describes the creation of a pooled, barcoded amplicon 454 library using a set of barcoded primer pairs. [The V13 and V35 primers were designed at the Broad Institute and used in the Human Microbiome Project's 16S survey of healthy subjects.]

8.2.3.2. Requirements

Materials/Equipment	Vendor	Catalog Number
AccuPrime™ Taq DNA Polymerase High Fidelity	Invitrogen	12346-086
Forward and Reverse Primers premixed	Operon	custom order
96 well thermocyler plate		
clear adhesive plate seals		
DNAse/RNAse free water	-	-
Thermo Cycler	-	-
Vortex	-	-
Pipettes	-	-
Aerosol resistant pipette tips MinElute PCR Purification Kit	Qiagen	28004
Ampure (SPRI) Beads (60mL kit)	Agencourt	A29152
1x low TE, pH 8.0		
Quant-IT ds DNA Assay, high sensitivity	Invitrogen/Molecular Probes	Q33120

8.2.3.3. Documentation

Quant-iT ds DNA Assay protocol (manufacturer's specifications)
SybrGreen Assay protocol (manufacturer's specifications)
poolingCalculator.xls
MinElute PCR Purification Kit Manual

8.2.3.4. Method

The PCR will be carried out using AccuPrime Taq High Fidelity. It is not necessary to setup this reaction on ice, however it is recommended.

PCR Primer Setup

Set up of 10uM primer plates (combining barcoded A primer with non-barcoded B primer- see appendix below for primer & tag sequences):

- 1:10 dilution of the 100uM stocks using EB buffer.
- Forward primers are in a tube [1ml each].
- Tagged reverse primers are in the 96-well plate (10ul of final concentration 10uM).

8.2.3.4.1. PCR Setup - Mastermix

1. MasterMix contains the following amounts per sample:

13.85ul	RNAse/DNAse free water
2ul	10X AccuPrime PCR Buffer II
0.15ul	Accuprime Taq Hifi
1ul	Forward Primer
---	---
17uL	Total Volume

2. Multiply all volumes above by the amount of reactions needed plus 4.
3. Combine reagents in a 2mL micro centrifuge tube and mix. If more than 100 reactions are needed a 15mL tube should be used.
4. Using an automated pipette transfer 17uL of master mix into individual wells in the 96 well reaction plate.

8.2.3.4.2. PCR Setup

1. Transfer 1uL of barcoded primers from primer plate to corresponding wells in 96 well PCR plate.
2. Transfer 2uL DNA sample at ~2-3ng/ul to their respective reaction wells.
3. Securely seal with clear adhesive plate seal and mix.
4. Spin briefly at 2000 rpm in a centrifuge.
5. Place in thermo cycler and cycle as follows:

95°C	2 min	
95°C	20 sec	} 30 cycles
50 or 56°C*	30 sec	
72°C	5min	
4°	forever	

*** 56oC for V3-1, 50oC for V5-3 and V9-6**

8.2.3.5. PCR Gel Analysis

(E-gel alternative using 1ul of PCR product - faster) - we will know from the Quantification step below if we have product so this step is actually optional.

1. In a new reaction plate add 1uL PCR product to 1uL 6X loading dye
2. Cover, vortex to mix, briefly centrifuge to collect sample at the bottom of the well.
3. Prepare a 1% agarose 1X TAE gel with EtBr.
4. Load samples and run approximately 1 hour at 100V.
5. Capture gel image on gel-doc and retain for analysis.
6. Alternative: 1.2% Flash Gel run for 5-6 minutes at 275v

8.2.3.5.B. Clean PCR Products

B.1 Clean PCR products using Agencourt AmPure Beads (use Agencourt protocol → 1.8x volume beads (36ul beads) – follow manufacturer's specificiations.

B.3 Elute beads with 25ul 1x low TE, pH 8.0 and transfer to new 96 well plate.

8.2.3.6. PCR Product Quantification

Quantify PCR product using SYBR-Green Quantification or Quant-IT ds DNA high sensitivity assay according to the manufacturer's specifications. [WashU Genome Institute uses the latter Qubit protocol here.]

8.2.3.7. PCR Pooling

1. Using values from the SYBR Green or Quant-IT quantification, calculate pooling amounts using the poolingCalculator.xls or according to the following formula:
   Amount (uL) of each sample = ((vol/2)*(min)) / sampleconc
   where:
   Vol = total volume of each sample
   Min = concentration in ng/ul of the sample with the lowest concentration
   Sampleconc = concentration in ng/uL of target sample

2. Pool samples using a minimum transfer volume of 0.5uL.
3. Using a Qiagen minElute column, purify the pool according to the manufacturer's protocol.
   a. Elute column with 35ul warm EB.
   b. Let column sit for 5 mins after EB addition before elution.

(The Broad normalizes by converting all concentrations to molecules/ul. Determine which sample has the lowest concentration and then dilute all other samples to the same concentration. Pool equal volume of each (5-10ul) sample and then concentrate using a Qiagen MinElute columen (elution with 1x low TE, pH 8.0).

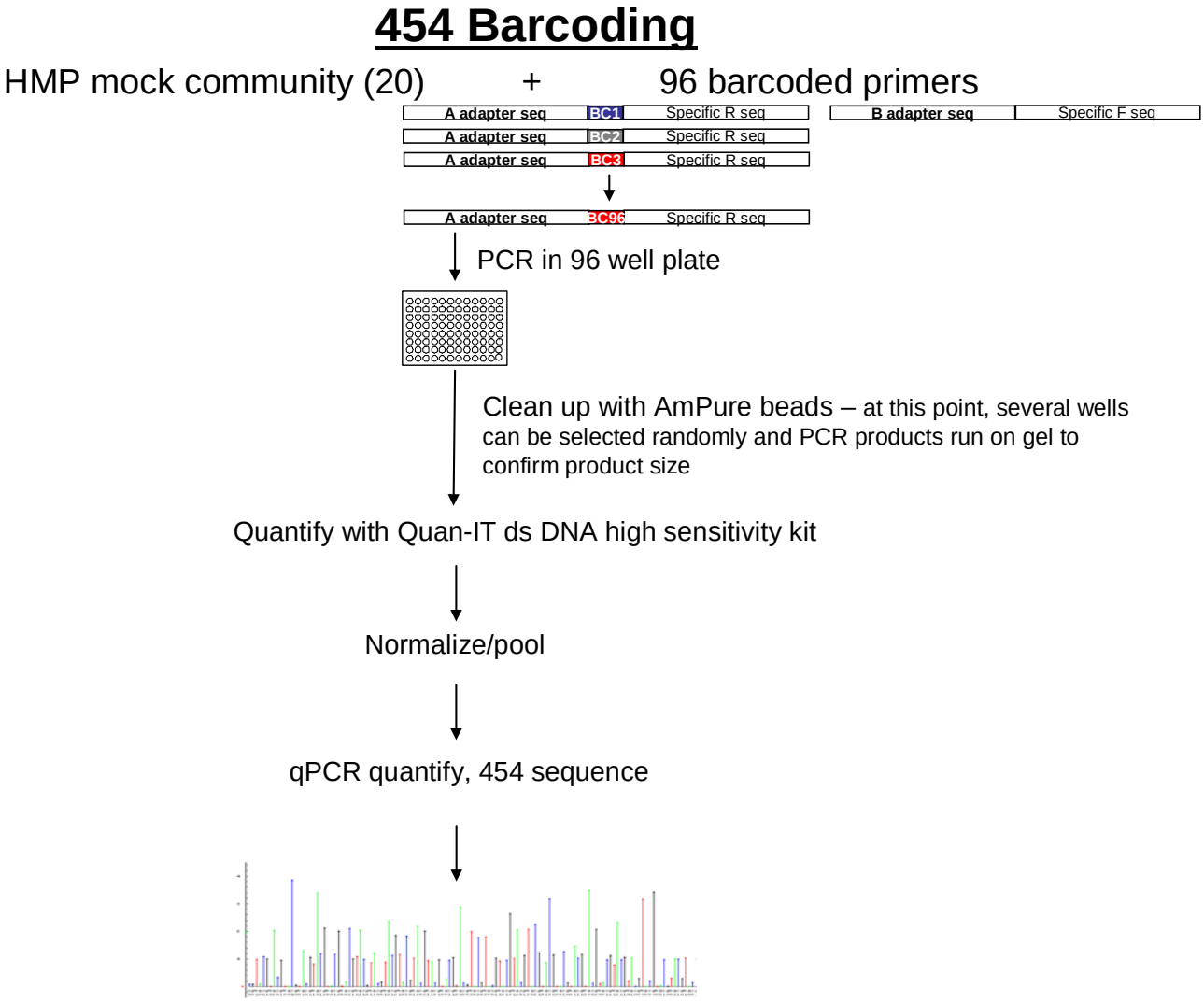
8.2.3.8. Sample Transfer for 454 Library Completion

1. Proceed directly to the qPCR library step.
2. Enter emPCR using ¼ the recommended primer concentration to avoid too many molecules amplified on bead. This results in high signal intensities during run, which leads to higher mixed reads and shorter read lengths.

8.2.3.9. Appendix: BROAD INSTITUTE Primer sequences including Tags

8.2.3.9.1. Purpose

In order to multiplex 16S rRNA community samples for sequencing, we need to barcode our samples. In this approach, we will 454 barcode sequences designed internally by the Broad (Pablo Alvarez and Will Brockman) between the A adapter and primer specific sequence (see picture below in Approach Section). Barcoded primer sets for 3, 16S rRNA gene regions (V3→1, V5→V3 and V9→V6) were tested alongside a primer set without the barcode sequence. We want to make sure that the addition of the barcode in the amplification primers does not influence or bias the diversity of the community being tested. An HMP mock provided by Sarah Highlander was.



Primer Sequences:

Primers were obtained from Operon reconstituted in TE for concentration of 100uM (100pmol/ul)

Variable regions V3 --> V1

"B" adapter for XLR + V1-3 27F
5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG

plate position	barcode name	barcode sequence	Barcode city name	primer name	A barcoded adapter for XLR system + barcode + V1-3 534R primer
A1	v2bBar8L	CACGC	Kabul	XLR_534R_v2bBar8L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCATTACCGCGGCTGCTGG
A2	v2bBar23L	CGCAAC	Tirana	XLR_534R_v2bBar23L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCAACATTACCGCGGCTGCTGG
A3	v2bBar174L	TGAAGC	Algiers	XLR_534R_v2bBar174L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAAGCATTACCGCGGCTGCTGG
A4	v2bBar602L	ACTTGC	Canberra	XLR_534R_v2bBar602L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTTGCATTACCGCGGCTGCTGG
A5	v2bBar212L	TCACAC	Vienna	XLR_534R_v2bBar212L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACACATTACCGCGGCTGCTGG
A6	v2bBar25L	CGTGAC	Baku	XLR_534R_v2bBar25L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACATTACCGCGGCTGCTGG
A7	v2bBar622L	ACGCGC	Nassau	XLR_534R_v2bBar622L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGCATTACCGCGGCTGCTGG
A8	v2bBar72L	CCTCTC	Bridgetown	XLR_534R_v2bBar72L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTCTCATTACCGCGGCTGCTGG
A9	v2bBar600L	ACTCAC	Minsk	XLR_534R_v2bBar600L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCACATTACCGCGGCTGCTGG
A10	v2bBar559L	AGACAC	Brussels	XLR_534R_v2bBar559L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACACATTACCGCGGCTGCTGG
A11	v2bBar31L	CGACTC	Sarajevo	XLR_534R_v2bBar31L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTCATTACCGCGGCTGCTGG
A12	v2bBar551L	AGCTTC	Rio	XLR_534R_v2bBar551L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTTCATTACCGCGGCTGCTGG
B1	v2bBar1149L	AAGCCGC	Sofia	XLR_534R_v2bBar1149L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCCGCATTACCGCGGCTGCTGG
B2	v2bBar15L	CAAGAAC	Ottawa	XLR_534R_v2bBar15L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAAGAACATTACCGCGGCTGCTGG
B3	v2bBar556L	AGTTGGC	Bangui	XLR_534R_v2bBar556L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTTGGCATTACCGCGGCTGCTGG
B4	v2bBar144L	TATCAAC	Santiago	XLR_534R_v2bBar144L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAACATTACCGCGGCTGCTGG
B5	v2bBar575L	AGGCGGC	Beijing	XLR_534R_v2bBar575L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCGGCATTACCGCGGCTGCTGG
B6	v2bBar48L	CGGTATC	Bogota	XLR_534R_v2bBar48L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTATCATTACCGCGGCTGCTGG
B7	v2bBar166L	TGACGAC	Kinshasa	XLR_534R_v2bBar166L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACGACATTACCGCGGCTGCTGG
B8	v2bBar613L	ACAAGGC	Brazzaville	XLR_534R_v2bBar613L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACAAGGCATTACCGCGGCTGCTGG
B9	v2bBar560L	AGACCTC	Zagreb	XLR_534R_v2bBar560L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACCTCATTACCGCGGCTGCTGG
B10	v2bBar741L	ATACCAC	Havana	XLR_534R_v2bBar741L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATACCACATTACCGCGGCTGCTGG
B11	v2bBar228L	TCGCGGC	Nicosia	XLR_534R_v2bBar228L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGGCATTACCGCGGCTGCTGG
B12	v2bBar807L	ATCTTAC	Prague	XLR_534R_v2bBar807L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTTACATTACCGCGGCTGCTGG
C1	v2bBar1273L	AACCAGC	Copenhagen	XLR_534R_v2bBar1273L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCAGCATTACCGCGGCTGCTGG
C2	v2bBar441L	TTCGAGC	Djibouti	XLR_534R_v2bBar441L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGCATTACCGCGGCTGCTGG
C3	v2bBar1174L	AAGGTGC	Quito	XLR_534R_v2bBar1174L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGTGCATTACCGCGGCTGCTGG
C4	v2bBar209L	TCTTGGC	Cairo	XLR_534R_v2bBar209L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGGCATTACCGCGGCTGCTGG
C5	v2bBar153L	TAATCTC	Suva	XLR_534R_v2bBar153L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAATCTCATTACCGCGGCTGCTGG
C6	v2bBar213L	TCACCTC	Helsinki	XLR_534R_v2bBar213L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACCTCATTACCGCGGCTGCTGG
C7	v2bBar298L	TCCGCTC	Paris	XLR_534R_v2bBar298L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGCTCATTACCGCGGCTGCTGG
C8	v2bBar146L	TATTGAC	Berlin	XLR_534R_v2bBar146L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTGACATTACCGCGGCTGCTGG
C9	v2bBar554L	AGTCGAC	Accra	XLR_534R_v2bBar554L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCGACATTACCGCGGCTGCTGG
C10	v2bBar646L	ACGGCTC	Athens	XLR_534R_v2bBar646L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGGCTCATTACCGCGGCTGCTGG
C11	v2bBar158L	TGCGTTC	Guatemala	XLR_534R_v2bBar158L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTTCATTACCGCGGCTGCTGG
C12	v2bBar207L	TCTCGAC	Conakry	XLR_534R_v2bBar207L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGACATTACCGCGGCTGCTGG
D1	v2bBar77L	CCAGGAC	Bissau	XLR_534R_v2bBar77L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGGACATTACCGCGGCTGCTGG
D2	v2bBar601L	ACTCCTC	Budapest	XLR_534R_v2bBar601L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCCTCATTACCGCGGCTGCTGG
D3	v2bBar481L	TTCCTGC	Jakarta	XLR_534R_v2bBar481L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCATTACCGCGGCTGCTGG
D4	v2bBar419L	TTCATAC	Tehran	XLR_534R_v2bBar419L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCATACATTACCGCGGCTGCTGG
D5	v2bBar26L	CGTCGTC	Baghdad	XLR_534R_v2bBar26L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCGTCATTACCGCGGCTGCTGG
D6	v2bBar1172L	AAGGCAC	Dublin	XLR_534R_v2bBar1172L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCACATTACCGCGGCTGCTGG
D7	v2bBar1210L	AACAACTC	Jerusalem	XLR_534R_v2bBar1210L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACAACTCATTACCGCGGCTGCTGG
D8	v2bBar606L	ACACGGAC	Rome	XLR_534R_v2bBar606L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACACGGACATTACCGCGGCTGCTGG
D9	v2bBar159L	TGCCGAAC	Kingston	XLR_534R_v2bBar159L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCGAACATTACCGCGGCTGCTGG
D10	v2bBar147L	TATTCGTC	Tokyo	XLR_534R_v2bBar147L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTCGTCATTACCGCGGCTGCTGG
D11	v2bBar141L	TAGGAATC	Amman	XLR_534R_v2bBar141L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGAATCATTACCGCGGCTGCTGG
D12	v2bBar119L	CCGGCCAC	Nairobi	XLR_534R_v2bBar119L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGCCACATTACCGCGGCTGCTGG
E1	v2bBar1379L	AATGGTAC	Tarawa	XLR_534R_v2bBar1379L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAATGGTACATTACCGCGGCTGCTGG
E2	v2bBar208L	TCTCCGTC	Pyongyang	XLR_534R_v2bBar208L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCCGTCATTACCGCGGCTGCTGG
E3	v2bBar1267L	AACCTGGC	Seoul	XLR_534R_v2bBar1267L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTGGCATTACCGCGGCTGCTGG
E4	v2bBar637L	ACGAAGTC	Bishkek	XLR_534R_v2bBar637L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAAGTCATTACCGCGGCTGCTGG
E5	v2bBar435L	TTCGTGGC	Riga	XLR_534R_v2bBar435L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGGCATTACCGCGGCTGCTGG
E6	v2bBar1202L	AACACAAC	Beirut	XLR_534R_v2bBar1202L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACACAACATTACCGCGGCTGCTGG
E7	v2bBar413L	TTCTTGAC	Maseru	XLR_534R_v2bBar413L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGACATTACCGCGGCTGCTGG
E8	v2bBar289L	TCCAAGTC	Monrovia	XLR_534R_v2bBar289L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGTCATTACCGCGGCTGCTGG
E9	v2bBar433L	TTCGCGAC	Tripoli	XLR_534R_v2bBar433L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGCGACATTACCGCGGCTGCTGG
E10	v2bBar121L	CCGGTCGC	Vaduz	XLR_534R_v2bBar121L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGTCGCATTACCGCGGCTGCTGG
E11	v2bBar669L	ACCTGAAC	Vilnius	XLR_534R_v2bBar669L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTGAACATTACCGCGGCTGCTGG
E12	v2bBar1156L	AAGAGTTC	Luxembourg	XLR_534R_v2bBar1156L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGTTCATTACCGCGGCTGCTGG
F1	v2bBar370L	TTGACAAC	Bamako	XLR_534R_v2bBar370L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGACAACATTACCGCGGCTGCTGG
F2	v2bBar281L	TCCAGAAC	Valletta	XLR_534R_v2bBar281L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAGAACATTACCGCGGCTGCTGG
F3	v2bBar49L	CGGTCTTC	Kishinev	XLR_534R_v2bBar49L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTCTTCATTACCGCGGCTGCTGG
F4	v2bBar1173L	AAGGCCTC	Monaco	XLR_534R_v2bBar1173L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCCTCATTACCGCGGCTGCTGG
F5	v2bBar599L	ACTAATTC	Rabat	XLR_534R_v2bBar599L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAATTCATTACCGCGGCTGCTGG
F6	v2bBar167L	TGACCGTC	Maputo	XLR_534R_v2bBar167L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACCGTCATTACCGCGGCTGCTGG
F7	v2bBar161L	TGTCGGAC	Kathmandu	XLR_534R_v2bBar161L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCGGACATTACCGCGGCTGCTGG
F8	v2bBar580L	AGGTTGTC	Amsterdam	XLR_534R_v2bBar580L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGTTGTCATTACCGCGGCTGCTGG
F9	v2bBar629L	ACGAGAAC	Wellington	XLR_534R_v2bBar629L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGAACATTACCGCGGCTGCTGG
F10	v2bBar184L	TGGTGAAC	Managua	XLR_534R_v2bBar184L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTGAACATTACCGCGGCTGCTGG
F11	v2bBar233L	TCGTTGTC	Abuja	XLR_534R_v2bBar233L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTTGTCATTACCGCGGCTGCTGG
F12	v2bBar364L	TTGTGTTC	Oslo	XLR_534R_v2bBar364L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGTGTTCATTACCGCGGCTGCTGG

G1	v2bBar78L	CCACGGTC	Muscat	XLR_534R_v2bBar78L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCACGGTCATTACCGCGGCTGCTGG
G2	v2bBar393L	TTGGAGGC	Islamabad	XLR_534R_v2bBar393L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCATTACCGCGGCTGCTGG
G3	v2bBar350L	TTATCGGC	Asuncion	XLR_534R_v2bBar350L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTATCGGCATTACCGCGGCTGCTGG
G4	v2bBar1164L	AAGAAGAC	Lima	XLR_534R_v2bBar1164L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAAGACATTACCGCGGCTGCTGG
G5	v2bBar1196L	AACTGTTC	Manila	XLR_534R_v2bBar1196L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACTGTTCATTACCGCGGCTGCTGG
G6	v2bBar411L	TTCTCAAC	Warsaw	XLR_534R_v2bBar411L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCAACATTACCGCGGCTGCTGG
G7	v2bBar6L	CTTCCTTC	Lisbon	XLR_534R_v2bBar6L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCTTCATTACCGCGGCTGCTGG
G8	v2bBar1031L	ATTCGTAC	Doha	XLR_534R_v2bBar1031L	CCATCTCATCCCTGCGTGTCTCCGACTCAGATTCGTACATTACCGCGGCTGCTGG
G9	v2bBar76L	CCTTCCGC	Moscow	XLR_534R_v2bBar76L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTCCGCATTACCGCGGCTGCTGG
G10	v2bBar555L	AGTCCGTC	Kigali	XLR_534R_v2bBar555L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCCGTCATTACCGCGGCTGCTGG
G11	v2bBar378L	TTGAACTC	Riyadh	XLR_534R_v2bBar378L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAACTCATTACCGCGGCTGCTGG
G12	v2bBar1225L	AACGAGGC	Dakar	XLR_534R_v2bBar1225L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGAGGCATTACCGCGGCTGCTGG
H1	v2bBar99L	CCGTTCAC	Belgrade	XLR_534R_v2bBar99L	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGTTCACATTACCGCGGCTGCTGG
H2	v2bBar236L	TCGAGGAAC	Singapore	XLR_534R_v2bBar236L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGGAACATTACCGCGGCTGCTGG
H3	v2bBar731L	ACCGGAAGC	Bratislava	XLR_534R_v2bBar731L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGGAAGCATTACCGCGGCTGCTGG
H4	v2bBar628L	ACGTTCCAC	Mogadishu	XLR_534R_v2bBar628L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTTCCACATTACCGCGGCTGCTGG
H5	v2bBar1250L	AACGGAGTC	Pretoria	XLR_534R_v2bBar1250L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGGAGTCATTACCGCGGCTGCTGG
H6	v2bBar438L	TTCGTTATC	Madrid	XLR_534R_v2bBar438L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTTATCATTACCGCGGCTGCTGG
H7	v2bBar693L	ACCGTAATC	Colombo	XLR_534R_v2bBar693L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGTAATCATTACCGCGGCTGCTGG
H8	v2bBar672L	ACCTTGGTC	Khartoum	XLR_534R_v2bBar672L	CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTTGGTCATTACCGCGGCTGCTGG
H9	v2bBar355L	TTAAGATTC	Stockholm	XLR_534R_v2bBar355L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGATTCATTACCGCGGCTGCTGG
H10	v2bBar187L	TGGTTGGTC	Bern	XLR_534R_v2bBar187L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTTGGTCATTACCGCGGCTGCTGG
H11	v2bBar162L	TGTCCGGTC	Damascus	XLR_534R_v2bBar162L	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCGGTCATTACCGCGGCTGCTGG
H12	v2bBar1292L	AACCGTGTC	Taipei	XLR_534R_v2bBar1292L	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCGTGTCATTACCGCGGCTGCTGG

Variable regions V5 --> V3

"B" adapter for XLR + V3-5 357F
5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCCTACGGGAGGCAGCAG

plate position	barcode name	barcode sequence	Barcode city name	primer name	"A" barcoded adapter for XLR system + barcode + V3-5 926R primer
A1	v2bBar8L	CACGC	Kabul	XLR_926R_v2bBar8L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCCCGTCAATTCMTTTRAGT
A2	v2bBar23L	CGCAAC	Tirana	XLR_926R_v2bBar23L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCAACCCGTCAATTCMTTTRAGT
A3	v2bBar174L	TGAAGC	Algiers	XLR_926R_v2bBar174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAAGCCCGTCAATTCMTTTRAGT
A4	v2bBar602L	ACTTGC	Canberra	XLR_926R_v2bBar602L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTTGCCCGTCAATTCMTTTRAGT
A5	v2bBar212L	TCACAC	Vienna	XLR_926R_v2bBar212L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACACCCGTCAATTCMTTTRAGT
A6	v2bBar25L	CGTGAC	Baku	XLR_926R_v2bBar25L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACCCGTCAATTCMTTTRAGT
A7	v2bBar622L	ACGCGC	Nassau	XLR_926R_v2bBar622L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGCCCGTCAATTCMTTTRAGT
A8	v2bBar72L	CCTCTC	Bridgetown	XLR_926R_v2bBar72L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTCTCCCGTCAATTCMTTTRAGT
A9	v2bBar600L	ACTCAC	Minsk	XLR_926R_v2bBar600L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCACCCGTCAATTCMTTTRAGT
A10	v2bBar559L	AGACAC	Brussels	XLR_926R_v2bBar559L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACACCCGTCAATTCMTTTRAGT
A11	v2bBar31L	CGACTC	Sarajevo	XLR_926R_v2bBar31L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTCCCGTCAATTCMTTTRAGT
A12	v2bBar551L	AGCTTC	Rio	XLR_926R_v2bBar551L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTTCCCGTCAATTCMTTTRAGT
B1	v2bBar1149L	AAGCCGC	Sofia	XLR_926R_v2bBar1149L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCCGCCCGTCAATTCMTTTRAGT
B2	v2bBar15L	CAAGAAC	Ottawa	XLR_926R_v2bBar15L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCAAGAACCCGTCAATTCMTTTRAGT
B3	v2bBar556L	AGTTGGC	Bangui	XLR_926R_v2bBar556L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTTGGCCCGTCAATTCMTTTRAGT
B4	v2bBar144L	TATCAAC	Santiago	XLR_926R_v2bBar144L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAACCCGTCAATTCMTTTRAGT
B5	v2bBar575L	AGGCGGC	Beijing	XLR_926R_v2bBar575L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCGGCCCGTCAATTCMTTTRAGT
B6	v2bBar48L	CGGTATC	Bogota	XLR_926R_v2bBar48L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTATCCCGTCAATTCMTTTRAGT
B7	v2bBar166L	TGACGAC	Kinshasa	XLR_926R_v2bBar166L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACGACCCGTCAATTCMTTTRAGT
B8	v2bBar613L	ACAAGGC	Brazzaville	XLR_926R_v2bBar613L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACAAGGCCCGTCAATTCMTTTRAGT
B9	v2bBar560L	AGACCTC	Zagreb	XLR_926R_v2bBar560L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACCTCCCGTCAATTCMTTTRAGT
B10	v2bBar741L	ATACCAC	Havana	XLR_926R_v2bBar741L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATACCACCCGTCAATTCMTTTRAGT
B11	v2bBar228L	TCGCGGC	Nicosia	XLR_926R_v2bBar228L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGGCCCGTCAATTCMTTTRAGT
B12	v2bBar807L	ATCTTAC	Prague	XLR_926R_v2bBar807L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTTACCCGTCAATTCMTTTRAGT
C1	v2bBar1273L	AACCAGC	Copenhagen	XLR_926R_v2bBar1273L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCAGCCCGTCAATTCMTTTRAGT
C2	v2bBar441L	TTCGAGC	Djibouti	XLR_926R_v2bBar441L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGCCCGTCAATTCMTTTRAGT
C3	v2bBar1174L	AAGGTGC	Quito	XLR_926R_v2bBar1174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGTGCCCGTCAATTCMTTTRAGT
C4	v2bBar209L	TCTTGGC	Cairo	XLR_926R_v2bBar209L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGGCCCGTCAATTCMTTTRAGT
C5	v2bBar153L	TAATCTC	Suva	XLR_926R_v2bBar153L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAATCTCCCGTCAATTCMTTTRAGT
C6	v2bBar213L	TCACCTC	Helsinki	XLR_926R_v2bBar213L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACCTCCCGTCAATTCMTTTRAGT
C7	v2bBar298L	TCCGCTC	Paris	XLR_926R_v2bBar298L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGCTCCCGTCAATTCMTTTRAGT
C8	v2bBar146L	TATTGAC	Berlin	XLR_926R_v2bBar146L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTGACCCGTCAATTCMTTTRAGT
C9	v2bBar554L	AGTCGAC	Accra	XLR_926R_v2bBar554L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCGACCCGTCAATTCMTTTRAGT
C10	v2bBar646L	ACGGCTC	Athens	XLR_926R_v2bBar646L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGGCTCCCGTCAATTCMTTTRAGT
C11	v2bBar158L	TGCGTTC	Guatemala	XLR_926R_v2bBar158L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTTCCCGTCAATTCMTTTRAGT
C12	v2bBar207L	TCTCGAC	Conakry	XLR_926R_v2bBar207L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGACCCGTCAATTCMTTTRAGT
D1	v2bBar77L	CCAGGAC	Bissau	XLR_926R_v2bBar77L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGGACCCGTCAATTCMTTTRAGT
D2	v2bBar601L	ACTCCTC	Budapest	XLR_926R_v2bBar601L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCCTCCCGTCAATTCMTTTRAGT
D3	v2bBar481L	TTCCTGC	Jakarta	XLR_926R_v2bBar481L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCCCGTCAATTCMTTTRAGT
D4	v2bBar419L	TTCATAC	Tehran	XLR_926R_v2bBar419L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCATACCCGTCAATTCMTTTRAGT
D5	v2bBar26L	CGTCGTC	Baghdad	XLR_926R_v2bBar26L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCGTCCCGTCAATTCMTTTRAGT
D6	v2bBar1172L	AAGGCAC	Dublin	XLR_926R_v2bBar1172L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCACCCGTCAATTCMTTTRAGT
D7	v2bBar1210L	AACAACTC	Jerusalem	XLR_926R_v2bBar1210L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACAACTCCCGTCAATTCMTTTRAGT
D8	v2bBar606L	ACACGGAC	Rome	XLR_926R_v2bBar606L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACACGGACCCGTCAATTCMTTTRAGT
D9	v2bBar159L	TGCCGAAC	Kingston	XLR_926R_v2bBar159L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCGAACCCGTCAATTCMTTTRAGT
D10	v2bBar147L	TATTCGTC	Tokyo	XLR_926R_v2bBar147L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTCGTCCCGTCAATTCMTTTRAGT
D11	v2bBar141L	TAGGAATC	Amman	XLR_926R_v2bBar141L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGAATCCCGTCAATTCMTTTRAGT
D12	v2bBar119L	CCGGCCAC	Nairobi	XLR_926R_v2bBar119L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGCCACCCGTCAATTCMTTTRAGT

E1	v2bBar1379L	AATGGTAC	Tarawa	XLR_926R_v2bBar1379L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAATGGTACCCGTCAATTCMTTTRAGT
E2	v2bBar208L	TCTCCGTC	Pyongyang	XLR_926R_v2bBar208L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCCGTCCCGTCAATTCMTTTRAGT
E3	v2bBar1267L	AACCTGGC	Seoul	XLR_926R_v2bBar1267L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTGGCCCGTCAATTCMTTTRAGT
E4	v2bBar637L	ACGAAGTC	Bishkek	XLR_926R_v2bBar637L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAAGTCCCGTCAATTCMTTTRAGT
E5	v2bBar435L	TTCGTGGC	Riga	XLR_926R_v2bBar435L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGGCCCGTCAATTCMTTTRAGT
E6	v2bBar1202L	AACACAAC	Beirut	XLR_926R_v2bBar1202L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACACAACCCGTCAATTCMTTTRAGT
E7	v2bBar413L	TTCTTGAC	Maseru	XLR_926R_v2bBar413L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGACCCGTCAATTCMTTTRAGT
E8	v2bBar289L	TCCAAGTC	Monrovia	XLR_926R_v2bBar289L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGTCCCGTCAATTCMTTTRAGT
E9	v2bBar433L	TTCGCGAC	Tripoli	XLR_926R_v2bBar433L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGCGACCCGTCAATTCMTTTRAGT
E10	v2bBar121L	CCGGTCGC	Vaduz	XLR_926R_v2bBar121L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGTCGCCCGTCAATTCMTTTRAGT
E11	v2bBar669L	ACCTGAAC	Vilnius	XLR_926R_v2bBar669L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTGAACCCGTCAATTCMTTTRAGT
E12	v2bBar1156L	AAGAGTTC	Luxembourg	XLR_926R_v2bBar1156L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGTTCCCGTCAATTCMTTTRAGT
F1	v2bBar370L	TTGACAAC	Bamako	XLR_926R_v2bBar370L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGACAACCCGTCAATTCMTTTRAGT
F2	v2bBar281L	TCCAGAAC	Valletta	XLR_926R_v2bBar281L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAGAACCCGTCAATTCMTTTRAGT
F3	v2bBar49L	CGGTCTTC	Kishinev	XLR_926R_v2bBar49L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTCTTCCCGTCAATTCMTTTRAGT
F4	v2bBar1173L	AAGGCCTC	Monaco	XLR_926R_v2bBar1173L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCCTCCCGTCAATTCMTTTRAGT
F5	v2bBar599L	ACTAATTC	Rabat	XLR_926R_v2bBar599L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAATTCCCGTCAATTCMTTTRAGT
F6	v2bBar167L	TGACCGTC	Maputo	XLR_926R_v2bBar167L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACCGTCCCGTCAATTCMTTTRAGT
F7	v2bBar161L	TGTCGGAC	Kathmandu	XLR_926R_v2bBar161L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCGGACCCGTCAATTCMTTTRAGT
F8	v2bBar580L	AGGTTGTC	Amsterdam	XLR_926R_v2bBar580L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGTTGTCCCGTCAATTCMTTTRAGT
F9	v2bBar629L	ACGAGAAC	Wellington	XLR_926R_v2bBar629L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGAACCCGTCAATTCMTTTRAGT
F10	v2bBar184L	TGGTGAAC	Managua	XLR_926R_v2bBar184L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTGAACCCGTCAATTCMTTTRAGT
F11	v2bBar233L	TCGTTGTC	Abuja	XLR_926R_v2bBar233L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTTGTCCCGTCAATTCMTTTRAGT
F12	v2bBar364L	TTGTGTTC	Oslo	XLR_926R_v2bBar364L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGTGTTCCCGTCAATTCMTTTRAGT
G1	v2bBar78L	CCACGGTC	Muscat	XLR_926R_v2bBar78L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCACGGTCCCGTCAATTCMTTTRAGT
G2	v2bBar393L	TTGGAGGC	Islamabad	XLR_926R_v2bBar393L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCCGTCAATTCMTTTRAGT
G3	v2bBar350L	TTATCGGC	Asuncion	XLR_926R_v2bBar350L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTATCGGCCCGTCAATTCMTTTRAGT
G4	v2bBar1164L	AAGAAGAC	Lima	XLR_926R_v2bBar1164L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAAGACCCGTCAATTCMTTTRAGT
G5	v2bBar1196L	AACTGTTC	Manila	XLR_926R_v2bBar1196L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACTGTTCCCGTCAATTCMTTTRAGT
G6	v2bBar411L	TTCTCAAC	Warsaw	XLR_926R_v2bBar411L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCAACCCGTCAATTCMTTTRAGT
G7	v2bBar6L	CTTCCTTC	Lisbon	XLR_926R_v2bBar6L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCTTCCCGTCAATTCMTTTRAGT
G8	v2bBar1031L	ATTCGTAC	Doha	XLR_926R_v2bBar1031L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATTCGTACCCGTCAATTCMTTTRAGT
G9	v2bBar76L	CCTTCCGC	Moscow	XLR_926R_v2bBar76L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTCCGCCCGTCAATTCMTTTRAGT
G10	v2bBar555L	AGTCCGTC	Kigali	XLR_926R_v2bBar555L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCCGTCCCGTCAATTCMTTTRAGT
G11	v2bBar378L	TTGAACTC	Riyadh	XLR_926R_v2bBar378L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAACTCCCGTCAATTCMTTTRAGT
G12	v2bBar1225L	AACGAGGC	Dakar	XLR_926R_v2bBar1225L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGAGGCCCGTCAATTCMTTTRAGT
H1	v2bBar99L	CCGTTCAC	Belgrade	XLR_926R_v2bBar99L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGTTCACCCGTCAATTCMTTTRAGT
H2	v2bBar236L	TCGAGGAAC	Singapore	XLR_926R_v2bBar236L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGGAACCCGTCAATTCMTTTRAGT
H3	v2bBar731L	ACCGGAAGC	Bratislava	XLR_926R_v2bBar731L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGGAAGCCCGTCAATTCMTTTRAGT
H4	v2bBar628L	ACGTTCCAC	Mogadishu	XLR_926R_v2bBar628L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTTCCACCCGTCAATTCMTTTRAGT
H5	v2bBar1250L	AACGGAGTC	Pretoria	XLR_926R_v2bBar1250L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGGAGTCCCGTCAATTCMTTTRAGT
H6	v2bBar438L	TTCGTTATC	Madrid	XLR_926R_v2bBar438L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTTATCCCGTCAATTCMTTTRAGT
H7	v2bBar693L	ACCGTAATC	Colombo	XLR_926R_v2bBar693L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGTAATCCCGTCAATTCMTTTRAGT
H8	v2bBar672L	ACCTTGGTC	Khartoum	XLR_926R_v2bBar672L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTTGGTCCCGTCAATTCMTTTRAGT
H9	v2bBar355L	TTAAGATTC	Stockholm	XLR_926R_v2bBar355L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGATTCCCGTCAATTCMTTTRAGT
H10	v2bBar187L	TGGTTGGTC	Bern	XLR_926R_v2bBar187L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTTGGTCCCGTCAATTCMTTTRAGT
H11	v2bBar162L	TGTCCGGTC	Damascus	XLR_926R_v2bBar162L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCGGTCCCGTCAATTCMTTTRAGT
H12	v2bBar1292L	AACCGTGTC	Taipei	XLR_926R_v2bBar1292L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCGTGTCCCGTCAATTCMTTTRAGT

Variable regions V9 --> V6

	"B" adapter for XLR + V6-9 968F
	5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAACGCGAAGAACCTTAC

plate position	barcode name	barcode sequence	Barcode city name	primer name	"A" barcoded adapter for XLR system + barcode + V6-9 1492R primer
A1	v2bBar8L	CACGC	Kabul	XLR_1492R_v2bBar8L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCACGCTACGGYTACCTTGTTAYGACTT
A2	v2bBar23L	CGCAAC	Tirana	XLR_1492R_v2bBar23L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGCAACTACGGYTACCTTGTTAYGACTT
A3	v2bBar174L	TGAAGC	Algiers	XLR_1492R_v2bBar174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAAGCTACGGYTACCTTGTTAYGACTT
A4	v2bBar602L	ACTTGC	Canberra	XLR_1492R_v2bBar602L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTTGCTACGGYTACCTTGTTAYGACTT
A5	v2bBar212L	TCACAC	Vienna	XLR_1492R_v2bBar212L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACACTACGGYTACCTTGTTAYGACTT
A6	v2bBar25L	CGTGAC	Baku	XLR_1492R_v2bBar25L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGACTACGGYTACCTTGTTAYGACTT
A7	v2bBar622L	ACGCGC	Nassau	XLR_1492R_v2bBar622L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGCTACGGYTACCTTGTTAYGACTT
A8	v2bBar72L	CCTCTC	Bridgetown	XLR_1492R_v2bBar72L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTCTCTACGGYTACCTTGTTAYGACTT
A9	v2bBar600L	ACTCAC	Minsk	XLR_1492R_v2bBar600L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCACTACGGYTACCTTGTTAYGACTT
A10	v2bBar559L	AGACAC	Brussels	XLR_1492R_v2bBar559L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACACTACGGYTACCTTGTTAYGACTT
A11	v2bBar31L	CGACTC	Sarajevo	XLR_1492R_v2bBar31L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGACTCTACGGYTACCTTGTTAYGACTT
A12	v2bBar551L	AGCTTC	Rio	XLR_1492R_v2bBar551L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTTCTACGGYTACCTTGTTAYGACTT
B1	v2bBar1149L	AAGCCGC	Sofia	XLR_1492R_v2bBar1149L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCCGCTACGGYTACCTTGTTAYGACTT
B2	v2bBar15L	CAAGAAC	Ottawa	XLR_1492R_v2bBar15L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCAAGAACTACGGYTACCTTGTTAYGACTT
B3	v2bBar556L	AGTTGGC	Bangui	XLR_1492R_v2bBar556L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTTGGCTACGGYTACCTTGTTAYGACTT
B4	v2bBar144L	TATCAAC	Santiago	XLR_1492R_v2bBar144L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATCAACTACGGYTACCTTGTTAYGACTT
B5	v2bBar575L	AGGCGGC	Beijing	XLR_1492R_v2bBar575L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCGGCTACGGYTACCTTGTTAYGACTT
B6	v2bBar48L	CGGTATC	Bogota	XLR_1492R_v2bBar48L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTATCTACGGYTACCTTGTTAYGACTT
B7	v2bBar166L	TGACGAC	Kinshasa	XLR_1492R_v2bBar166L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACGACTACGGYTACCTTGTTAYGACTT
B8	v2bBar613L	ACAAGGC	Brazzaville	XLR_1492R_v2bBar613L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACAAGGCTACGGYTACCTTGTTAYGACTT
B9	v2bBar560L	AGACCTC	Zagreb	XLR_1492R_v2bBar560L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACCTCTACGGYTACCTTGTTAYGACTT
B10	v2bBar741L	ATACCAC	Havana	XLR_1492R_v2bBar741L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATACCACTACGGYTACCTTGTTAYGACTT
B11	v2bBar228L	TCGCGGC	Nicosia	XLR_1492R_v2bBar228L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCGGCTACGGYTACCTTGTTAYGACTT
B12	v2bBar807L	ATCTTAC	Prague	XLR_1492R_v2bBar807L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATCTTACTACGGYTACCTTGTTAYGACTT

C1	v2bBar1273L	AACCAGC	Copenhagen	XLR_1492R_v2bBar1273L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCAGCTACGGYTACCTTGTTAYGACTT
C2	v2bBar441L	TTCGAGC	Djibouti	XLR_1492R_v2bBar441L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGCTACGGYTACCTTGTTAYGACTT
C3	v2bBar1174L	AAGGTGC	Quito	XLR_1492R_v2bBar1174L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGTGCTACGGYTACCTTGTTAYGACTT
C4	v2bBar209L	TCTTGGC	Cairo	XLR_1492R_v2bBar209L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGGCTACGGYTACCTTGTTAYGACTT
C5	v2bBar153L	TAATCTC	Suva	XLR_1492R_v2bBar153L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAATCTCTACGGYTACCTTGTTAYGACTT
C6	v2bBar213L	TCACCTC	Helsinki	XLR_1492R_v2bBar213L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACCTCTACGGYTACCTTGTTAYGACTT
C7	v2bBar298L	TCCGCTC	Paris	XLR_1492R_v2bBar298L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGCTCTACGGYTACCTTGTTAYGACTT
C8	v2bBar146L	TATTGAC	Berlin	XLR_1492R_v2bBar146L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTGACTACGGYTACCTTGTTAYGACTT
C9	v2bBar554L	AGTCGAC	Accra	XLR_1492R_v2bBar554L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCGACTACGGYTACCTTGTTAYGACTT
C10	v2bBar646L	ACGGCTC	Athens	XLR_1492R_v2bBar646L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGGCTCTACGGYTACCTTGTTAYGACTT
C11	v2bBar158L	TGCGTTC	Guatemala	XLR_1492R_v2bBar158L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCGTTCTACGGYTACCTTGTTAYGACTT
C12	v2bBar207L	TCTCGAC	Conakry	XLR_1492R_v2bBar207L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCGACTACGGYTACCTTGTTAYGACTT
D1	v2bBar77L	CCAGGAC	Bissau	XLR_1492R_v2bBar77L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGGACTACGGYTACCTTGTTAYGACTT
D2	v2bBar601L	ACTCCTC	Budapest	XLR_1492R_v2bBar601L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTCCTCTACGGYTACCTTGTTAYGACTT
D3	v2bBar481L	TTCCTGC	Jakarta	XLR_1492R_v2bBar481L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCTGCTACGGYTACCTTGTTAYGACTT
D4	v2bBar419L	TTCATAC	Tehran	XLR_1492R_v2bBar419L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCATACTACGGYTACCTTGTTAYGACTT
D5	v2bBar26L	CGTCGTC	Baghdad	XLR_1492R_v2bBar26L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCGTCTACGGYTACCTTGTTAYGACTT
D6	v2bBar1172L	AAGGCAC	Dublin	XLR_1492R_v2bBar1172L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCACTACGGYTACCTTGTTAYGACTT
D7	v2bBar1210L	AACAACTC	Jerusalem	XLR_1492R_v2bBar1210L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACAACTCTACGGYTACCTTGTTAYGACTT
D8	v2bBar606L	ACACGGAC	Rome	XLR_1492R_v2bBar606L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACACGGACTACGGYTACCTTGTTAYGACTT
D9	v2bBar159L	TGCCGAAC	Kingston	XLR_1492R_v2bBar159L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCGAACTACGGYTACCTTGTTAYGACTT
D10	v2bBar147L	TATTCGTC	Tokyo	XLR_1492R_v2bBar147L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTATTCGTCTACGGYTACCTTGTTAYGACTT
D11	v2bBar141L	TAGGAATC	Amman	XLR_1492R_v2bBar141L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGAATCTACGGYTACCTTGTTAYGACTT
D12	v2bBar119L	CCGGCCAC	Nairobi	XLR_1492R_v2bBar119L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGCCACTACGGYTACCTTGTTAYGACTT
E1	v2bBar1379L	AATGGTAC	Tarawa	XLR_1492R_v2bBar1379L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAATGGTACTACGGYTACCTTGTTAYGACTT
E2	v2bBar208L	TCTCCGTC	Pyongyang	XLR_1492R_v2bBar208L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCCGTCTACGGYTACCTTGTTAYGACTT
E3	v2bBar1267L	AACCTGGC	Seoul	XLR_1492R_v2bBar1267L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTGGCTACGGYTACCTTGTTAYGACTT
E4	v2bBar637L	ACGAAGTC	Bishkek	XLR_1492R_v2bBar637L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAAGTCTACGGYTACCTTGTTAYGACTT
E5	v2bBar435L	TTCGTGGC	Riga	XLR_1492R_v2bBar435L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGGCTACGGYTACCTTGTTAYGACTT
E6	v2bBar1202L	AACACAAC	Beirut	XLR_1492R_v2bBar1202L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACACAACTACGGYTACCTTGTTAYGACTT
E7	v2bBar413L	TTCTTGAC	Maseru	XLR_1492R_v2bBar413L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGACTACGGYTACCTTGTTAYGACTT
E8	v2bBar289L	TCCAAGTC	Monrovia	XLR_1492R_v2bBar289L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGTCTACGGYTACCTTGTTAYGACTT
E9	v2bBar433L	TTCGCGAC	Tripoli	XLR_1492R_v2bBar433L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGCGACTACGGYTACCTTGTTAYGACTT
E10	v2bBar121L	CCGGTCGC	Vaduz	XLR_1492R_v2bBar121L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGTCGCTACGGYTACCTTGTTAYGACTT
E11	v2bBar669L	ACCTGAAC	Vilnius	XLR_1492R_v2bBar669L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTGAACTACGGYTACCTTGTTAYGACTT
E12	v2bBar1156L	AAGAGTTC	Luxembourg	XLR_1492R_v2bBar1156L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGTTCTACGGYTACCTTGTTAYGACTT
F1	v2bBar370L	TTGACAAC	Bamako	XLR_1492R_v2bBar370L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGACAACTACGGYTACCTTGTTAYGACTT
F2	v2bBar281L	TCCAGAAC	Valletta	XLR_1492R_v2bBar281L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAGAACTACGGYTACCTTGTTAYGACTT
F3	v2bBar49L	CGGTCTTC	Kishinev	XLR_1492R_v2bBar49L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGTCTTCTACGGYTACCTTGTTAYGACTT
F4	v2bBar1173L	AAGGCCTC	Monaco	XLR_1492R_v2bBar1173L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGCCTCTACGGYTACCTTGTTAYGACTT
F5	v2bBar599L	ACTAATTC	Rabat	XLR_1492R_v2bBar599L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACTAATTCTACGGYTACCTTGTTAYGACTT
F6	v2bBar167L	TGACCGTC	Maputo	XLR_1492R_v2bBar167L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACCGTCTACGGYTACCTTGTTAYGACTT
F7	v2bBar161L	TGTCGGAC	Kathmandu	XLR_1492R_v2bBar161L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCGGACTACGGYTACCTTGTTAYGACTT
F8	v2bBar580L	AGGTTGTC	Amsterdam	XLR_1492R_v2bBar580L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGTTGTCTACGGYTACCTTGTTAYGACTT
F9	v2bBar629L	ACGAGAAC	Wellington	XLR_1492R_v2bBar629L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGAACTACGGYTACCTTGTTAYGACTT
F10	v2bBar184L	TGGTGAAC	Managua	XLR_1492R_v2bBar184L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTGAACTACGGYTACCTTGTTAYGACTT
F11	v2bBar233L	TCGTTGTC	Abuja	XLR_1492R_v2bBar233L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTTGTCTACGGYTACCTTGTTAYGACTT
F12	v2bBar364L	TTGTGTTC	Oslo	XLR_1492R_v2bBar364L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGTGTTCTACGGYTACCTTGTTAYGACTT
G1	v2bBar78L	CCACGGTC	Muscat	XLR_1492R_v2bBar78L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCACGGTCTACGGYTACCTTGTTAYGACTT
G2	v2bBar393L	TTGGAGGC	Islamabad	XLR_1492R_v2bBar393L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCTACGGYTACCTTGTTAYGACTT
G3	v2bBar350L	TTATCGGC	Asuncion	XLR_1492R_v2bBar350L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTATCGGCTACGGYTACCTTGTTAYGACTT
G4	v2bBar1164L	AAGAAGAC	Lima	XLR_1492R_v2bBar1164L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAAGACTACGGYTACCTTGTTAYGACTT
G5	v2bBar1196L	AACTGTTC	Manila	XLR_1492R_v2bBar1196L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACTGTTCTACGGYTACCTTGTTAYGACTT
G6	v2bBar411L	TTCTCAAC	Warsaw	XLR_1492R_v2bBar411L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCAACTACGGYTACCTTGTTAYGACTT
G7	v2bBar6L	CTTCCTTC	Lisbon	XLR_1492R_v2bBar6L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCTTCTACGGYTACCTTGTTAYGACTT
G8	v2bBar1031L	ATTCGTAC	Doha	XLR_1492R_v2bBar1031L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGATTCGTACTACGGYTACCTTGTTAYGACTT
G9	v2bBar76L	CCTTCCGC	Moscow	XLR_1492R_v2bBar76L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTCCGCTACGGYTACCTTGTTAYGACTT
G10	v2bBar555L	AGTCCGTC	Kigali	XLR_1492R_v2bBar555L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTCCGTCTACGGYTACCTTGTTAYGACTT
G11	v2bBar378L	TTGAACTC	Riyadh	XLR_1492R_v2bBar378L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAACTCTACGGYTACCTTGTTAYGACTT
G12	v2bBar1225L	AACGAGGC	Dakar	XLR_1492R_v2bBar1225L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGAGGCTACGGYTACCTTGTTAYGACTT
H1	v2bBar99L	CCGTTCAC	Belgrade	XLR_1492R_v2bBar99L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGTTCACTACGGYTACCTTGTTAYGACTT
H2	v2bBar236L	TCGAGGAAC	Singapore	XLR_1492R_v2bBar236L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAGGAACTACGGYTACCTTGTTAYGACTT
H3	v2bBar731L	ACCGGAAGC	Bratislava	XLR_1492R_v2bBar731L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGGAAGCTACGGYTACCTTGTTAYGACTT
H4	v2bBar628L	ACGTTCCAC	Mogadishu	XLR_1492R_v2bBar628L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACGTTCCACTACGGYTACCTTGTTAYGACTT
H5	v2bBar1250L	AACGGAGTC	Pretoria	XLR_1492R_v2bBar1250L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACGGAGTCTACGGYTACCTTGTTAYGACTT
H6	v2bBar438L	TTCGTTATC	Madrid	XLR_1492R_v2bBar438L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTTATCTACGGYTACCTTGTTAYGACTT
H7	v2bBar693L	ACCGTAATC	Colombo	XLR_1492R_v2bBar693L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCGTAATCTACGGYTACCTTGTTAYGACTT
H8	v2bBar672L	ACCTTGGTC	Khartoum	XLR_1492R_v2bBar672L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGACCTTGGTCTACGGYTACCTTGTTAYGACTT
H9	v2bBar355L	TTAAGATTC	Stockholm	XLR_1492R_v2bBar355L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGATTCTACGGYTACCTTGTTAYGACTT
H10	v2bBar187L	TGGTTGGTC	Bern	XLR_1492R_v2bBar187L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGTTGGTCTACGGYTACCTTGTTAYGACTT
H11	v2bBar162L	TGTCCGGTC	Damascus	XLR_1492R_v2bBar162L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCGGTCTACGGYTACCTTGTTAYGACTT
H12	v2bBar1292L	AACCGTGTC	Taipei	XLR_1492R_v2bBar1292L	5' CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCGTGTCTACGGYTACCTTGTTAYGACTT

8.2.4. S004 – University of Colorado, Boulder

<Not included>

8.2.5. S005 – University of California at San Francisco PhyloChip Protocol

<Not included>

8.2.6. S007 – University of Pittsburgh

<Not included>

9. FORM INSTRUCTIONS

9.1. General Form Information

Each form has certain key items at the top which uniquely identify that form. Fill in these items on all forms. These items include the clinical center number, the LHMP Participant ID (when known), the local site participant ID, and visit identification. There are at least two types of forms which may be completed.

1. Forms which are completed and retained at the center and are not sent to the DACC. These are for the center's internal use only and should be filed in the participant's record.
2. Forms which are designed to be transferred from the center to the DACC by entry into MIDAS, the internet data entry site; or by sending electronic files of the data to the DACC's secure FTP (File Transfer Protocol) server for the LHMP database.

There are several forms used for phenotype data collection including the Basic Patient Information (BASE) form, LHMP Blood Collection Form (BLOOD), LHMP Bronchoalveolar Lavage Form (BALFORM), LHMP Consent Record Form (CONSENT), LHMP Demographics Form (DEMOFORM), LHMP Diagnosis Form (DIAGNOSI), LHMP Oral Wash Collection Form (OW) and the LHMP Pulmonary HIV Questionnaire (PULQUEST). All forms including the accompanying Administrative Guides are found in the Appendices.

9.2. General Instructions

- Each center will work with the DACC to adapt the LHMP forms to the needs of the individual center.
    1. Questions labeled as **(CORE)** are questions that the LHMP study group would like completed, but they are not required. On the form, these questions are **not** highlighted in grey.
    - Questions labeled as **(OPTIONAL**) are questions which each site can select to include or not; they are not part of the core questions. On the form, these questions are highlighted in grey.
    - The DACC will send the center a final version of the center's revised form for approval and that final version will be kept on file at the DACC. If the center desires any additional changes the DACC is to be notified as soon as possible to ensure that all the questions and skip patterns are aligned in the database and to maintain current records at the DACC.
- To print the center's version of the form for study use, make sure that the OPTIONAL questions are un-highlight and delete the blue instruction box at the beginning of the form.
- The Administrative Guide should only be printed for by study staff.

9.2.1. Minimum Data Requirements

While all centers are strongly encouraged to complete all questions labeled as **CORE** questions, it is understood that there are occasional unusual circumstances where this is not possible. In an effort to be as flexible and inclusive as possible, the following questions require responses for the participant to be included as an LHMP participant, (and only when it is not possible to obtain additional information):

- Demographics Form:
    - Question I.A.: What is your date of birth?
    - Question I.B.: What is your sex?
    - Question I.C.: Are you Hispanic or Latino/Latina?

        - Question I.D.: Which one of the following (race) best describes you?
- Pulmonary Questionnaire Version 2 (and Follow-up Pulmonary when the question is asked):
    - Question 1: Are you infected with HIV?
    - Question 2: Before entering this study, had you ever been on any antiretroviral medications?
    - Question 3: Have you been on any antiretroviral medications in the last 6 months?
    - Question 5: Are you currently (within the last week) on any antiretroviral medicines?
    - Question 58: Have you smoked at least 100 cigarettes in your entire life?
    - Question 60: On average, how many cigarettes do you now smoke a day?
- Note: some of these questions include a skip pattern, when a response indicates that a question can be skipped, then the response is not required. For example if the response to Question 1 "Are you infected with HIV?" is "No", then Questions 2, 3 and 5 are skipped.

9.2.2. Header Information

- Clinic: Record the number that corresponds to the institutional listed below.

C001	University of Michigan, Ann Arbor
C002	University of Pennsylvania
C005	University of California, San Francisco
C006	University of Colorado, Denver
C007	University of Pittsburgh
C008	Indiana University

- LHMP Participant ID: Record the LHMP ID number of the participant, when known.
- Site Participant ID: Record the ID number used by the center to identify the participant in their own database. Fill in the preceding boxes with zeros when the Site Participant ID requires fewer boxes than provided.
- Visit: Record the Visit identification code using three characters. Each center will use their own coding system, and are requested to implement the code consistently, to provide the DACC with information on the format, and for those centers with follow-up visits to also provide the increments that will be used for those subsequent visits.

9.2.3. Form Completion

The underlying principle in evaluating and analyzing data at the DACC is to process the information as submitted without interpretation or second guessing what the recorder intended. The providers of the data are requested to follow simple rules as they complete the form:

- All forms are completed as clearly and legibly as possible using ink (preferably blue or black but NEVER red).
- The staff member completing the form can also write notes and comments in the margins and on the back to help recall and clarify special circumstances.
- Once completed, have another staff member review the form (by 'eyeballing') for completeness, legibility, miscodes, correct skip patterns, and outliers.
- Resolve any problems prior to data entry or file transfer.

- Centers may want to initiate a system of indicating that a form has been reviewed and is ready for data entry, such as writing in a corner '√ by XY'.

9.2.4. Making Edits and Corrections on a Data Form

All edits for phenotype data and specimen characterization data must be made in the location where the data was entered. If the center is entering data into MIDAS, that is where those edits are made; if the center is sending electronic files, the data must be edited in the originating database at the center.

Occasionally corrections, changes, or clarifications are required to data originally marked on a form. All data changes must be documented. When making any change on a data form:

- DO NOT USE WHITE OUT.
- Use red ink or another colored ink different from the one used to record original data (forms should NEVER be initially completed in red or in pencil).
- Cross out the wrong response with one line and write the correct response above or below the wrong response.
- All data changes MUST be initialed and dated next to the correct response.
- A brief explanatory note may also be written next to the correct response or a sticky (Post-it) note attached to the form (if using a sticky note, staple it to the form so it does not get separated).
- A note in the file may also be used to describe corrections which are too long or detailed for the form or sticky note.
- Any data corrections after a form is key entered should be made not only on the form, but in MIDAS as well.

9.3. Specimen Inventory – Characterization Data

9.3.1. Specimen Identification

Each laboratory specimen is labeled with a unique specimen ID number, which may be independent of the Site Participant ID number or an extension of that number. Forms to collect this data for the bronchoalveolar lavage, blood and oral wash samples are located in the appendices.

9.4. Form Change Procedures

There will be no changes to the LHMP form questions without the appropriate review and approval. An LHMP form is one which the study group, as a whole, has agreed to use in whole or in part – LHMP forms currently contain both core questions and optional questions.

9.4.1. Changes to LHMP Forms

Changes to the LHMP forms may come about during the implementation of the LHMP as either additional information is required or specific core questions require clarification to collect the specific information be sought.

- The Coordinators will be asked to review any of these questions or issues that arise. They will be able to review whether the same issues are occurring at more than one center.
- Requests for changes to LHMP forms will then be referred to the originating or designing working group for review.
- DACC will work the appropriate working group to implement the changes: to the paper forms, in MIDAS and in the mapping for the electronic file transfers. These changes will be documented appropriately to for later analysis purposes.

- Changes or additions to forms may occur twice per year in October and April, for a one month open season.

9.4.2. Addition of Center Specific Questions

<Not Included>

9.4.2.1. Re-numbering and re-ordering questions on forms:

<Not included>

9.4.2.2. Other Forms/Center Specific Questions – Important Considerations

Forms that are not being used by the LHMP will not be added to the DACC MIDAS data base.

The DACC will not accept responsibility for editing or cleaning the single center variables.

If upon review, other centers may decide to include these additional questions, during the time period mentioned above and or when more than 25% of the centers have adopted a specific additional question, that question will be designated at that time either a “core” question or an optional question. Once adopted as part of the LHMP form, the question will retain its original definition, as defined when implemented by the first center and will not be redefined, to protect the integrity of the data initially collected.

10. DATA TRANSFER PROCEDURES

10.1. Phenotype Data

<Not included>

10.2. Sequencing Data

<Not included>

10.3. Requests for Data

<Not included>

11. QUALITY ASSURANCE

11.1. Data Quality Monitoring

Data is entered into the DACC's web based database system, MIDAS (Multimodal Integrated Data Acquisition System) by certified keyers at each center. This system includes programmed data editing features. Weekly data checks are run to check for data entry errors, verify data which falls outside of expected ranges, incorrect skip patterns and conflicting data. Additionally SAS audits are run as needed to cross check for inconsistent data between forms.

11.2. Site Visits

The purpose of an LHMP site visit is to ensure the quality and consistency of the data collected under the LHMP Overview Protocol or any of the collaborative protocols. As stated in the LHMP Overview Protocol, all clinical and sequencing centers will receive at least one site visit during the course of the project.

11.2.1. Types and Purpose of Visits

There are three types of visits; an in-person site visit, site review and visits for cause. Procedures for site visits will remain fairly constant between centers; however, procedures for some visits, i.e. sites visited for cause, will be tailored to address specific concerns at particular centers.

An in-person site visit or a site review will be conducted to confirm that the LHMP policies and procedures, as stated in the LHMP Manual of Operations sections on data collection, have been followed and to confirm the quality of the data. Visits for cause are conducted to address center performance issues.

11.2.2. Timing of Visits

Centers participating in the LHMP are at different stages in their individual center protocols, therefore the first three centers will be selected for a site visit based on how far along they are in their contributions to the collaborative LHMP collaborative protocols. Visits will also be determined in part by geographical location in order to minimize travel costs.

11.2.3. Procedures

These procedures are intended to determine if:

- The LHMP Overview Protocol is being followed.
- The original and any changes to the protocol have been approved by the IRB and/or reported to the sponsor and the IRB.
- Accurate, complete, and current records are being maintained.
- The investigator is carrying out the agreed-upon activities and has not delegated them to other previously unspecified staff.
- The facilities used by the investigator are acceptable for purposes of the study.

Guidelines for Data Quality Assurance in Clinical Trials and Observational Studies (NHLBI)

11.2.4. General Procedures for conducting a site visit

1. The center will work with the DACC to develop the agenda and logistics of the in-person site visit and a visit for cause. The DACC will coordinate with the center to schedule the site

review including the conference call(s). It is important to note that under NIH regulations the center should not be providing food or entertainment for the site visitors.

a) The regularly scheduled in-person site visit:
    i. Will be attended by
        - Two DACC representatives
        - One NHLBI Program Officer may also attend.
    ii. The visit will consist of (details are outlined below):
        - Introduction and descriptions of roles of center personnel
        - Tour of the facilities and description of participant, data and specimen flow
        - Discussion of written responses
        - Discussion of recruitment efforts to date
        - Review of paperwork and data entry
        - Review documentation.
        - Closed meeting with PI and Coordinator
        - Summary and feedback to PI and Coordinator. Thanks & goodbye.

b) The site review visit:
    i. Will be conducted by the DACC and will involve the following steps:
        - The LHMP Site Visit Questionnaire will be sent to the center on the agreed 5 weeks prior to the scheduled site visit conference call and can be found in Appendix 13 LHMP Site Visit Questionnaire . The response to the questionnaire is to be completed and returned to the DACC a minimum of five days prior to the scheduled site visit conference call.
        - A list of participant IDs will be sent to the center at the same time to enable the center to send all those participant's LHMP forms to the DACC. These must be received a minimum of five days prior to the scheduled site visit conference call. The requested files can be sent via email, fax or hard copies by mail. The data audit will be conducted at the DACC.
        - The DACC will review the written responses and the results of the data audit.
    ii. The conference call date/time will be coordinated with the center when arranging the site review. This will be attended by the:
        - LHMP Clinical and Sequencing Center PI's and Coordinators
        - Two DACC representatives
        - One NHLBI Program Officer may also attend.

c) Sites visited for cause, will be tailored to address specific concerns at particular centers.

d) The DACC will provide a written report to the center within four weeks of the visit with recommendations and guidelines and a recommended timeframe for any problem resolution. A copy of the report will be provided to the center's PIs, Coordinator and NHLBI Program Officer.

11.2.5. LHMP In-person Site Visit Checklist

1. Introductions and descriptions of roles of center personnel
    a. Clinical center management
        i. What is the degree of institutional support and commitment for the LHMP clinical center?
        ii. How often are staff meetings held and who attends them?

iii. How is information disseminated to staff (i.e., protocol changes, form changes, etc.)?
iv. How is clinic flow organized? How are participant visits organized from initial contact to screening to first visit to follow-up visits?

b. Sequence center management
i. What is the degree of institutional support and commitment for the LHMP sequencing center?
ii. How often are staff meetings held and who attends them?
iii. How is information disseminated to staff (i.e. procedure changes)
iv. What is standard staff human subject training?
v. What are the certification procedures?

c. Personnel
i. Who are the personnel at your clinical center who are involved with LHMP? Please include their names, percent commitment on LHMP, major responsibilities, and how long they have been involved in the study.
ii. Please also include the training received by each person involved and the resources (e.g., computer, office space) provided to each.
iii. This information should be provided to site visitors 1 week prior to the site visit.

2. Tour of facilities and description of participant flow
a. Patient waiting area
b. Area for providing study information and informed consent (understand the procedures followed at the center)
c. Exam rooms
i. BAL room
ii. Pulmonary function testing room
iii. Blood drawing area
d. Clinical Lab
i. Sample processing area (including freezer)
ii. Review of procedures and certifications
iii. Sample storage procedures
iv. Sample transfer procedures and transfer/receipt forms
e. Sequencing Lab
i. Sample receipt and tracking procedures
f. Paperwork processing area
i. Participant files and CRFs
ii. File storage and organization
iii. Electronic data entry (when post visit and by whom?)
iv. Data queries processing flow; receipt, resolution and sign off responsibilities and specific staff involved.
v. Records Retention
g. Sequencing Lab
i. Sample documentation
ii. Lab quality, any contamination issues
iii. Data transfer to DACC (repository?) procedures
iv. Repository sample storage and transfer procedures
v. List of specimens with BioLINCC IDs for repository

3. Discussion of recruitment and retention
4. Review of paperwork (Data Audit)
a. Regulatory documents
i. Informed consent (current version on file and being used?)
ii. Documentation of current IRB approval on file

iii. Current protocol and LHMP Overview Protocol and all versions under which participants were enrolled
iv. Staff training and certification for human subject protection.

b. Participants' Case Report Forms (CRFs) and source documents
i. How is data flow managed? How is form completion and data entry completion monitored?
ii. Does the center have separate source documents? If so, how handled differently than CRFs?
iii. Participant chart and CRF review—capture Pt ID, Issues noted
iv. Go over issues with the person doing the documentation
v. Who monitors data integrity (i.e., reviewing source documents, informed consents, edits, etc.)?

5. Labs, outcomes
i. What is your system to monitor Local Lab results and track follow-up when applicable
6. Closed meeting with PI and their lead Coordinator (if acceptable to PI)
a. Commitment to date
b. Barriers/challenges
c. Organizational issues
d. Plans and commitment for future
7. Summary and feedback to PI and Coordinator. Thanks and good-bye.
8. Post site visit feedback (including report referenced above)

11.3. Data Audit Procedures

11.3.1. Goal of the Data Audit

There are three major goals for the data audit:

- Assess clinic staff's performance in completing and updating Case Report Forms (CRFs) per DACC instructions.
- Assess clinic staff's performance entering and updating data in the appropriate data management system (either a local system or MIDAS) as per DACC instructions.
- Give feedback to clinic staff so they can improve their future performance in any areas requiring improvement.

11.3.2. Procedures for Data Audit

To meet the goals of a data audit the DACC representative(s) will:

- Bring a copy of all data in the DACC database for randomly selected participants at the center being visited.
- When conducting a virtual site visit, a list of the participant ID's will be sent to the center approximately 5 weeks prior to the scheduled site visit conference call. The requested files can be sent via email, fax or hard copies by mail. The data audit will be conducted at the DACC.
- Compare database values to the CRFs stored at the center, flagging and noting any discrepancies.
- Look over CRFs for any additional notes, markings, or answers that are not entered into the DACC database.
- Compile a list of common discrepancies and/or errors found in the audit and review this list with the PC or other center staff responsible for data.

11.4. Retention of Study Documentation

The length of time all study files are maintained is specified in this section. NIH policy requires that studies conducted under a grant retain participant forms for three years, while studies conducted under contract must retain participant forms for seven years. Individual IRBs, institutions, states, and countries may have different requirements for record retention. Investigators should adhere to the most rigorous requirements and should retain forms and all other study documents for the longest applicable period.

11.5. Standard Operating Procedures

One aspect of center quality control is a set of Standard Operating Procedures (SOPs). SOPs describe a center's generic procedures that may have been developed to assist with standardization across studies. SOPs may include clinical and sequencing laboratory and pharmacy procedures, and storage of study documents. As relevant, SOPs should be developed by a center to ensure quality studies and study staff should be trained on them, the local center's SOPs should be in accordance with the LHMP SOPs located in this MOP. The SOPs should be located in a central location and made easily available to staff for reference.

Note: Printed versions of SOPs should be limited in order to maintain version control.

11.6. Study Protocol

The Overview Protocol also serves to document the infrastructure by which the collaborative development of protocols, data acquisition, standardization and sharing of data (phenotype, sequencing) and specimens will occur including the planned contribution to data and specimen repositories. The Overview Protocol of the LHMP includes, as appendices, the protocols for all center-specific studies and the cross-center collaborative studies which contribute to the LHMP. All participants in the LHMP will work collaboratively to ensure the clinical and scientific integrity and success of the project. Version 2.3 of the LHMP Protocol has been provided to each repository.