Manual of Procedures

for

Human Microbiome Project

Core Microbiome Sampling
Protocol A
HMP Protocol # 07-001

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1 INTRODUCTION

The Manual of Procedures (MOP) is to be used as a reference document for current National Institutes of Health (NIH) policies and procedures as they apply to the Human Microbiome Project (HMP) Core Microbiome Sampling study.

All staff members participating in the conduct of this study at participating institutions should have access to the MOP and be familiar with its contents. The current version of the MOP and archived versions are posted to the study web site:

https://web.emmes.com/study/dnt/hmp/index.htm

1.1 NIH-Sponsored Studies of the Human Microbiome

The Human Microbiome Project, a program initiated under the NIH Roadmap, is sponsoring studies to characterize the human microbiome and analyze its role in human health and disease.

1.2 Sponsoring Institution and Study Support

The NIH Human Microbiome Project is sponsoring this study. The HMP Core Microbiome Sampling clinical protocol team leaders at the National Institute of Dental and Craniofacial Research (NIDCR) and the National Human Genome Research Institute (NHGRI) will work with the study sites to develop and implement the study in accordance with Good Clinical Practices. Contact information for these individuals and other facilities providing study support is listed on the protocol roster and in Appendix B of this MOP.

1.3 Composition and Responsibilities of the Study Steering Committee

The Study Steering Committee is composed of the Study Chair, the Principal Investigator at each of the two clinical sites, and representatives from the NIH.

Study Management lies primarily in the hands of the Steering Committee. The Principal Investigators (responsibilities listed in Section 1.4) will make decisions regarding conduct of the trial with the endorsement and approval of the Steering Committee.

The Steering Committee will hold regular discussions via conference calls on the overall strategy and progress of the project. The information discussed on these calls will be used to generate reports used to document the overall progress of the study. The Steering Committee will review the progress of specimen collection, DNA isolation, and analysis of the sequence data.

The Steering Committee has the responsibility for developing protocol amendments. Protocol amendments will only be implemented after a formally amended version of the protocol has been approved and written IRB approval for the amendment is obtained (see Section 1.5).

The Steering Committee has responsibility for publishing the results of this study. The publication will be in accordance with the publication policy listed in Section 1.6.

1.4 Responsibilities of the Clinical Principal Investigator at Each Core Clinical Site

- To ensure that he/she has sufficient time to conduct and complete the study and have adequate staff and appropriate facilities which are available for the duration of the study and to ensure that other studies do not divert essential subjects or facilities away from the study at hand.
- To submit an up-to-date curriculum vitae and other credentials (e.g. medical license number in the United States) to the NIH and, where required, to relevant authorities.
- To submit relevant IRB documentation and correspondence to the NIH.
- To acquire the normal ranges for laboratory tests performed locally and, if required by local regulations, obtain the Laboratory License or Certification.
- To conduct the study in compliance with the protocol and appendices.
- To cooperate with the clinical site monitors in the monitoring process of the study.

1.5 Protocol Amendments and Modifications

No changes to the study protocol will be allowed unless they have been discussed in detail and have received the concurrence of the Steering Committee.

Any amendment/modification to the protocol will be adhered to by the participating center(s) and will apply to all subjects. Written IRB approval of protocol amendments is required prior to implementation; modifications are submitted to the IRB for information only.

1.6 Publications and Confidentiality

Timely communication with the scientific community is one of the essential functions of the Steering Committee, and is accomplished by the publication of manuscripts in scientific literature and oral or poster presentations at scientific meetings. Several publications and presentations will emanate from the Steering Committee, but most are of the following types: 1) the core manuscripts reporting results of the study, i.e., those

that cover primary, secondary, and other objectives of the study (core protocol publications/presentations); 2) specialized laboratory studies or other investigations that are not central to the objectives of the study (ancillary study publications/presentations); and 3) general reviews.

The publication policy of the Steering Committee is meant to be flexible and to facilitate rapid and accurate publication of results. Investigators are responsible for drafting the publications and presentations with meaningful input from sponsors. Internal review of manuscripts and abstracts is deemed necessary to ensure accuracy and consistent representation of concepts and data from the clinical trials. The procedures outlined herein are guidelines and all publications of the Steering Committee must meet the criteria for authorship, disclosure, scientific integrity and other requirements of peer-reviewed scientific journals.

The Steering Committee members will be the authors of the core manuscripts. Other manuscripts and abstracts are drafted by writing teams whose members must meet guidelines pertinent to the scientific standards of authorship. Masthead authorship should reflect those individuals whose scientific contributions to the publication were essential to its conduct. Contribution of the laboratory, the Clinical Data Coordinating Center and NIH should also be reflected.

The Steering Committee has the primary responsibility for carrying out editorial review of publications. While the sponsors may require up to 30 days for review of proposed publications, efforts will be made to accommodate the timely review of manuscripts and abstracts.

Subject Study IDs are **not** to be used in any publications.

Media inquiries and press releases should be referred to the NHGRI Communications and Public Liaison Branch:

Geoff Spencer
National Human Genome Research Institute
National Institutes of Health

This office should approve any press releases and responses to inquiries. Local media activity should be coordinated with the NHGRI Office of Communications.

Manuscripts/publications derived from this study are to be sent to PubMed Central in accordance with NIH Public Access Policy (Consolidated Appropriations Act 2008).

1.7 MOP Distribution and Updates

This manual is prepared and distributed by the CDCC. Contact the Data Project Manager, Gina A. Gorgone Simone, at HMP@emmes.com, with any questions about this manual.

This manual will be reviewed at least annually and updated as necessary. When the MOP is updated, the new version will be posted on the study web site, accompanied by a memo describing the revisions. If there is a discrepancy between the current MOP and the protocol, refer to the current protocol and notify the NIH representative and the CDCC. See the study web site for previous archived versions of the MOP.

2 REGULATORY REQUIREMENTS

2.1 Study Conduct

This NIH-sponsored study will be conducted in accordance with its protocol, the policies and procedures described in this document, in compliance with Good Clinical Practice (GCP) as laid out in the International Conference on Harmonisation (ICH) E6 GCP Consolidated Guidance (ICH 1996), and in accordance with applicable regulatory requirements.

2.2 Protection of Human Subjects

NIH studies will be conducted according to the principles of respect for persons, beneficence, and justice as stated in the Belmont Report. This study will also embrace the principles set forth in the Declaration of Helsinki. Investigators will comply with the provisions for the protection of the rights and welfare of human research subjects set forth in the U.S. Code of Federal Regulations, Title 45 Part 46, and in compliance with determinations of all Institutional Review Boards (IRBs) overseeing the research. All institutions participating in the protocol will have in place a Federal Wide Assurance (FWA) with the DHHS Office for Human Research Protections (OHRP). The assurance documents the institution's commitment to the human subjects regulations.

The study will also be conducted under Good Clinical Practice (GCP) as laid out in the International Conference on Harmonisation (ICH) E6 GCP Consolidated Guidance (ICH 1996). Investigator responsibilities are set out in Section 4 of the E6 Guideline (as published in the Federal Register May 1997). Sponsor responsibilities are set out in Section 5 of the E6 ICH Guideline (as published in the Federal Register May 1997).

Investigators should understand their commitments set forth in these regulations and standards. Investigators should also be familiar with the reporting requirements of their IRBs. We recommend that investigators maintain a copy of their IRB policies as part of the study file.

The study protocol, informed consent documents and all types of subject recruitment or advertisement information must be submitted to the IRB for review and must be approved prior to study initiation. Any amendments to the protocol and/or consent form must also be approved by the IRB prior to implementing any changes in the study.

The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The investigator must also keep the IRB informed of any serious

adverse events, unanticipated problems, or protocol deviations resulting in serious or severe adverse events.

To protect the privacy of study subjects, the Study ID code list should be maintained in a secure location that is separate from the regulatory binder. Copies of any identifying documents, e.g., documents containing the subject's name, should also be maintained in a secure location that is separate from both the regulatory binder and the subjects' individual study binders.

2.3 IRB Submission

If a revised protocol or amendment is submitted to the IRB, the version number and date must be cited and the submission must be recorded in the study regulatory file. All protocol submissions must include the full protocol. Protocol submission should follow local IRB policies.

Any reports of SAEs or unanticipated problems received by the site from NIH must be submitted to the IRB.

2.4 Informed Consent

Informed consent is an ongoing process that begins with the first contact with a prospective subject and continues until the study is completed. The consent form provides information about the study, including the rights of the subject and the risks and benefits involved in participating in the study. The consent form also documents the subject's agreement to participate. All procedures, subject obligations, and subject rights should be explained to the subject in easily understood language. During the explanation of the study and during the actual study, the subject is entitled to privacy and respect. The investigator or a designee may present the information and administer the consent. The investigator/designee should be well versed in the protocol and able to answer questions about the study procedures. The investigator/designee presenting the study should encourage the prospective subject to ask questions during this introduction to the study and anytime during his/her participation. Following the information presentation, the administrator should feel confident that the subject understands the study before the consent form is signed and before final inclusion into the study.

A sample consent form is provided for the protocol. Sites may modify the sample consent form as necessary for submission to the local IRB, but no information from the risk section may be deleted from the sample consent form. The site specific consent form will be reviewed and approved by NIH, prior to submission to the site IRB.

A copy of the current IRB-approved consent form will be used to obtain informed consent from the subject. The consent form must be signed by the subject before participation in any study-related activities. A copy of the signed and dated consent form must be provided to the subject. Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at all times.

- Subjects should be re-consented if information is changed that might have an impact on their continued participation.
- The signature must be the subject's legal name.
- The subject should not use initials.
- The signature must be in ink.
- The subject must record the date of his/her own signature. It is not acceptable for the research staff to complete the date for the subject.

2.5 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, Good Clinical Practice (GCP), or protocol-specific Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH Good Clinical Practice:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Each investigator must adhere to the study protocol as detailed in the study protocol document. Each investigator will be responsible for enrolling only those subjects who have met all protocol eligibility criteria.

A physician may implement a deviation from, or a change in the protocol to eliminate immediate hazard(s) to study subjects without prior NIH and/or IRB approval. As soon as possible, the implemented deviation or change, the justification and if appropriate, the proposed protocol amendment(s) must be submitted to:

- The IRB for review and approval/favorable decision
- NIH for agreement; and if required
- The regulatory authority(ies)

All deviations from the protocol must be addressed in the study subject source document. The documentation should include the reasons for the deviation and all attempts to prevent or correct them. A completed copy of the Protocol Deviation (PD) Form must be maintained in the Regulatory File, as well as in the subject's source document file.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled Protocol-required activity.

Protocol deviations must also be reported to the IRB, following the local IRB's instructions, and documented in the study records. The site PI and study staff are responsible for knowing and adhering to their IRB requirements.

2.6 Study Site Regulatory Document Requirements

All required regulatory paperwork must remain at the study site (as indicated in the site registration packet received prior to study start) and must be accurately maintained and may be verified during study monitoring visits.

Site Regulatory Documents should include the following:

1. Study Personnel signature/responsibility list
2. Monitor Log and Monitoring Reports
3. Subject Screening/Enrollment log
4. Study ID Code List
5. Original Protocol and Revisions/Amendments
6. Consent Forms (all versions)
7. Blank Case Report Forms (CRFs)
8. Advertisements and Subject Information Materials
9. MOP
10. CVs and Practitioner Licenses
11. IRB Approval - Regulatory Review History
12. Copies of SAE Reports and Unanticipated Problem Reports
13. Copies of Protocol Deviation Reports
14. Laboratory normal values and accreditations
15. Specimen Retention Records
16. Sponsor Correspondence
17. Internal Correspondence
18. Notes to File

2.7 Study Task Delegation and Staff Signature Lists

The Study Task Delegation and Staff Signature List should be updated regularly for the duration of the study. The objective of this form is to identify site personnel, to describe the responsibilities that the investigator delegates to each person, to establish dates of

participation for every person involved in the study, to document signatures and initials of study personnel, and to document the proper training of each team member. The initials of personnel providing training should also be recorded. All personnel listed on the Investigator of Record Form must be listed on this form. Changes include additions or changes in site staff or changes in the responsibilities of already existing staff. More than one person can be responsible for a given task. The original, completed forms should be kept in the site regulatory binder until the closeout of the study. At study close out, a copy of this form should be sent to NIH.

2.8 Retention of Records

Records and documents pertaining to the conduct of the study, including CRFs, source documents, consent forms, and laboratory test results must be retained by the investigator for a minimum of seven years after the investigation is discontinued or until the NIH authorizes transfer or destruction of study records. No study records will be destroyed without prior authorization from the NIH.

2.9 Site Monitoring Visit

For the purpose of compliance with Good Clinical Practice and Regulatory Agency Guidelines, it may be necessary for NIH or their designees to conduct a site monitoring visit. This may occur at any time from the start of the study to after conclusion of the study.

2.9.1 Site Visit Expectations

- Study staff should be available to facilitate the site visit.
- Data entry into the CDCC data management system should be current to within 72 hours of the site visit and have had an internal quality review in accordance with the site's internal quality management plan.
- It is recommended that all study records be collected in one place to increase efficiency, and to alleviate the need for the monitor to interrupt staff for additional information.
- In addition to telephone access, the monitor will need a computer and a high-speed Internet line with workspace to access the database for source document verification against data entry.

The following activities should be anticipated, but may be altered based upon protocol needs, as determined by NIH:

- An assessment of general site adequacy will be done at each site visit.
- A full Regulatory file review will be done at the 1st interim monitoring visit and close-out; at other visits, the monitors will check for IRB approvals of new protocols/amendments/consents and SAEs.
- A Research Lab review will be done at the 1st interim monitoring visit and close-out; an abbreviated review will be done at the other visits, to check that specimens are labeled/stored appropriately; specimens are identifiable and easily retrievable.
- The informed consent forms and source documents will be reviewed at each visit.
- Clinical activity observations will be made at least once during a study, if activity is ongoing during the visit period (e.g., observation of consent process). No clinical observations should be allowed without the subject's agreement.
- If any issues are identified during any of the reviews, they will be followed until resolution.

The monitor will discuss the findings of the site visit with the study staff at each visit. Attempts will be made to resolve data problems while at the site.

The Principal Investigator (PI) should be available for a debriefing with the monitor at the conclusion of each site visit.

2.9.2 Site Visit Reports

Following each visit, the PI will receive a report and a letter from the monitor. The site will be expected to follow-up and respond to the issues noted in the site visit report.

3 DATA MANAGEMENT

3.1 Source Documentation

The source document is defined as the first place the data are recorded. The CDCC will provide source documents derived from the eCRFs. Blank source documents are posted to the study web site. In some instances, staff might need documentation from their own or other institutions (e.g., laboratory reports or a hospital report for an SAE). In this case, please request a copy of the record from the institution. It is also recommended that copies of records from outside the clinical research site be added to the subject's binder.

All source documents should be completed by the clinician (or other appropriate study personnel). Data entries into source documents should be made in blue or black ink. Corrections should be made with a single line through the entry and the change initialed and dated. Original entries should remain legible (i.e., they should never be erased or covered with correction fluid to obscure the original entry). Late entries, e.g., laboratory results on the Eligibility Checklist, should be initialed and dated at the time entered.

Data should be handled in accordance with GCP, U.S. federal regulations, local regulations (if applicable), and instructions from NIH. All source documents should be filled out completely by the examining personnel or the study coordinator and should be signed by the person collecting the data on that form. The source documents are reviewed, signed and dated by the principal investigators or study staff designated by the principal investigators.

Source documents for subjects who are screened but not enrolled must be retained following the same guidelines as other study source documents.

3.2 Case Report Forms

Data will be entered electronically over the Internet by site study staff into AdvantageEDC, developed and maintained by The EMMES Corporation. Instructions for use of the system and completion of the data screens (eCRFs) for each study are included in the AdvantageEDC User's Guide, which is posted to the study web site. Data entry should be current to within 72 hours of the last visit for each subject unless otherwise specified.

3.3 Form Submission Schedule

AdvantageEDC includes an individual Forms Grid, which indicates the current status of forms submitted for each subject. In addition, a Forms Submission Schedule is posted for each protocol on the study web site.

3.4 Data Review and Quality

The CDCC reviews data for quality and provides a number of quality assurance reports to ensure that study data are clean and complete. Quality assurance reports will include, but are not limited to, the following: missing forms, missing and out of range values, automated data queries, and targeted manual reviews of study data. Data queries will be posted to the study web site and must be addressed within 1 week after posting unless otherwise specified.

3.5 Archiving of Data

Records and documents pertaining to the conduct of this study, including CRFs, source documents, consent forms, and laboratory test results, must be retained by the investigator for a minimum of 7 years after study completion. Electronic data including clinical information, LIMS (Laboratory Information System) and sequencing data will be transferred to the database of Genotype and Phenotype (dbGaP). No study records shall be destroyed without prior authorization from NIH. The CDCC will provide a copy of the locked data set to NIH upon study completion.

3.6 Handling of Data to Ensure Confidentiality

In order to ensure confidentiality of data the following procedures will be followed:

- Label both the source documents and the specimens with code numbers.
- Enter data from the source documents in a controlled-access database on the Internet, identified only by code number. Only researchers who have been approved to look at the information by an NIH Data Access Committee will be able to see this information.
- Store all source documents and consent forms in a locked file cabinet. Only members of the study team will have access to this file cabinet.

4 UNANTICIPATED PROBLEMS AND SERIOUS ADVERSE EVENTS

The methods of specimen collection in the Core Microbiome Sampling study pose only minimal risk to the study subjects. As defined in 45 US Code of Federal Regulations (CFR) 46.102 (i), "Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." The minimal physical risks associated with the sampling procedures are described in the protocol and in the informed consent document.

The results of research cannot be foreseen, so it is possible that unanticipated problems may arise in the study. In addition to unexpected adverse events, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. The investigator or designee is responsible for the detection and documentation of unanticipated problems and Serious Adverse Events (SAE) in persons participating in the study. Unanticipated problems and SAEs should be reported to the CDCC and to the IRB as outlined in the following sections.

4.1 Definition of an Unanticipated Problem

The Office for Human Research Protections (OHRP), Department of Health and Human Services (DHHS) considers unanticipated problems, in general, to include any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions to protect the safety, welfare, or rights of subjects or others. Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include:

- changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects;
- modification of inclusion or exclusion criteria to mitigate the newly identified risks;
- implementation of additional procedures for monitoring subjects;
- suspension of enrollment of new subjects;
- suspension of research procedures in currently enrolled subjects;
- modification of informed consent documents to include a description of newly recognized risks; and
- provision of additional information about newly recognized risks to previously enrolled subjects.

4.2 Reporting of Unanticipated Problems

Institutions engaged in human subjects research conducted or supported by DHHS must have written procedures for ensuring prompt reporting to the IRB, appropriate institutional officials, and any supporting department or agency head of any unanticipated problem involving risks to subjects or others (45 CFR 46.103(b)(5)). Furthermore, for research covered by an assurance approved for federal wide use by OHRP, DHHS regulations at 45 CFR 46.103(a) requires that institutions promptly report any unanticipated problems to OHRP.

Incidents or events that meet the OHRP criteria for unanticipated problems require the completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB:

- appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- a detailed description of the adverse event, incident, experience, or outcome;
- an explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;

- a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

For multicenter research protocols, if a local investigator at one institution engaged in the research independently proposes changes to the protocol or informed consent document in response to an unanticipated problem, the investigator should consult with the study sponsor or coordinating center regarding the proposed changes because changes at one site could have significant implications for the entire research study.

Forms describing unanticipated problems will be submitted to the CDCC and the IRB per site requirements. The CDCC will notify the NIH study representative of the unanticipated problem. Unanticipated problems will be reported immediately to the NIH Clinical Study Oversight Committee (CSOC). The CSOC may convene an *ad hoc* meeting for review of the unanticipated problem and consideration of corrective actions. Other supporting documentation of the problem may be requested by the CSOC and should be provided as soon as possible. A summary of the CSOC meeting and proposed actions will be provided to the study Steering Committee. The study PIs will then submit the CSOC review and any protocol changes to the IRBs.

Investigators will be responsible for reporting unanticipated problems from the time that the first subject is enrolled until one year after the final subject is enrolled.

4.3 Definition of a Serious Adverse Event

The OHRP, DHHS defines an Adverse Event (AE) as 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 (modified from the definition of adverse events in the 1996 International Conference on Harmonisation E-6 Guidelines for Good Clinical Practice). A Serious Adverse Event/Experience (SAE) is any adverse event/experience that meets any of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in congenital anomaly/birth defect

- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

For those events meeting the previously described definition of Serious Adverse Events, the completion of an SAE form is required.

4.4 SAE Reporting Requirements

OHRP considers adverse events that are unexpected, related or possibly related to participation in research, and *serious* to be the most important subset of adverse events representing unanticipated problems, because such events always suggest that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized, and routinely warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects. Furthermore, OHRP notes that IRBs have authority to suspend or terminate approval of research that, among other things, has been associated with unexpected serious harm to subjects (45 CFR 46.113). In order for IRBs to exercise this important authority in a timely manner, they must be informed promptly of those adverse events that are unexpected, related or possibly related to participation in the research, and serious (45 CFR 46.103(b)(5)).

All serious adverse events will be:

- recorded on the appropriate Serious Adverse Event case report form and faxed to the CDCC at 301-251-1355. [The CDCC will notify the NIH study representative of the serious adverse event within one business day.]
    - All deaths and immediately life-threatening events, whether related or unrelated, will be reported via fax within 24 hours of site awareness.
    - Serious adverse events other than death and immediately life-threatening events, regardless of relationship, will be reported via fax within 72 hours of site awareness.
- followed until satisfactory resolution or until the Principal Investigator or Sub Investigator deems the event to be chronic or the subject to be stable;
- reported to the IRB per site requirements;
- entered in AdvantageEDC on the appropriate eCRF;
- reported to and evaluated by the Independent Safety Monitor (ISM).

SAEs that are related to study participation will be reported immediately to the NIH CSOC. The CSOC may convene an ad hoc meeting for review of the SAE and consideration of corrective actions. Other supporting documentation of the event may be requested by the CSOC and should be provided as soon as possible. A summary of the CSOC meeting and proposed actions will be provided to the study Steering Committee. The study PIs will then submit the CSOC review and any protocol changes to the IRBs.

Serious adverse events that are considered unrelated to study participation will be provided to the CSOC in a line listing to be reviewed at regularly scheduled meetings.

This study will employ an unsolicited SAE reporting system. Subjects will be counseled to report SAEs as listed in Section 4.3.

Questions about SAE reporting can be referred to Wendy Fanaroff, the NIDCR Safety Coordinator or Holli Hamilton, MD, Medical Monitor. Contact information for these individuals and for the study site independent safety monitors (ISM) follows:

Wendy Fanaroff RN, MSN
Nurse Consultant
Office of Clinical Trials Operations & Management,
National Institute of Dental and Craniofacial Research (NIDCR), NIH

Holli Hamilton, MD
Senior Medical Officer
Division of Extramural Research
National Institute of Dental and Craniofacial Research (NIDCR), NIH

ISM - Baylor College of Medicine
Stephen Baruch Greenberg, MD

ISM - Washington University
Thomas Charles Bailey, MD

5 STUDY OVERSIGHT AND SAFETY MONITORING

5.1 Roles and Responsibilities of the Clinical Study Oversight Committee

The Clinical Study Oversight Committee (CSOC), an independent group of experts, will advise NIH and study investigators on this study. The responsibilities of the CSOC are to 1) monitor human subject safety by reviewing and evaluating the accumulated study data, 2) review study conduct and progress, and 3) make recommendations to NIH concerning the continuation, modification, or termination of the study. The CSOC considers study-specific data as well as relevant background information about applicable procedures and progress of the study.

Prior to the first data review and preferably prior to study initiation, the CSOC will define its deliberative processes. These may include event triggers or a process that would call for an *ad hoc* review, milestones expectations, endpoint analysis, and voting procedures. The CSOC is responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it.

The CSOC will review the protocol, including the oversight and risk monitoring plan, and identify any major concerns prior to implementation. During the study the CSOC will review:

- Real-time and cumulative safety data for evidence of procedure-related serious adverse events
- Unanticipated problems involving risks to subjects or others (for additional information, see the online guidance document from the Office for Human Research Protections, available at http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm)
- Adherence to the protocol
- Factors that might affect the study outcome or compromise the study data, such as protocol violations, losses to follow-up, breach of subject confidentiality
- Unexpected barriers, if any, to study progress or completion, such as slow enrollment, new data or findings, other milestones, change in resources, futility of endpoints, etc.

The CSOC will conclude each review with each member's recommendation to NIH as to whether the study should continue, be modified, or be terminated. Recommendations regarding modification of the design and conduct of the study may include corrective actions when performance is unsatisfactory, recommendations to suspend or terminate enrollment, or recommendations to modify consent documents.

Confidentiality must always be maintained during all phases of CSOC review and deliberations.

5.2 CSOC Membership

The membership of the CSOC will reflect the disciplines and clinical specialties, such as dental, medical, and nursing, necessary to interpret the data from the clinical study and to fully evaluate subject safety. The CSOC will consist of at least three voting members. Membership will include an Independent Safety Monitor (ISM) from each of the participating sites, an individual with expertise in the clinical aspects of the subject population being studied or procedures being performed, and an individual with expertise in current clinical research conduct and methodology.

Consideration will be given to including other voting or *ad hoc* members, such as a bioethicist, if it is considered that the study design or subject population would benefit from this expertise. CSOC and *ad hoc* members may be from the principal investigator's institution or from other participating sites, but will not be directly involved with the study or under the supervision of the study investigator. Furthermore, the CSOC members should be in a different organizational group than the Principal Investigator (PI).

NIH staff not involved in the study may also participate as voting members. NIH staff involved in the study may participate as *ex officio,* non-voting members. Individuals with vested interests in the outcome of the study are not eligible to serve on the CSOC as *ex officio* or voting members.

The voting members of the CSOC for this study are:

Martin Rosenberg, PhD, Chairman

Stephen Baruch Greenberg, MD, ISM for Baylor

Thomas Charles Bailey, MD, ISM for Washington University

See Section 4.4 for contact information for the ISMs.

5.2.1 Conflict of Interest

No member of the CSOC will have direct involvement in the conduct of the study. Furthermore, no member will have or appear to have financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the CSOC. Letters of invitation to prospective CSOC and *ad hoc* members will include the following: "Acceptance of this invitation to serve on the HMP Core Microbiome CSOC confirms

that I do not have any financial or other interest with any of the organizations involved in the study that constitute a potential conflict of interest." In addition, all CSOC and *ad hoc* members will sign a Conflict of Interest certification to that effect at the time they are asked to participate. At the beginning of every CSOC meeting, NIH program staff or the CSOC Chair will reconfirm that no conflict of interest exists for CSOC members. Interests that may create a potential conflict of interest should be disclosed to the CSOC prior to any discussion. The CSOC will determine how to handle such potential conflict. The CSOC can require that a member with a potential conflict not vote or take other means deemed appropriate. NIH may dismiss a member of the CSOC in the event of unmanageable potential conflict or appearance of conflict.

5.2.2 Selection and Invitation to Participate

The NIH holds primary responsibility for the formation of the CSOC, and is responsible for developing the roster of potential CSOC members. Recommendations for proposed members may be solicited from many sources. The proposed roster of members must be submitted to the Chief, Office of Clinical Trials Operations and Management (OCTOM) and the NIH Medical Monitor for review and approval before invitations are issued.

The NIH is responsible for identifying the CSOC Chair. NIH staff may either select the Chair directly or ask CSOC voting members to select the Chair.

5.3 CSOC Meetings

The initial CSOC meeting will occur before the start of the study or as soon thereafter as possible. NIH staff may discuss the NIH perspective on and expectations for the study at this initial meeting. At this meeting the CSOC will discuss the protocol, set triggers for data review, define a quorum, and establish guidelines for monitoring the study. The CSOC will decide which member(s) should receive reports of serious adverse events and unanticipated problems in real time and determine if on-site review of clinical records might be needed. Guidelines for stopping the study for concerns of risk or other factors will be established. At this meeting, the CSOC will also develop procedures for conducting business (e.g., data required for review, voting rules, attendance, etc.). Teleconference calls may be an appropriate means for conducting meetings.

Based on initial discussions, the CSOC will decide whether to meet on a regular basis or only with the occurrence of serious adverse events or unanticipated problems involving risks to subjects. It may be appropriate for meetings to be convened on an *ad hoc* basis, in response to the occurrence of serious adverse events or unanticipated problems involving risks to subjects. Scheduling of meetings will be based on the

magnitude of the perceived risks, rate of subject enrollment, or problems that occur during the progress of the study.

The NIH is responsible for convening meetings or conference calls as needed. However, meetings may be requested for cause by any member of the CSOC, the investigators, IRB, or NIH. The investigators will be responsible for ensuring the distribution of materials for review to CSOC members and other meeting participants.

5.3.1 CSOC Meeting Format

The recommended meeting format may consist of the following sessions: Open Session and Closed Executive Session.

Open Session: Occurrences of serious adverse events, unanticipated problems involving risks to subjects, enrollment and endpoints are reviewed. Issues relating to the general conduct and progress of the study will also be considered. CSOC members, voting and *ex officio* members, NIH staff members, and *ad hoc* experts attend and participate in this session. The lead investigator and study statistician, if applicable, should attend and participate to present results and respond to questions. This session is open to study investigators, coordinating center staff, and NIH staff.

Closed Executive Session: This final session involves only voting members to ensure complete independence for making decisions and formulating independent recommendations.

5.3.2 Voting

A quorum, as defined by the CSOC in the initial meeting, must be present either in person or by conference call. After a thorough discussion of CSOC members' opinions and rationale and a joint attempt to reach clarity regarding individual recommendations, the final recommendations of each CSOC member will be solicited in Closed Executive Session (*ex officio* members shall not vote and shall not be present at this voting session). A consensus opinion or recommendation among members is not required; each member may have individual opinions. The final recommendations are recorded and either identified as majority or minority positions or accompanied by actual vote tallies for each divergent recommendation, i.e., as number of votes for or against a particular action, e.g., continue study, terminate study, etc.

5.4 Study Reports for CSOC Review

It is the responsibility of the PI to ensure that the CSOC is apprised of all new safety and risk information relevant to the study. Summary safety data, enrollment data, and other progress reports (e.g., protocol deviations, site monitoring summaries) will be forwarded periodically to the CSOC. The CSOC will receive all protocol revisions and may receive other documents relating to the study.

Reports will be prepared by the study investigators. The general content for reports to the CSOC is as determined by the CSOC at the initial meeting. The CSOC and NIH must also review and approve the actual data elements to be presented. At each meeting, additions or modifications to these reports may be directed by the CSOC on a one-time or continuing basis. Distribution of written reports should allow sufficient time for review.

Reports for meetings of the CSOC will consist of the Open Session Report. Open Session reports are distributed to CSOC members, selected NIH staff, and other appropriate persons as directed by the CSOC.

5.5 Other Reports of Study Progress

Safety and enrollment data will be forwarded periodically to all CSOC members. The CSOC will also receive all protocol revisions and may receive other documents relating to the study, such as annual reports, protocol deviations, manuscripts, and newsletters.

5.6 Reports from the CSOC

Verbal Report: At the conclusion of a CSOC meeting, the CSOC will discuss its findings and recommendations with NIH representatives and the study investigators. If NIH is not represented at the meeting, the CSOC Chair will contact NIH immediately after the meeting to brief the NIH staff.

Summary Report: The CSOC will periodically issue a written summary report that identifies topics discussed by the CSOC and describes their individual findings, overall safety assessment, and recommendations. This would generally occur after each meeting, but CSOCs that meet on a more frequent basis may summarize more than one meeting in each report. The rationale for recommendations will be included when appropriate. This report will not include confidential information. The CSOC Chair or designee is responsible for preparing and distributing the report. Unless otherwise specified, the summary report will be forwarded through the NIH to a designated study team representative (usually the Principal Investigator) and to other appropriate NIH staff. The study team representative is responsible for disseminating the CSOC

summary report to site investigators. Site investigators must, in turn, submit the reports to their respective Institutional Review Boards (IRB) in accordance with local IRB policy.

Immediate Action Report: The CSOC Chair will notify the NIH study representative of any findings of a serious and immediate nature, such as if the CSOC recommends that all or part of the study be discontinued. The NIH study representative will immediately inform appropriate NIH staff, including the Director of OCTOM and the NIH Medical Monitor. In addition to verbal communications, recommendations to discontinue or substantially modify the design or conduct of a study must be conveyed to NIH in writing on the day of the CSOC meeting. This written, confidential briefing may contain supporting data and should include the CSOC members' rationale for its recommendations. The written briefing should be submitted to OCTOM.

5.7 Independent Safety Monitor

5.7.1 ISM Roles and Responsibilities

The ISM or Local Medical Monitor is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. For the Human Microbiome study, this will be accomplished by review of serious adverse events and unanticipated problems immediately after they occur, with follow-up through resolution or stabilization. The ISM evaluates individual and cumulative subject data when making recommendations regarding the safe continuation of the study.

Each site participating in the Human Microbiome protocol must identify an ISM for the study. The ISM will perform this role as a member of the study CSOC.

5.7.2 Study Materials for ISM Review

The primary focus of the ISM is to independently review all serious adverse events and unanticipated problems and thoroughly investigate them. Clinical and laboratory data, clinical records, and other study-related records should be made available for ISM review. If necessary, special reports are prepared by the investigator.

It is the responsibility of the PI to ensure that the ISM is appraised of all new safety information relevant to the study. Summary safety and enrollment data should be forwarded periodically to the ISM. The ISM should receive all protocol revisions and may receive other documents relating to the study.

5.7.3 ISM Selection and Invitation to Participate

The ISM will be selected based on relevant study-related expertise. Participation is for the duration of the study. The ISM should be readily accessible to review subject records in real time. He/she will be a member of the participating institution's staff. The ISM should not be under the direct supervision of the investigator and should preferably be from a different organizational group.

5.7.4 ISM Review Report

According to pre-specified criteria agreed upon by the NIH, the ISM should communicate in writing his/her findings, any concerns and recommendations to NIH representatives and the study investigators.

Unless otherwise specified, the written report will be forwarded through the NIH to a designated study team representative (usually the PI) and to other appropriate NIH staff including the Director of OCTOM and the NIH Medical Monitor. The study team representative is responsible for disseminating the ISM summary report to any other site investigators and each investigator must, in turn, submit the report as per local IRB policy.

5.8 Relationship between the CSOC and IRBs

Once the CSOC is established, each of the relevant IRBs will be informed of the operating procedures with regard to data and safety monitoring (e.g., who, what, when, and how monitoring will take place). This information will serve to assure the IRB that the safety of the research subjects is appropriately monitored. If the IRB is not satisfied with the monitoring procedures, it should request modifications. While it is recognized that it may not be possible to satisfy every IRB completely, IRB comments should be considered seriously.

6 SCREENING, ENROLLMENT AND STUDY ID ASSIGNMENT

6.1 Subject Study ID

A Screening and Enrollment Log with the list of valid subject Study IDs will be provided to the site by the Clinical Data Coordinating Center (CDCC). Subjects who have documented informed consent by signing a study Consent Form will be assigned study IDs sequentially as they are screened. The Study ID includes a protocol identifier, a site identifier, and a subject identifier.

Examples: 01DBA0001, 01DBA0002

- Protocol identifier: 01D
- Site identifier: Baylor College of Medicine: BA
- Subject identifier: 0001, 0002, 0003, etc.

The Study ID must be entered on all source documents and will be used to identify all subject data records in AdvantageEDC^{SM}. Subject names or other personally identifying information should NOT be recorded on any documents sent to NIH, the Coriell Institute, or the CDCC (e.g., supporting documents for reporting adverse events).

6.2 Screening and Re-screening Schedule

Screening must take place between 2 and 30 days before sampling commences. The time from the first date of screening to the last date of screening may not exceed 30 days. The time from the first date of screening to the date of the final body site specimen collection may not exceed 44 days (allowing 14 days to complete all specimen collections).

- The subject screening visit may occur over multiple days; in this event the date of the first visit should be used for entry into the eligibility checklist as the screening date; study enrollment must occur within 30 days of this date.
- Subjects who fail any screening criterion that, in the opinion of the clinician, could be remediated within the allowed 30-day window between first date of screening and first date of sampling, may be rescreened for that parameter within 30 days. If found eligible, the subject may be enrolled and appointed for sample collection.
    - Example 1: A subject who has untreated dental caries may obtain the necessary dental treatment and return to the study clinic within 30 days.

In this case, only the oral screening examination would need to be repeated to determine eligibility for the subject.

- Example 2: At initial screening, a female subject has a posterior fornix pH of 4.6. The clinician may feel that proximity to the tail-end of menstruation contributes to this pH and that a repeat measurement within the next 30 days has a high probability of falling in the required range of pH below 4.5, thereby making the subject eligible for enrollment and sample collection.

In both of the above examples, the clinician may elect to defer all of the screening for this subject, or may allow screening of other body sites and schedule a return screening visit as soon as practical (but within the 30-day window) to repeat the screening exam of the body site(s) that failed.

Appendix M contains a list of topics that should be discussed with female subjects prior to scheduling the screening visit, in order to minimize the number of screening failures related to vaginal pH.

- Subjects whose first date of screening is more than 30 days prior to enrollment must be re-screened before they can be enrolled; consent to participate in the study must be re-confirmed.
- Subjects who fail screening due to time-limited exclusion criteria may be re-screened at a later date to determine eligibility for enrollment; consent to participate in the study must be re-confirmed.

Subjects will be assigned a new Study ID if they are re-screened outside of the allowed 30-day window for completion of screening.

6.3 Screening Procedures

The subject screening process will verify a subject's eligibility based on all inclusion and exclusion criteria listed in the protocol, and will include the following procedures:

6.3.1 Screening for Pregnancy*

A. Urine will be collected from female subjects of child bearing potential, at the screening visit and at each sampling visit, into an appropriate receptacle that is labeled with the subject's Study ID. The urine specimen will be given to study personnel for pregnancy testing. Female subjects who are of child bearing potential will only be enrolled into the research study if their pregnancy test is negative on both the day of screening and the day of baseline sampling.

B. Urine pregnancy test form header will be completed entirely including date, complete name of test kit which includes the brand, test kit lot number and expiration date.

C. The subject's Study ID will be written in the first column of the test form.

D. Personnel will put on gloves and will prepare the test kit as directed by package instructions and will set the timer for required time.

E. When the timer alarms, the clinic personnel will read the pregnancy test and complete the remaining columns on the test form. This includes the results of the positive (the appearance of a blue procedural control line) and negative (a clear background in the test result window) internal controls, the test result, the initials of authorized personnel conducting the test and comments, if applicable.

F. Urine pregnancy test results will be recorded in the subject's research record as required.

* Refer to site specific SOPs.

Women are considered to be of non-child bearing potential if they are surgically sterile for > 6 months.

6.3.2 Screening for Viral Markers*

A. Ask the subject to sit in the phlebotomy chair or in the chair next to the phlebotomy table and place their arm comfortably on the table. If the phlebotomist prefers, he/she can ask the subject to place their opposite hand in a fist position and place it under the elbow of the arm from which the blood will be drawn. This positioning will help straighten the arm and stabilize the vein.

B. Assemble the needle into the vacutainer holder and place the tourniquet on the subject's arm.

C. Locate an appropriate vein by palpating the subject's arm. When an appropriate vein is identified, put on gloves.

D. Wipe the area where the needle is to be inserted with an alcohol prep pad and dry with a cotton ball.

E. Insert the needle, bevel side up into the skin and vein and push the appropriate blood collection tubes* onto the needle in the vacutainer holder.

** Baylor and Washington University require use of Serum Separator Tubes for screening of viral markers.*

F. If attempted blood draw is unsuccessful, ask the subject if a second attempt can be made. If the subject agrees, then clinic personnel can try a second time to complete the blood draw or can ask another clinic staff member to draw the blood. Do not ask the subject to allow more than two attempts at drawing their blood. If the subject offers for a third attempt to be made then the clinic personnel can proceed.

G. The required volume of blood for the viral marker testing is 10 mL. If multiple tubes are required, then as each tube fills with blood, change the tubes until all that are required are full.

H. After all required tubes are filled with blood, remove the tourniquet from the arm and quickly withdraw the needle out of the arm. Immediately place a cotton ball over the venipuncture site and ask the subject to hold pressure on the cotton ball.

I. Dispose of the needle in an appropriate sharps container without recapping the needle.

J. Place a Band-Aid over the cotton ball over the venipuncture site. Remove and dispose of gloves.

K. Label the blood tube(s) with subject's Study ID and collection date.

L. Document blood draw in the subject's research record as required.

M. Maintain the blood at room temperature and take the tube(s) to the reference laboratory in a rack, box or Ziploc bag as soon as possible.

N. Blood samples will be submitted to a reference laboratory (see below) to test for infection with HIV (as measured by viral antibody), HCV (as measured by viral antibody), and HBV (as measured by HbsAg). Results will be available in 2-3 days.

Reference laboratories:

Baylor College of Medicine Laboratory Corporation of America 7207 North Gessner Houston, Texas 77040	**Washington University** Barnes-Jewish Hospital Laboratories, One Barnes-Jewish Hospital Plaza, St. Louis, MO 63110

* Refer to site specific SOPs

6.3.3 Oral Cavity Screening

A. At the time of the screening visit, subjects will be excluded if they have:

- Chronic dry mouth, as assessed through questioning of the subject by an experienced clinician
- Periodontal pockets $\geq$ 4 mm
- Untreated cavitated carious lesions or oral abscesses
- Evidence of precancerous or cancerous lesions
- Evidence of candidiasis
- Clinically meaningful halitosis as determined by organoleptic assessment by an experienced clinician
- More than 8 missing teeth, with missing teeth accounted for by third molar extractions, teeth extracted for orthodontic purposes, teeth extracted because of trauma, or teeth that are congenitally missing
- More than 10% of sites with BOP

B. Screening procedures require a standard dental chair with good lighting and an examination kit containing at least one long-handled mouth mirror, dental explorer, and calibrated periodontal probe (Michigan-O with Williams markings, Hu-Friedy Manufacturing Co., Chicago, IL).

C. Obtain a careful dental and oral history including recent history of oral infections, inflammation, pustules, canker sores, benign or malignant masses. Record length of time since last oral prophylaxis or periodontal treatment.

D. Perform a complete soft tissue examination: gingivae, oral mucous membranes, tongue, pharynx, palatine tonsils. Record findings on the oral screening examination form.

E. Note number of teeth. Subjects should have at least 24 teeth with missing teeth accounted for by 3rd molar extractions and/or teeth extracted for orthodontic reasons and/or for trauma and/or teeth that are congenitally missing.

F. Note number of untreated cavitated carious teeth and restored teeth. Only obvious carious lesions are anticipated to be detected by this examination.

G. Periodontal examination should include 6 surfaces on all teeth: (1) mesiofacial; (2) midfacial; (3) distofacial; (4) mesiolingual; (5) midlingual; (6) distolingual.

H. Periodontal measures will include full mouth:

- Probing depth – PD
- Bleeding on probing – BOP

Oral sampling kits are cleaned and sterilized in accordance with the CDC's MMWR recommendations refer to:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5217a1.htm

6.3.4 Skin Examination

A. At the time of the screening visit, subjects will be excluded if they have:

- used topical antibiotic or topical steroid on the face, scalp, neck, arms, forearms or hands in the previous 7 days. (These subjects could be deferred for screening and sampling when they meet the exclusion time window for topical use of antibiotics or steroids);
- acne at sites other than on the face, chest, back or shoulders;
- multiple blisters, pustules, boils, abscesses, erosions or ulcers on the scalp, face, neck, arms, forearms or hands;
- a single blister, pustule, boil, abscess, erosion, ulcer, scab, cut, crack or pink/hyperpigmented patch or plaque at or within 4 cm of the sampling sites;
- more than one pink/red scaly patch/plaque anywhere on the body (suggestive of psoriasis or eczema);
- uniformly thickened, cracking, “dry” skin on bilateral palms and/or soles;
- scalp dandruff that does not clear up with over-the-counter dandruff shampoos used daily for 2 weeks;
- disseminated rash (at multiple body sites or extending throughout a broad body area).

B. The dermatologic examination will be conducted with good lighting and will include a general survey of the skin (entire body) and a detailed examination of areas that will be sampled.

C. The retroauricular crease (behind left and right ears) and the antecubital fossa (inner elbow) of both arms will be scrutinized carefully by visual examination. Individuals with any single blister, pustule, boil, abscess, erosion, ulcer, scab, cut, crack or pink/hyperpigmented patch or plaque visible skin lesions more than 4 cm away from the sampling site are still considered to be eligible for inclusion in the study.

6.3.5 Nasal Examination

A. Obtain a careful history of any recent sinusitis, nasal inflammation or upper respiratory tract infections, nasal discharge, or nasal polyps/masses. The history should include any recent exploration or surgery involving the nasal cavities. If a subject has recently received a nasally-delivered live, attenuated influenza

vaccine, nasal sampling must not be completed until 28 days after administration of the vaccine.

B. Perform a clinical examination of nares (especially anterior nares) in order to examine surfaces for any evidence of inflammation, polyps/masses, or nasal discharge.

6.3.6 Gastrointestinal Examination

A. At the time of the screening visit, subjects will be excluded if they have had:

- major surgery of the GI tract, with the exception of cholecystectomy and appendectomy, in the previous 5 years.
- any major bowel resection at any time.

B. At the time of the screening visit, subjects will be excluded if they have a history of active uncontrolled gastrointestinal disorders or diseases including:

- inflammatory bowel disease (IBD), including ulcerative colitis (mild-moderate-severe), Crohn's disease (mild-moderate-severe), or indeterminate colitis;
- irritable bowel syndrome (IBS) (moderate-severe);
- persistent, infectious gastroenteritis, colitis or gastritis; persistent or chronic diarrhea of unknown etiology; *Clostridium difficile* infection (recurrent) or *Helicobacter pylori* infection (untreated);
- chronic constipation;

C. Obtain a careful history of any gastric or intestinal ulcerations/GI bleeding, abdominal pain, dyspepsia, tenesmus or difficulties with defecation, gastrointestinal or colonic polyps or masses or dysplasia or cancer, and diarrhea or infectious gastroenteritis.

D. Record on the Concomitant Medications Form the use of bismuth subsalicylate-containing products, including Pepto-Bismol, Kaopectate, Maalox Total Relief, Bismatrol, Pink Bismuth, Stomach Relief, Stress, Walgreens Soothe, and other similar bismuth subsalicylate medications.

E. Perform a limited clinical examination if history suggests specific conditions such as abdominal tenderness, pain or anal disease.

6.3.7 Vaginal Examination and pH Determination

A. At the time of the screening visit, subjects will be excluded if they have:

- a history of regular urinary incontinence;
- condyloma or human papillomavirus (HPV) diagnosis within the previous 2 years;
- treatment for or suspicion of ever having had toxic shock syndrome;
- history of candidiasis, urinary tract infections, or active STD (specifically Chlamydia, gonorrhea, syphilis, genital herpes, trichomoniasis) within the previous 2 months;
- history of vulvar, vaginal or cervical dysplasia within the previous 5 years;
- history of hysterectomy.

B. Perform clinical examination to check for any visible vaginal or cervical lesions including genital warts (or papillomas), vaginal discharge, evidence of vulvar/vaginal inflammation or signs of vulvar/vaginal irritation. Subjects will be excluded if they have evidence of vulvar or vaginal irritation at the time of screening.

C. Determine vaginal pH.

Vaginal Introitus:

- Spread labia to visualize vaginal introitus immediately posterior to hymenal ring/tissue.
- Gently apply the microelectrode pH meter (Oakton, model pH Spear) to the vaginal mucosa at the center most point of the vaginal introitus.
- Take at least two separate recordings (digital recordings of pH), and derive a mean pH. Record the pH readings. The introitus pH is not an exclusion criterion. (The screening pH at the vaginal posterior fornix must be $\leq$ 4.5 and this measure does serve as an exclusion criterion. Subjects with posterior fornix pH >4.5 are not eligible to participate in the study.)

Posterior fornix

- Insert prewarmed speculum (either Medium Pederson or Large Pederson). It is preferable that the speculum be used without lubricant or water. If, in the opinion of the clinician, it is not feasible to insert the dry speculum without causing extreme discomfort to the subject, the exterior of the tip of the speculum may be moistened with tap water, taking care to use as little water as possible to avoid any impact on pH measurement.

- Visualize cervix and posterior fornix.
- With complete visualization, gently apply the microelectrode pH meter (Oakton, model pH Spear) to the vaginal mucosa at the highest point of the posterior fornix.
- Take at least two separate recordings (digital recordings of pH), and determine a mean pH. If the mean pH is ≤4.5, then eligibility is confirmed with regard to vaginal pH.
- If the mean pH at the posterior fornix is >4.5 at screening, the subject is not eligible for study enrollment.

Pending clinical judgment, subjects may be re-screened at a future time.

Note: Cidex® may be used for cleaning of instruments between subjects. See Appendix L for Disinfection of the Oakton pH Spear.

6.4 Enrollment and Screening Failures

All subjects who are screened for this study should be recorded on the Screening and Enrollment Log provided on the Study Materials page of the study web site. All screened subjects will be entered into the AdvantageEDC Internet Data Entry System (IDES) screening segment. Those who will participate in the study will be enrolled into the protocol in AdvantageEDC; those who will not participate will be entered as "Screening Failures" in AdvantageEDC.

6.4.1 Screening

Enrollment for each subject is done over the Internet using the enrollment module in AdvantageEDC (refer to the AdvantageEDC User's Guide for instructions). Enrollment should take place just prior to sampling, but after the subject has signed the IRB-approved consent form and is undergoing the screening process.

Section 6.2 describes procedures for rescreening subjects who have a transient health issue that can be remediated to permit enrollment (e.g., vaginal pH outside of allowed range).

6.4.2 Enrollment

Subjects who have met all of the inclusion and none of the exclusion criteria should be enrolled in AdvantageEDC as close as possible to the actual time of study intervention, to have an accurate accrual account to facilitate coordination between multiple clinical sites.

Clinic staff will enter the subject's Study ID (from the Screening and Enrollment Log) into AdvantageEDC at the time of enrollment. AdvantageEDC requires the entry of demographic information and completion of the Eligibility Checklist to confirm that the subject has met all of the inclusion criteria and none of the exclusion criteria.

6.4.3 Screening Failures

Individuals who have documented informed consent by signing a study Consent Form and who are screened for participation in the protocol but are considered ineligible, or will otherwise not enroll, are entered into the "Screening Failures" segment of the AdvantageEDC system. The process is similar to the enrollment process in that demographic information is completed but then, instead of choosing to enroll into the active study, the subject is enrolled into a "Screening Failures" segment, which is protocol-specific and is selected from the drop-down list on the protocol selection screen during the Enrollment process.

The Eligibility Checklist (for Screening Failures) is then completed.

- This form is completed to the degree to which the screening process was completed. If during the screening process, study staff identify that a potential subject does not meet a particular inclusion/exclusion criterion, the remainder of the screening process is not required. The Screening Failures Eligibility Checklist should be completed to the fullest extent possible; un-assessed criteria may be left blank.
- If all criteria are met, but the subject does not participate in the study, indicate the reason in the "Eligible But Not Enrolled" section near the bottom of the form.
- If a subject is re-screened outside of the allowed 30-day window for completion of screening, select a new Study ID from the Screening and Enrollment Log.

No other forms are submitted in IDES for individuals who fail screening.

Demographics and Screening Checklist data for these individuals are used to generate reports of screening activity, i.e., rates and assessments of failed criteria, and will not otherwise be included in analyses or reports for the protocol.

7 BIOLOGICAL SPECIMENS

This section provides information to the investigators and study personnel regarding the collection, processing, and transportation of clinical specimens to the proper destination. It is essential that these guidelines be followed without deviation to ensure that specimen integrity is maintained.

Biological specimens are to be collected in accordance with protocol specifications, as outlined in the Detailed Description of Study Procedures in the protocol. It may not be possible to collect all of the required specimens from a subject on one day. Therefore, the specimen collections for the Baseline Sampling Visit and the Re-Sampling Visits may take place over periods that do not exceed 14 days. For the Baseline Sampling Visit, specimen collection must commence within 30 days of the first date of screening, and sampling from all body sites must be completed within a 14-day window, such that the time from the first screening date to the final specimen collection date does not exceed 44 days. If specimen collection occurs over multiple days, it is important to ascertain that the subject continues to observe the windows for avoidance of activities and products listed in Appendix C.

7.1 Safety Precautions

Follow all safety guidelines of your local safety committee for collection and handling of all biological specimens. Observe Universal Precautions guidelines, including the wearing of gloves, safety glasses or face shield, and a lab coat.

7.2 Collection of Specimens for Screening

7.2.1 Urine Pregnancy Test

A urine specimen will be collected from all female subjects of childbearing potential (Women are considered to be of non-child bearing potential if they are surgically sterile for > 6 months.) at the Screening Visit and at each sampling visit, to perform a pregnancy test. (See section 6.3.1 for procedure.)

7.2.2 Viral Markers Screening

Blood (10 mL) will be collected at the Screening Visit to perform HIV, HBV and HCV testing. (See section 6.3.2 for procedure.)

7.3 Collection of Clinical Specimens

If a subject has a body piercing within the area from which a specimen will be obtained (e.g., tongue, nose), the subject should remove any piercing jewelry or hardware on the day of specimen collection. Study staff should record the presence of a piercing in the applicable comments section of the visit documentation form.

During sampling, **each** specimen collection tube and container (See App. K) will be labeled with the appropriate, body site-specific, pre-printed clinic label bearing the following information (See App. N for an example of a single subject label set.):

> Subject study ID # e.g. 01DBA0001
>
> Visit 01 / 02 (*circled as appropriate)*
>
> DT (Date of visit) ____/___/____ (dd/MMM/yyyy).
>
> *Coded Body Site** e.g. RAC_L

Site staff will write the subject's study ID number and visit date, and circle the appropriate visit number in the space provided.

A similar set of labels designed specifically for Visit 03 will be used for specimens from subjects who return for a third sampling.

Body Site		Specimen	Acronym	Collection Tube
Oral Cavity		1. Saliva	SAL	Screw-top, conical 50 mL tube
	Soft	2. Tongue Dorsum	TONG	750 µL MoBio buffer in MoBio tubes, 2 mL
		3. Hard Palate	HPAL	
		4. Buccal Mucosa	BUCC	
		5. Keratinized Gingiva (attached)	GING	
		6. Palatine Tonsils	PTON	
		7. Throat	THRO	
	Hard	8. Supragingival Plaque	SUPRA	
		9. Subgingival Plaque	SUB	
Skin		10. Retroauricular Crease – Left	RAC_L	750 µL MoBio buffer in MoBio tubes, 2 mL
		11. Retroauricular Crease – Right	RAC_R	
		12. Antecubital Fossa – Left	ACF_L	
		13. Antecubital Fossa – Right	ACF_R	
Nasal Cavity		14. Anterior Nares (L & R pooled)	NASAL	750 µL MoBio buffer in MoBio tube, 2 mL

GI Tract	15. Stool	STOOL	Stool kit
Vagina	16. Vaginal Introitus	INTRO	750 µL MoBio buffer in MoBio tubes, 2 mL
	17. Posterior Fornix	PFORN	
	18. Mid-Vagina	MIDVAG	
Blood	Whole Blood	COR-1 COR-2	10mL Yellow Tops with ACD Solution A (2)
	Blood for Immune assays	SERUM	10mL Red Top Vacutainer Tube with Anticoagulant

7.3.1 Specimen Collection from the Oral Cavity

7.3.1.1 Sampling Schedule

Specimens will be collected from the oral cavity of each subject at the Baseline Sampling Visit and at the Re-Sampling Visits.

7.3.1.2 Specimen Labeling

Label the specimen collection tubes with the appropriate body site-specific, pre-printed clinic label**.**

7.3.1.3 Materials Needed

1. Standard dental chair with good lighting.
2. Examination kit (See App. J for kit contents) containing at least one long-handled mouth mirror, dental explorer, surgical scissors, cotton pliers, 2 x 2 gauze pads, cotton rolls, cotton pledgets, wooden tongue depressor.
3. Small (2 mL) screw-top (external thread) MoBio tubes containing 750 µL of specimen collection fluid (MoBio buffer).
4. 50 mL sterile conical polypropylene tube (Falcon) for saliva collection.
5. Body site-specific, pre-printed clinic label set for all oral cavity sites to be collected.
6. Sterile Catch-All™ Specimen Collection Swabs (Epicentre Biotechnologies, Madison WI) to collect specimens from soft tissue surfaces.
7. Multiple sterile Micro Mini Five Gracey curettes (one curette will be used to sample and pool the supragingival plaque from the index teeth, and one curette will be used to sample and pool subgingival plaque from the same index teeth; additional sterile curettes should be available in case they are needed).
8. Stopwatch with second hand.

9. Ziploc plastic bag to hold the MoBio tubes containing the collected specimens.
10. Small ice chest with ice for transport to the clinical laboratory for processing.

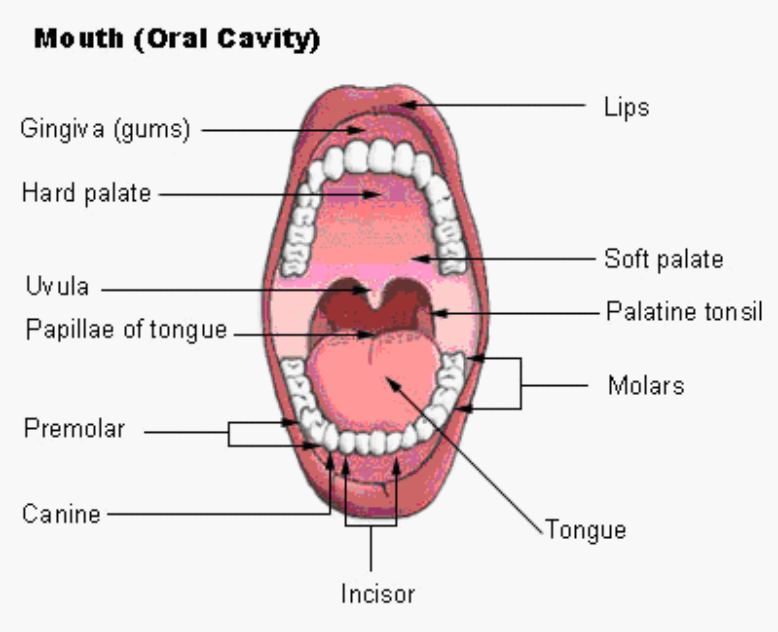
7.3.1.4 Collection Methods

Aseptic technique will be used for collection of all specimens (See App. O).

Sequence of collection:

1. Saliva
2. Soft tissue sites*
3. Hard tissue sites

* Sampling of the throat is difficult because of the gag reflex. It is suggested that this sample be obtained as the very last specimen, after all the other soft tissue and hard tissue sites have been sampled.



[Figure from Cochrane Systematic Reviews on Oral Cancer, May 6, 2007]

After each specimen is collected, place the tube in a Ziploc bag and place over ice. Within 4 hours of collection, take to the clinical lab for processing.

7.3.1.5 Saliva

A. Subject is asked to let saliva collect in the mouth for at least 1 minute. The subject is then asked to drool into the labeled 50 mL collection tube (Falcon, sterile conical polypropylene tube with flat-top screw cap). This process may be repeated multiple times in order to collect larger volumes of saliva (2-5 mL).

B. Alternatively, saliva production can be stimulated by administration of a standard piece of unflavored gum base (1.0 –1.5 g; Wrigley Co., Peoria, IL). This piece of gum may be placed in the subject's mouth. The subject is asked to swallow any accumulated saliva and instructed to chew the gum at a regular rate. Upon sufficient accumulation of saliva in the oral cavity, the saliva is drooled into the labeled collection tube. This process may be repeated multiple times in order to collect larger volumes of saliva (2-5 mL).

C. Put the tube in a Ziploc bag and place over ice. Within approximately 4 hours take to the clinical lab for processing.

7.3.1.6 Soft tissue sites

A. All soft tissue sites are sampled using Catch-All™ Sample Collection Swabs. Immediately after swabbing, each swab must be swirled in 750 µL of MoBio buffer in the MoBio tube. The swab sponge should be pressed against the tube wall multiple times for 20 seconds to ensure transfer of bacteria from swab to solution. The specimen in the MoBio buffer should be kept cold until processing.

   a. Tongue dorsum:

   Swab 1 cm^2 of the center of the tongue for 5 seconds.

   b. Hard palate:

   Swab the entire hard palate for 10 seconds.

   c. Buccal mucosa:

   Swab the entire area of both left and right buccal mucosa for 10 seconds each. Take care not to touch the teeth.

   d. Keratinized (attached) gingiva:

   Swab the maxillary anterior attached gingiva for 10 seconds.

   e. Palatine Tonsils:

   Swab left and right palatine tonsils for 5 seconds each, concentrating on fissures and pits.

   f. Throat :

This site is difficult because of the gag reflex. It is suggested that this sample be obtained as the very last specimen, after all the other soft tissue and hard tissue sites have been sampled. Have the subject open mouth and relax tongue; insert depressor all the way to the rear of tongue and depress entire tongue out of the way. Gently swab across the rear of the oropharynx for up to 5 seconds. Do not swab tonsils.

Each of the above soft tissue sites is sampled and vialed separately and labeled with the appropriate body site-specific, pre-printed clinic label.

7.3.1.7 Hard tissue sites

A. Supragingival plaque

The goal is to sample supragingival plaque from the 6 index teeth: 2 molar teeth (#3 and #19), 2 premolar teeth (#12 and #28) and 2 incisor teeth (#9 and #25).

Molar teeth: 2 molar teeth must be sampled: #3 – first molar in upper right quadrant, and #19 – first molar in lower left quadrant. If the index first molar has an unrestored mesial surface, sample this tooth. If the index first molar has a restored mesial surface, and the second molar in the same quadrant has an unrestored mesial surface, the second molar should be sampled instead, as the goal is to sample unrestored surfaces as often as possible. If both molars in an index molar quadrant have restored mesial surfaces, then sample the index first molar.

Premolar teeth: 2 premolar teeth must be sampled: #12 – first premolar in upper left quadrant, and #28, the first premolar in the lower right quadrant. If the index premolar has an unrestored mesial surface, sample this tooth. If the index first premolar has a restored mesial surface, and the second premolar in the same quadrant has an unrestored mesial surface, the second premolar should be sampled instead, as the goal is to sample unrestored surfaces as often as possible. If both premolars in an index premolar quadrant have restored mesial surfaces, then sample the index first molar. If a premolar is missing because of orthodontic extraction, sample the residual premolar regardless of whether the mesial surface is restored or not.

Incisor teeth: 2 incisor teeth must be sampled: #9 – central incisor in the upper left quadrant, and #25 – central incisor in the lower right quadrant.

Procedure: For the first tooth, isolate the site to be sampled with cotton rolls and dry with a gentle stream of air from an air-water syringe. With a Gracey curette remove all of the supragingival plaque from the mesial surface of the selected index tooth with as many strokes as necessary. Immerse the curette tip in the MoBio buffer in the MoBio tube for 4-5 seconds. Wipe off the face of the curette on the inside edge of the collection tube. The site can be immediately sampled again using the same procedure. Repeat the process for all the selected index teeth.

If the subject has little plaque, the arch counterparts to the index molar and premolar teeth should also be sampled. For example, #14 – first molar in upper left quadrant, and # 30 – first molar in lower right quadrant (mesial to distal ends) and #5 – first premolar in upper right quadrant, and #21 – first premolar in lower left quadrant should also be collected (a total of 10 teeth). Replace the lid on the MoBio tube and shake for 4-5 seconds in an attempt to maximize dispersion of the specimen in the fluid.

Record on the CRF the resulting selection of teeth for sampling.

Put the tube in a Ziploc bag and place over ice. Within approximately 4 hours take to the clinical lab for processing.

B. Subgingival plaque

Prior to collection of the subgingival specimens, place cotton rolls, dry the area, and wipe off any residual supragingival plaque. Collect subgingival plaque from the mesio-buccal surface of the selected index teeth with a sterile Gracey curette. Immerse the curette tip in the MoBio buffer in the MoBio tube vial for 4-5 seconds. Wipe off the face of the curette on the inside edge of the collection tube. Discard any specimens with significant amounts of blood, based on clinical judgment (i.e., if flooding occurs). Because of the risk of bleeding, immediate re-sampling of the same site is not recommended. If the pooled plaque is considered insufficient (from subjects with very clean mouths), sample the counterpart index teeth as described above. If the pooled plaque is still considered insufficient, then sample mesio-buccal subgingival plaque of second molars and pool these specimens with the first collected specimens. Replace the cap on the tube and shake for 4-5 seconds in an attempt to maximize dispersion of the specimen in the fluid. Put the tube in a Ziploc bag and place over ice. Within approximately 4 hours take to the clinical lab for processing.

The supragingival plaque samples are pooled in one MoBio tube. The subgingival plaque samples are pooled in a second tube. Each tube is labeled with the appropriate body site-specific, pre-printed clinic label.

7.3.2 Specimen Collection from the Skin and Nasal Cavity

7.3.2.1 Sampling Schedule

Specimens will be collected from the skin and nasal cavity of each subject at the Baseline Sampling Visit and at the Re-Sampling Visits.

7.3.2.2 Specimen Labeling

Label the specimen collection tubes with the appropriate body site-specific, pre-printed clinic label**.**

7.3.2.3 Materials Needed

1. Small (2 mL) screw-top (external thread) MoBio collection tubes containing 750 µL of specimen collection fluid (MoBio buffer)
2. Pre-printed clinic label set for all body sites to be collected
3. Sterile Catch-All™ Sample Collection Swabs (Epicentre Biotechnologies, Madison WI)
4. Vial of sterile SCF-1 (50 mM Tris buffer [pH 7.6], 1 mM EDTA [pH 8.0], and 0.5% Tween-20) to pre-moisten Catch-All swabs prior to collection
5. Ziploc plastic bag to hold the MoBio collection tubes containing the collected specimens
6. Small ice chest with ice

7.3.2.4 Collection Methods

Aseptic technique will be used for collection of all specimens (See App. O). A single pair of gloves may be worn to collect all of the skin and nasal cavity specimens from a subject, with care taken to avoid contamination of the gloves. If the gloved hand used to stretch the skin for sampling comes in contact with the sampling area, the glove(s) should be changed before sampling the next site.

Sequence of collection:

Retroauricular crease (behind ears, left & right sides to be kept separate)
Antecubital fossa (inner elbow, left & right sides to be kept separate)
Anterior nares (left & right sides to be combined)

Note: It is helpful for the subject to be comfortably lying down for these sampling procedures.

7.3.2.5 Retroauricular crease

The area of interest begins where the top of the ear joins the face and extends to where the ear lobe connects to the face. Skin surface specimens will be collected with a Catch-All™ Sample Collection Swab, moistened with sterile SCF-1 solution. To access the site, fold the ear forward with one hand to expose the crease. With the other hand, hold the shaft of the swab parallel to the surface of the skin and rub the swab back and forth along the retroauricular crease approximately 50 times, applying firm pressure (this requires approximately 30 seconds). Insert the swab head into the tube containing MoBio buffer. The head of the swab should then be aseptically cut from the handle, and the tube cap should be screwed back in place.

Note: To obtain optimal skin surface specimens, observe the key aspects of the sampling technique: use of the moistened swab, application of firm pressure, and consistency in rubbing (50 times back and forth on the sampling site during 30 seconds).

Right and left sides are sampled and stored separately and labeled with the appropriate body site-specific, pre-printed clinic label**.** Put the tubes in a Ziploc bag and place over ice. Within approximately 2 hours, take to the clinical lab for processing.

7.3.2.6 Antecubital fossa (inner elbow)

This area is located exactly at the bend of the inner elbow at the junction of the arm and the forearm. Skin surface specimens will be collected with a Catch-All™ Sample Collection Swab, moistened with sterile SCF-1 solution. With one hand, stretch the antecubital skin taut. With the other hand, hold the swab so the shaft is parallel to the skin surface and rub the swab back and forth approximately 50 times along the antecubital crease, applying firm pressure (this requires approximately 30 seconds). Insert the swab head into the tube containing MoBio buffer. The head of the swab should then be aseptically cut from the handle, and the tube cap should be screwed back in place.

Note: To obtain optimal skin surface specimens, observe the key aspects of the sampling technique: use of the moistened swab, application of firm

pressure, and consistency in rubbing (50 times back and forth on the sampling site during 30 seconds).

Right and left sides are sampled and stored separately and labeled with the appropriate body site-specific, pre-printed clinic label**.** Put the tubes in a Ziploc bag and place over ice. Within approximately 2 hours, take to the clinical lab for processing.

7.3.2.7 Anterior nares

With a twisting motion, gently rub the mucosal surfaces of the anterior nares with a sterile Catch-All specimen collection swab, going round the area 2 times.

Right and left sides are sampled and pooled together as a combined specimen and labeled with the appropriate body site-specific, pre-printed clinic label**.**

Immediately after swabbing, each swab must be swirled in 750 µL of MoBio buffer in the tube. The swab sponge should be pressed against the tube wall multiple times for 20 seconds to ensure transfer of bacteria from swab to solution.

Put the tubes in a Ziploc bag and place over ice. Within approximately 2 hours take to the clinical lab for processing.

7.3.3 Specimen Collection from the Gastrointestinal Tract

7.3.3.1 Sampling Schedule

Subjects will be provided with a stool specimen collection kit at the screening visit (for baseline sample collection) and at sampling visits (as needed for re-sampling collection). Subjects will collect stool specimens within a 24-hour period before each sampling visit. Subjects will bring the specimens to the clinic staff at the time of the visit.

7.3.3.2 Specimen Labeling

- Label the specimen collection containers with the appropriate body site-specific, pre-printed clinic label at the time of provision to the subject**.** (This label should be placed on the side wall of the container, near the tapered bottom. Avoid placing the label in the area where the container will be seated in the collection frame.)
- Label the specimen transport box with the pre-printed stool box label bearing the following information at the time of provision to the subject*:

e.g.

Subject ID #: 01DBA0001

Below to be completed by subject

Stool collection date: 09/25/08 (dd/MMM/yyyy)

Stool collection time: 0830 AM or PM

Below to be completed by clinical staff

Visit date: 09/26/08 (dd/MMM/yyyy)

Time of receipt: ___ ___: ____ ____ (24 hour clock)

Staff Initials: _____

- Upon return of the specimen, site staff will write the date and time the specimen is received and <u>confirm that receipt is within the 24 hour period of collection</u>.

7.3.3.3 Materials Needed

1. One stool collection kit with two stool collection containers (one is for back-up)
2. One Ziploc bag
3. One Thermosafe shipping container
4. Eight to ten polar packs for transport of specimen
5. One roll of packing tape
6. Specimen collection instructions
7. Body site-specific, pre-printed clinic label
8. Stool box label

7.3.3.4 Collection Methods

The following information and the kit will be provided to the subjects. The stool collection containers will be labeled with a body site-specific, pre-printed clinic label at the time of provision to the subject**.** The containers will be inspected for integrity and cleanliness prior to clinic labeling.

Instructions For Stool Specimen Collection

****Before you collect your specimen, please place all gel packs in your freezer for at least 12 hours. ****

Specimen should be collected no more than 24 hours before your clinic visit.

STEP 1. Raise the toilet seat. Place the stool collection frame on the back of the toilet bowl (see Figure 1). All four corners of the collection frame should be supported by the toilet bowl. Place collection bowl in frame (see Figure 2).

Figure 1

Figure 2

STEP 2. Place toilet seat down (see Figure 3). Do **not** urinate into the collection container*. Deposit your stool directly into the collection container.
If accidental urination occurs, discard the stool and collect a new sample using the second (back-up) container provided.

Figure 3

STEP 3. After collecting your specimen, remove the container from the frame (see Figure 4). Place the container on a flat surface and firmly press the lid closed (see Figures 5 and 6).

Figure 4

Figure 5

Figure 6

STEP 4. Place the closed container into the Ziploc bag (see Figure 7) and seal the bag.


Figure 7

STEP 5. Discard collection frame in trash.

STEP 6. Package your specimen immediately following the instructions below. Write the date and time of collection on the outer box label, and bring it in with you for your baseline sampling visit or re-sampling visit. Your specimen may be stored in the Styrofoam box with gel packs (see below) up to a maximum of 24 hours, prior to your clinic visit.

Stool Packaging Instructions

1. Place all seven gel packs in your freezer (see Figure 1). Do not collect your specimen until the gel packs have been in your freezer for at least 12 hours.

FIGURE 1

2. Remove the gel packs from your freezer and place two of the gel packs in the bottom of the Styrofoam box (see Figure 2).

FIGURE 2

3. Place the sealed Ziploc bag containing your stool specimen on top of the two frozen gel packs in the Styrofoam container (see Figure 3).

FIGURE 3

4. Place four of the frozen gel packs around the specimen container so that the container is completely surrounded (see Figures 4 and 5).

FIGURE 4

FIGURE 5

5. Place one gel pack on top of the container (see Figure 6).

FIGURE 6

6. Place the Styrofoam lid on the Styrofoam container (see Figure 7) and close cardboard box.

FIGURE 7

7. Using the tape dispenser provided, seal the middle of the box and both sides of the box (see Figures 8 and 9).

FIGURE 8

FIGURE 9

8. Bring the box with you to your sample collection appointment. [Note: Subject ID# and body site-specific, pre-printed clinic label will be applied to the container at the time of provision to the subject]

7.3.3.5 Specimen Receipt

When subject arrives in the clinic for the specimen collection visit, the stool collection container should be retained in the Styrofoam box with the gel packs while being transported to the clinical lab.

7.3.4 Specimen Collection from the Vagina

7.3.4.1 Sampling Schedule

Specimens will be collected from the vagina of each female subject at the Baseline Sampling Visit and at the Re-Sampling Visits.

7.3.4.2 Specimen Labeling

Label the specimen collection tubes with the appropriate body site-specific, pre-printed clinic label**.**

7.3.4.3 Materials Needed

1. Standard gynecologic exam table with good lighting
2. Examination kit containing at least one medium Pederson and one large Pederson speculum, microelectrode pH meter (Oakton, model pH Spear) and 3 sterile Catch-All™Sample collection swabs (Epicentre Biotechnologies, Madison WI)
3. Small (2 mL) screw-top (external thread) MoBio collection tubes containing 750 µL of specimen collection fluid (MoBio buffer)
4. Pre-printed clinic label set for all body sites to be collected
5. Stopwatch with second hand
6. Ziploc Biohazard-labeled plastic bag to hold the MoBio collection tubes containing the collected specimens
7. Small ice chest with ice

7.3.4.4 Collection Methods

Aseptic technique will be used for collection of all specimens (See App. O).

1. Collect information regarding menstrual cycle by inquiring about last menstrual period (LMP) and cycle length.
2. Record use of all contraception within the preceding 12 months on the Concomitant Medications or Medical History forms as applicable:
    a. For oral contraceptives, record brand name of oral contraceptive, date initiated (month/year), and whether it is used “continuous dose” (placebo pills not taken, such that menstruation does not occur) or “traditional dose.”

b. For intrauterine devices, record type of device used (Mirena® versus Paragard® versus other) and date of insertion (month/year).

c. For progesterone-based injections and insertion rods, record delivery device (Depo-Provera® versus Implanon™ versus other) and date of insertion or last injection (month/year).

d. For permanent sterilization methods (tubal ligation and Essure® device), record type and date of procedure (month/year).

3. Spread labia to visualize vaginal introitus immediately posterior to hymenal ring/tissue.

4. Repeat vaginal pH determination of vaginal introitus immediately prior to sampling (each and every time sampling is performed). Follow the same procedure used for screening subjects, as described in Section 6.3.7 Vaginal Examination and pH Determination.

5. Proceed with obtaining vaginal introitus specimen: place one Sterile Catch-All™ Sample Collection Swab (Epicentre Biotechnologies, Madison WI) at the vaginal introitus posterior to the hymenal ring/tissue and rotate swab along the lumen with a circular motion five times. Immediately after swabbing, the swab is swirled in 750 µL of MoBio buffer in the labeled specimen collection tube. The swab should be pressed against the tube wall multiple times for 20 seconds to ensure transfer of bacteria from swab to solution.

6. Insert pre-warmed speculum. It is preferable that the speculum be used without lubricant or water. If, in the opinion of the clinician, it is not feasible to insert the dry speculum without causing extreme discomfort to the subject, the exterior of the tip of the speculum may be moistened with tap water, taking care to use as little water as possible to avoid diluting the vaginal fluids being collected and to avoid any impact on pH measurement.

7. Visualize cervix and posterior fornix.

8. Determine pH of posterior fornix immediately prior to sampling (each and every time sampling is performed). Follow the same procedure used for screening subjects, as described in Section 6.3.7 Vaginal Examination and pH Determination. If posterior fornix pH>4.5 at sampling visit but was $\leq$4.5 at screening, proceed with sampling the posterior fornix.

9. Proceed with obtaining posterior fornix specimen: place one Sterile Catch-All™ Sample Collection Swab (Epicentre Biotechnologies, Madison WI) in the posterior fornix and rotate the swab along the lumen with a circular motion five times.

Immediately after swabbing, the swab is swirled in 750 µL of MoBio buffer in an appropriately labeled specimen collection tube. The swab should be pressed against the tube wall multiple times for 20 seconds to ensure transfer of bacteria from swab to solution.

10. After obtaining posterior fornix specimen, proceed with collecting vaginal midpoint specimen using one Sterile Catch-All™ Sample Collection Swab (Epicentre Biotechnologies, Madison WI) to gently rub the mid-vaginal wall. Immediately after swabbing, the swab is swirled in 750 µL of MoBio buffer in an appropriately labeled specimen collection tube. The swab should be pressed against the tube wall multiple times for 20 seconds to ensure transfer of bacteria from swab to solution.
11. Store all the tubes on ice and deliver to the clinical laboratory within approximately two hours.

Avoid sampling of the vaginal area where there has been contact with the speculum.

Vaginal introitus, Posterior fornix and Mid-vagina areas are sampled and stored separately and labeled with the appropriate body site-specific, pre-printed clinic label.

7.3.5 Collection of Blood Specimens (Baseline collection only)

7.3.5.1 Sampling Schedule

Thirty mL of whole blood will be collected from all subjects at the Baseline Sampling Visit.

7.3.5.2 Specimen Labeling

The phlebotomist will label the specimen collection vials with the appropriate body site-specific, pre-printed clinic label**.**

NOTE: Subject's gender will be indicated on the Coriell samples by the addition of an M or F on the clinic label.

e.g.

01DBA Visit: 01 DT: **COR-1 M or F (indicate one)**

7.3.5.3 Materials Needed

- Blood collection kit from Coriell Institute for Medical Research containing two yellow top tubes
- Red top vacutainer tubes (without anticoagulant)
- Pre-printed clinic label set for all body sites to be collected

7.3.5.4 Collection Methods

Aseptic technique will be used for collection of all specimens (See App. O).

Coriell Specimens

1. 20 mL (2 x 10 mL) will be collected in the Coriell-provided yellow-top collection tubes (vacutainer with ACD Solution A), labeled with the pre-printed clinic label and stored at room temperature. These tubes will be sent to the clinical laboratory for bar-coding, packaging and same day shipment to the Coriell Institute for Medical Research. Follow preparation, packaging and shipping instructions as provided by Coriell Institute and contained in App H.
2. Blood will be processed and stored at the Coriell Institute for Medical Research for future genotyping, DNA sequencing, and creation of cell lines.

Serum Sample (Serum)

1. 10 mL will be collected in a red-top tube without anticoagulant (for future serum immune response measurements) and labeled with the pre-printed clinic label.
2. Serum will be processed (per site SOP). Samples (up to 5 x 1 mL) will be bar-coded by the clinical lab and stored at -20ºC in the clinical laboratory for future antibody assays.

7.4 Bar-code labeling of Clinical Specimens

Bar-code labeling and GlobalTrace data entry of all specimens will be done exclusively in the clinical laboratory at each site.

Labeling Supplies

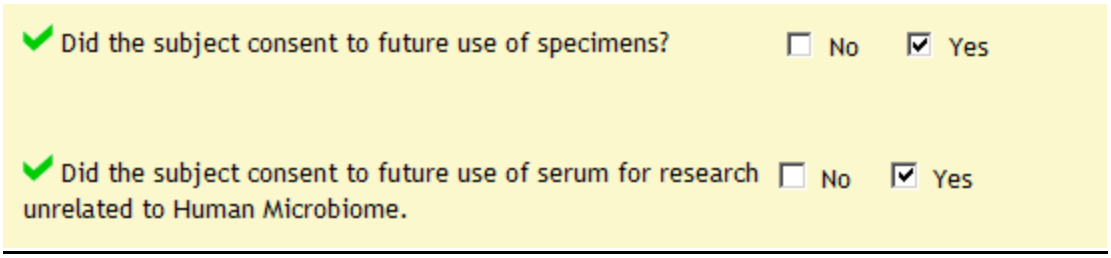
- Rolls of paired sets of identical barcodes (provided by the CDCC/EMMES)
- PC-compatible barcode scanner
- GlobalTrace Source Document (provided by the CDCC/EMMES)

Procedures

- Specimens will be transported (per site SOP) from the clinical site to the appropriate clinical laboratory personnel for processing within two hours of collection.
  - For each specimen, a GlobalTrace source document will be completed, capturing the subject's Study ID, visit number and date of collection, and YES to the question of future use.
- After the clinical lab has processed and/or aliquoted the specimen into the appropriate tube, the following procedure will be followed FOR EACH SPECIMEN:
  - One barcode label, of two identical barcodes found in pairs on the roll of barcode labels, will be applied to the tube. The other accompanying barcode will be placed on the GlobalTrace source document for that subject's specimen type. The source document will be signed and dated by the study personnel labeling the tube.
  - This labeling process will be repeated for each tube into which the sampling specimen has been collected/aliquoted. All of the subject's specimens of the same type should be included on the same GlobalTrace source document for any given visit.
    - Barcoded tubes (MoBio) will then be placed in the freezer for storage.
    - Barcoded yellow-top collection tubes of whole blood will be shipped to Coriell (per Coriell SOP).

7.5 Entering the Barcoded Specimens into the GTS Inventory

The barcodes from the GlobalTrace source document will be scanned into the GlobalTrace system and associated with the subject's Study ID, visit number, and visit date, noting the specimen type as well as the future use choice in the Purpose field. With the exception of the serum specimen, select Future Use as YES for all specimens for enrolled subjects in this study. For serum specimens, the subject may elect Future Use or No Future Use (for research unrelated to HMP).



For details on the GlobalTrace System, see the GlobalTrace System User's Guide.

Note: Specimens can be entered in GlobalTrace only for subjects who have already been enrolled in AdvantageEDC. Therefore, specimens collected at sampling visits will not be able to be entered into GlobalTrace until after the subject has been enrolled in AdvantageEDC at Visit 01.

7.6 Specimen Transport and Shipping

7.6.1 Blood specimens

Blood specimen shipment will occur between the clinical laboratory and the Coriell Institute for Medical Research (Camden NJ) on the same day as specimen collection. **Friday and Holiday shipments (see below) will not be permitted.** The blood specimens will be packaged and shipped according to the following instructions provided by the Coriell Institute:

To make shipment arrangements or to request additional transport materials, contact Barbara Frederick at the Repository

HOLIDAYS

Coriell Cell Repositories observes the major public holidays. Any holiday falling on Saturday or Sunday will be observed on the date (Friday or Monday) designated by the State of New Jersey as the State holiday. The Holidays are:

New Year's Day
Martin Luther King, Jr. Day
President's Day
Memorial Day
Independence Day
Labor Day
Thanksgiving Day
Day after Thanksgiving Day
Christmas Eve
Christmas
New Year's Eve

PLEASE DO NOT SCHEDULE SPECIMENS TO ARRIVE AT CORIELL ON THESE DAYS.

SHIPPING ADDRESS

CORIELL CELL REPOSITORIES
SUBMISSIONS: HUMAN MICROBIOME PROJECT

PRIOR NOTIFICATION OF SHIPMENT

Coriell will be contacted via email notification directly from the GlobalTrace system, when an electronic shipment is sent. If sites anticipate large or unusual shipments they should contact Coriell directly and provide the below information.

Prior notification of shipment was given by _____
Investigator

to _____
Coriell Staff Member

Date of Notification: _____

Planned Date of Shipment: _____

7.7 Initial Processing of Saliva and Stool Specimens

Aseptic technique will be used for processing of all specimens (See App. O).

7.7.1 Saliva

1. Centrifuge Falcon tubes containing collected saliva at 2600g for 15 minutes at room temperature. If the solids are not sufficiently separated, centrifuge again for 20 minutes.
2. Using a 1 mL Pipetman, transfer the saliva supernatant to two MoBio 2 mL tubes containing 750µL of lysis buffer.
3. Tubes will then be barcode labeled (see section 7.4) and frozen at -80ºC prior to DNA extraction.

7.7.2 Stool

1. From each sampling visit, four aliquots of stool lysate will be generated for purification of DNA and two aliquots of stool will be generated for future purification of RNA.
2. Upon receipt of the stool specimen, the clinical laboratory will aliquot approximately 2 mL of specimen into one 50 mL Falcon tube. Stool may be distributed using a disposable spatula or a metal spatula that is subsequently wiped clean and ethanol flamed for sterilization. 5 mL MoBio lysis buffer is added to the Falcon tube, and the specimen is homogenized by vortexing 30-40 seconds. Note that the

laboratory will need to have extra MoBio lysis buffer for this step, as this volume is not supplied with the DNA extraction kit.

3. Centrifuge the Falcon tube 5 minutes at 1500 rcf and pipet 1 mL of supernatant into 4 barcoded MoBio Garnet Bead tubes containing 750µL of MoBio buffer (this tube with buffer comes with the PowerSoil™ kit).
4. Heat at 65°C for 10 minutes, then at 95°C for 10 minutes. Specimens may be stored frozen at -80º C prior to DNA extraction.
5. Process per MoBio PowerSoil™ DNA Isolation Kit directions (see Section 7.9.2), and store the extracted and barcoded DNA at -80º C.
6. For the future RNA extraction, add 500 µL of stool specimen (as noted above) to each of two 2 mL external thread cryovials (Nunc or Nalgene). Add 1 mL of RNAlater® (Ambion, Inc., AM7020), which will help stabilize the nucleic acids during long-term storage. Barcode label, and freeze cryovial at -80º C.

7.8 Initial Processing of Clinical Specimens Stored in SCF-1 Buffer

Between 18 May 2009 and 15 June 2009, clinical specimens were collected in SCF-1 buffer instead of in MoBio buffer. For laboratory processing of these specimens, the following procedures will be followed:

1. Centrifuge the specimen at 13,000 rpm for 10 minutes and remove supernatant.
2. Use approximately 500 µL of MoBio Powersoil Bead Solution provided in the MoBio Bead tube to resuspend the pellet.
3. Transfer the resuspended specimen back to the MoBio Bead tube from which you took approximately 500 µL solution, and proceed with step one from the MoBio PowerSoil DNA Isolation Kit.

7.9 Specimen Processing for Extraction of Bacterial Genomic DNA

7.9.1 Materials

1. MoBio PowerSoil™ kit (Catalog # 12888-50, 50 Preps, or Catalog # 12888-100, 100 preps)
2. Microcentrifuge (10,000 x *g*)

3. Pipettors (50 µL - 500 µL)
4. Vortex
5. Vortex Adapter (MoBio Catalog # 13000-V1)

7.9.2 Procedures

The full MoBio PowerSoil™ DNA Isolation kit instructions can be accessed at http://www.mobio.com/files/protocol/12888.pdf.

NOTE: The only deviation from the MoBio kit protocol is in step 12: The tubes are centrifuged at room temperature for 2 minutes at 10,000 x *g*. Instructions in this section include this modification.

Please wear gloves at all times.

1. Specimens will arrive in the PowerBead Tubes provided, or specimen will need to be added to the tube after preprocessing (i.e., for stool, saliva).
2. Gently vortex to mix. In the case of skin specimens, aseptically remove the head of the Catch-All™ Sample Collection Swab from the PowerBead Tube. As it is removed, twist the swab head and press it against the side of the tube, in order to wring out as much as possible of the specimen-containing solution.
3. **Check Solution C1.** If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µL of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog No. 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
7. Transfer the supernatant to a clean 2 mL Collection Tube (provided). **Note**: Expect between 400 to 500 µL of supernatant. Supernatant may still contain some sample particles.
8. Add 250 µL of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*

10. Avoiding the pellet, transfer up to, but no more than, 600 µL of supernatant to a clean 2 mL Collection Tube (provided).
11. Add 200 µL of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes at room temperature for 2 minutes at 10,000 x *g.*
13. Avoiding the pellet, transfer up to, but no more than, 750 µL of supernatant into a clean 2 mL Collection Tube (provided).
14. Add 1200 µL of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675 µL onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µL of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required.
16. Add 500 µL of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
19. Carefully place Spin Filter in a clean 2 mL Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 100 µL of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog No. 17000-10).
21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.

7.10 Specimen Processing for Extraction of Microbial RNA

Aseptic technique will be used for processing of all samples (See App. O).

[THIS SECTION IS UNDER DEVELOPMENT]

7.10.1 Materials

[THIS SECTION IS UNDER DEVELOPMENT]

7.10.2 Procedures

[THIS SECTION IS UNDER DEVELOPMENT]

7.11 Bar-code labeling of Extracted Nucleic Acids

For each tube containing extracted nucleic acid sample, a GlobalTrace source document will be completed, capturing the subject's Study ID, visit number (of corresponding sampling material), date of processing, and YES to the question of future use.

After the clinical lab has processed and/or aliquoted the sample into the appropriate tube, the following procedure will be followed FOR EACH SAMPLE:

- One barcode label, of two identical barcodes found in pairs on the roll of barcode labels, will be applied to the tube. The other accompanying barcode will be placed on the GlobalTrace source document for that subject's sample type. The source document will be signed and dated by the study personnel labeling the tube.
- This labeling process will be repeated for each tube into which the sample has been collected/aliquoted. All of the subject's samples of the same type should be included on the same GlobalTrace source document for any given visit.
- Barcoded tubes will then be placed in the freezer for storage prior to shipment to a sequencing center.

7.12 Entering the Barcoded Nucleic Acid Samples into the GTS Inventory

The barcodes from the GlobalTrace source document will be scanned into the GlobalTrace system and associated as an **aliquot or pool** with the subject's source specimen material, Study ID, visit number, and visit date, noting the specimen type as well as the future use choice in the Purpose field. *Select Future Use as YES for all enrolled subjects in this study.*

For details on the GlobalTrace System and its aliquoting features, see the GlobalTrace System User's Guide.

7.13 Freezer Inventory of Clinical Specimens and Nucleic Acid Samples

Primary specimens are given 10-digit barcode numbers. All specimen/sample information is entered by barcode scanning and remote web-based graphical user interface into a database maintained by EMMES. Hard copies of records are stored at the clinical center and at the specimen processing laboratory. In addition to all specimen metadata, the EMMES records contain box/slot freezer location information for each specimen/sample for inventory purposes.

Clinical specimens and nucleic acid samples are tracked locally by an inventory sheet that includes primary specimen barcode, primary derivative nucleic acid barcode, two (or more) subaliquot nucleic acid barcodes, nucleic acid concentration (determined by A260), and physical location of each specimen/sample. The inventory also summarizes data per subject specimen set including date/time of processing, processing technician, and date of aliquot generation. Extracted DNA sample aliquots are stored in a secured and temperature-monitored freezer. Upon sample request via an EMMES-generated "pick list", sample aliquots are removed from the freezer and transferred or shipped with cold gel packs or dry ice to the sequencing centers for metagenomic sequencing.

When samples are identified for shipment to a sequencing center, a shipping manifest is created in the EMMES database. Each sample barcode number to be included in the shipment is scanned into the shipping manifest, which is sent electronically to the destination sequencing center. Once the shipment is received by the sequencing center, the center confirms that all physical samples were received using the QC Scan feature in the EMMES database and either accepts or rejects the shipment in the EMMES database. Any discrepancies are reconciled before the sequencing center officially accepts a shipment. Accepted samples are then electronically associated with the sequencing center to which they were shipped.

Each of the four sequencing centers uses similar procedures for tracking nucleic acid samples and associating sequence data with the original subject specimens. The following paragraphs provide details of these procedures for each sequencing center:

Washington University Genome Sequencing Center:

Samples are stored in a temporary freezer for 24 hours. One day after accepting the shipment the EMMES database is queried at https://web.emmes.com/study/dnt/hmp/dataentry/dataentry.html and the updated metadata (temporary subject ID → permanent RSID#) are downloaded in a 'Web Page, HTML only' file generated by EMMES [https://web.emmes.com/study/dnt/hmp/protocols/07001/reports.htm "Sequence Center Specimen Listing"], which is then directly uploaded into the Genome Center's database for the Laboratory Information Management System (LIMS) tracking system. Each tube is then checked into a secure freezer location for future use.

As specific experimental goals are defined, electronic work orders that detail the specific lab processes to be undertaken are constructed and entered into the local database. Since each sample is assigned a unique internal barcode, all operations on aliquots from the sample are tracked in LIMS and associated with the unique barcode.

When submitting DNA sequence data into the National Center for Biotechnology Information (NCBI) databases, the sequence file names are associated with internal

stored EMMES metadata fields (RSID#, specimen number and specimen type) in an accompanying text file. These three fields allow one to associate the DNA sequence files with the appropriate extended metadata maintained in the database of Genotypes and Phenotypes (dbGaP).

Baylor Human Genome Sequencing Center:

The sequencing files include the molecular barcode in the sff filename. The sequencing files are submitted with accompanying metadata files that map each molecular barcode to its corresponding EMMES Sample ID.

Broad Institute:

EMMES samples are registered in the Biological Samples Platform (BSP) database and assigned and bar coded with new BSP identification numbers. The BSP IDs are linked to EMMES IDs. All provided phenotypic data are uploaded and linked to the samples in the BSP database. Samples are scanned into a BSP container and then re-labeled with Genome Sequencing Sample Repository (GSSR) bar codes that are linked to the BSP and EMMES sample IDs. Sample and phenotypic data are exported to the GSSR database and sent over to GSSR for processing.

J. Craig Venter Institute:

When a member of the JCVI staff logs in to the EMMES website to accept the shipment of samples, the shipment manifest with barcodes is downloaded as an Excel file. The corresponding RSIDs for the samples are then downloaded from the EMMES webpage [https://web.emmes.com/study/dnt/hmp/protocols/07001/reports.htm "Sequence Center Specimen Listing"]. These files are merged and used to build a manifest file for importing into JCVI's LIMS. JCVI's workflow in JLIMS links the source tube barcode to the library, which is linked to additional downstream processing. For Illumina the linkage is as follows: source tube -> library -> library amplification -> library QC -> library dilution -> flowcell barcode and lane -> GAIIx sequencing run. For 454 sequencing the linkage is as follows: source tube -> library -> library QC -> library dilution -> emulsion PCR -> enriched beads -> PTP barcode -> FLX sequencing run.

7.14 Shipping Samples of Extracted Nucleic Acids

Nucleic acid samples will be stored in Tris-EDTA buffer and shipped to the sequencing centers in 1.5 – 2 mL sterile polypropylene tubes. The tubes will be packaged and shipped with cold gel packs (-20ºC) or dry ice to maintain the sample integrity and packages will be marked as required by the current regulations.

Nucleic acid sample aliquots will be distributed to the sequencing centers on a periodic basis. Each month, the CDCC will generate electronic pick lists, in which available

nucleic acid samples for any Subject/SN-type/Visit that have not already been distributed to a sequencing center will be identified for distribution for 454 sequencing.

Distribution among the four sequencing centers is determined as follows:

- Samples from 40% of subjects will be sequenced at Washington University. (Subjects will be selected exclusively from those enrolled at Washington University; for these subjects, all samples from all visits will be sequenced at the same site.)

Samples from the remaining Washington University subjects and samples from all subjects enrolled at Baylor College of Medicine will be distributed according to the plan below; for each subject, all samples from all visits will be sequenced at the same site:

- Samples from 7% of subjects will be sequenced at Baylor College of Medicine. (Subjects will be selected exclusively from those enrolled at Baylor College of Medicine.)
- Samples from 27% of subjects will be sequenced at Broad Institute.
- Samples from 27% of subjects will be sequenced at J. Craig Venter Institute.

Redundancy sequencing will be completed as follows:

- Samples from five subjects sequenced at Washington University will also be sequenced at Craig Venter Institute.
- Samples from one subject sequenced at Baylor College of Medicine will also be sequenced at Broad Institute.
- Samples from three subjects sequenced at Broad Institute will also be sequenced at Washington University.
- Samples from three subjects sequenced at J. Craig Venter Institute will also be sequenced at Baylor College of Medicine.

The CDCC will provide each sequencing center with a web report for acquisition of mapping information between the subjects' Random Subject ID# and all associated nucleic acid sample numbers. These numbers will be propagated within each sequencing center's LIMS system and contained within the sequencing sample trace file, such that a "link" may occur to reconnect sequencing data with subject clinical data for analysis by authorized users.

7.15 Nucleic Acid QC and Sequencing Methodologies

Extracted samples are aliquoted and stored at -70°C or colder in the clinical processing laboratories. Aliquots of the samples are transferred to the sequencing centers for metagenomic sequencing. The clinical labs manage requests to submit/transfer samples from these sites to the genome sequencing centers. The following paragraphs summarize the QC and sequencing procedures employed at each of the four sequencing centers:

Washington University Genome Sequencing Center:

After DNA extraction following the defined protocol, nucleic acid samples are quantified using a NanoDrop™ fiber optic spectrophotometer (Thermo Scientific) and 260 and 280 readings are recorded to estimate purity of the DNA. Samples containing undetected amounts of DNA are many times of utility in microbiome community constituent studies employing analysis of the 16S genes. Analysis that employs a whole genome shotgun (WGS) sequencing approach requires a minimum of 50-100ng.

Two experimental sequencing approaches are employed: sequencing 16S genes and sequencing random fragments of whole genome DNA (WGS). Protocols developed by the Jumpstart Consortium are posted on the DACC: http://www.hmpdacc.org/sops.php#16s_anchor. Otherwise, the most recent manufacturer's recommended protocols are employed for the sequencing platforms used. For 16S studies, the Sanger sequencing on ABI 3730 platform and 454/Roche pyrosequencing platform are used. The WGS data are produced on the Illumina platform as well as the 454/Roche pyrosequencing platform.

Baylor Human Genome Sequencing Center:

After extraction, nucleic acid samples are quantified using NanoDrop™ (Thermo Scientific) and 260/280 readings are recorded to estimate purity of the DNA. A 260/280 reading of 1.8 or greater is considered "pure" for DNA. While this reading is recorded, all specimens are sequenced regardless of the 260/280 readings. Amplification and sequencing methods used are as follows:

Sanger sequencing:

- Amplification of the full-length 16S rRNA gene: DACC PCR Method ID# 318
- 16S rRNA gene cloning: TOPO TA Cloning® Cloning Kit (pCR®4-TOPO® Invitrogen, cat # K4580)
- ABI 3730 sequencing platform

454 sequencing:

- Amplification of variable regions within the 16S rRNA gene: DACC PCR Method ID# 467 (variable regions 1-3) and ID# 468 (variable regions 3-5)
- Roche 454 FLX Titanium pyrosequencing platform

WGS sequencing:

- Roche 454 FLX Titanium pyrosequencing platform and Illumina platform

Broad Institute:

Genome Sequencing Sample Repository (GSSR) performs PicoGreen analysis of samples to evaluate DNA concentration and quality. Aliquots of each sample are created and handed off to the Sanger, 454 or Illumina lab for processing.

Sanger sequencing: Amplification of the full-length 16S rRNA gene is carried out according to the PCR Method Protocol, BI_HMP_Sanger_Default_v2.0. This protocol can be found on the HMP DACC website (http://hmp-dacc.lbl.gov). The amplified products are cloned using the TOPO TA Cloning® Cloning Kit (pCR®4-TOPO® Invitrogen, cat # K4580) and sequencing is performed using the ABI 3730 sequencing platform.

454 Titanium Sequencing: 16S amplification and sequencing is carried out according to HMP established protocols (www.hmpdacc.org). All transfer steps are automated on the Agilent Bravo™ platform and sample identification is tracked through the process using physically barcoded receptacles (tubes and microtiter plates) and sequence tagged amplification primers. Emulsion PCR and sequencing proceed according to manufacturer's protocols with the exception of a qPCR quantification of the multiplexed library for more accurate estimation of library template copies per DNA capture bead in the emulsion mix.

Illumina Sequencing on GAIIx: Library preparation is automated on the Agilent Bravo™ including all transfers, Agencourt AMPure XP bead clean-ups, reagent additions and QPCR setup for enriched library quantification. Gel size selection is excluded in order to maximize yields and molecular diversity of low input samples. Cluster amplification is performed by Illumina cBot Cluster Generation System prior to Flowcell loading on GAIIx.

JCVI:

454 Sequencing, Titanium platform:

Library construction, emPCR, enrichment and 454 sequencing are performed following the vendor's standard protocols with some modifications. Specifically, qPCR is used to estimate the number of molecules needed for emPCR. An automation system (BioMek

FX, Beckman Coulter) is used to "break" the emulsions after emPCR and butanol is used to enable easier sample handling during the breaking process. The Robotic Enrichment Module (REM e, Roche) is used to automate the bead enrichment process in the pipeline.

Illumina Sequencing, GAIIx platform:

Libraries are prepared following Illumina's protocol, with a few exceptions. DNA is sheared using the Covaris™ S2 or E210 System (Applied Biosystems). All clean-up steps are done using Agencourt AMPure XP beads, and the libraries are quantitated and quality-controlled using the Agilent High Sensitivity DNA Kit. Cluster generation and sequencing are done using Illumina's standard protocol.

7.16 Stored Specimens

When subjects sign the consent form to participate in the study, they agree to the storage and future use of all their specimens except serum, which the subject may elect not to store for future use.

If a subject later chooses to withdraw consent for future use of stored specimens, the PI at the site or the sponsor will notify the Coriell Institute for Medical Research and any clinical laboratories housing their specimens or extracted nucleic acids to discard all remaining materials from that subject. These materials will be discarded in the appropriate manner and the disposition will be documented. (See Appendix F.)

8 SECONDARY CONTACT INFORMATION

Check informed consent to see if subjects agree to provide this information.

Site study staff should seek to obtain secondary contact information for subjects enrolled in these studies. Ideally, the contact would be a close friend or family member who is aware of the subject's participation in the trial. If the site is unable to contact the subject after successive attempts, the secondary contact person should be contacted.

9 SOURCE DOCUMENT INSTRUCTIONS

9.1 General Instructions

The CDCC prepares source documents to match the AdvantageEDC eCRF screens. These forms are posted to the Study web site:

https://web.emmes.com/study/dnt/hmp/index.htm

The forms for each study are accessed under the Protocols tab, by protocol number, selecting Study Materials, then Source Documents. Sites should print the current version of the study source document visit forms only as needed to keep up with enrollment.

If a Study Status Change form has been completed, sites should not retain any blank source document visit forms for a subject beyond the effective date of the change form.

Blank visit forms related to an incomplete/partial visit or incomplete evaluations should have the page header completed, lined through, and initialed/dated.

9.1.1 General Guidelines for Completing All Forms

- Print all information legibly using a black or blue pen.
- Fill in all spaces with appropriate answers.
    - Some questions are skipped due to answers to previous questions. These questions are typically indented and begin with a qualifier such as “If Yes, specify…”
    - Other fields will be considered missing data and will be queried via the Missing Value Report in AdvantageEDC. If information is unknown or will never be available, indicate this circumstance on the source document. When entering the data in AdvantageEDC, click on the circle icon next to the field and, when prompted, indicate the reason the data are missing, e.g., ‘not done’ or ‘not recorded’.
- The subject’s Study ID, assigned at the time of screening, must be used on all forms provided by the CDCC.
- DO NOT provide any confidential subject identifying information, such as subject name, phone number, or address on source documents provided by the CDCC. If subject identifiers are written on documentation to be sent to the CDCC or NIH, remove the identifiers PRIOR TO sending the documents to the CDCC or NIH.

- For date fields, use the international convention (ddMMMyyyy). Include leading zeroes for the day (e.g., 07NOV2004). If any component of a date is unknown, provide an estimate. Use the midpoint of the month if the day is unknown; use the midpoint of the year if the month and day are both unknown. For example, if only the month and year are known (e.g., July 2004), record the date as 15JUL2004. If only the year is known (e.g., 2004), record the date as 30JUN2004. Unless a field is provided to note the date is an estimate, include a notation in the comments field indicating that the date is an estimate.
- Note: For text fields, e.g., specifying medical history, dates may be reported in format appropriate to the extent of information known. For example, “Hypertension since ‘03”.
- Time: Use a 24-hour clock (e.g., 6:30 p.m. should be reported as 18:30).
- For numbers, include a leading zero (0) in the source document. However, you do not need to include the leading zero when the data are entered in AdvantageEDC.

9.1.2 Error Correction

- Correction of errors on source documents will be done following GCP guidelines.
- If a mistake is made and a correction or modification to a source document is required, draw a single horizontal line through the error and write the correct data directly above or below the original entry. Original entries must remain legible. Initial and date all modifications. Never use correction fluid, erasures, correction tape, or blacking out to alter entries.

9.1.3 Documentation of Form Completion

- Study personnel entering data on source documents should sign and date them at the time the form is initially completed. Signature lines are included on each source document provided by the CDCC; additional lines may be added to the form if needed.
- Data added after a form has been largely completed, e.g., stop date for a medication, should be initialed and dated in a manner similar to that for corrections.
- Completed Eligibility Checklist, baseline Medical History, SAE forms, and Unanticipated Problems forms must be reviewed and signed by a physician investigator.

- Physician investigators and sub-investigators listed on the Investigator of Record form may sign the source documents when an investigator's signature is required.

- Subsequent update/correction to these forms do not require an investigator's re-review and signature unless there is a clinically significant change that would affect the subject's safety or eligibility to continue participating in the study.

- The Eligibility Checklist and Medical History forms completed for Screening Failures do NOT need to be signed by a Physician Investigator.

9.1.4 Supplemental Visit Numbering

- Supplemental visits that are not scheduled in the protocol are assigned a study number with the trailing alpha, "S" (for supplemental). For example:
  - If a subject has an unscheduled visit (e.g., return for additional sampling) that occurs between Visits 01 and 02, the visit would be numbered 01S.
  - If more than one supplemental visit occurs between two scheduled visits, the alpha designation proceeds from "S" to "Z" (e.g., three visits between the scheduled Visits 01 and 02 would be named 01S, 01T, and 01U, respectively).

If the clinical lab determines that insufficient or inadequate specimen material has been obtained, they may request that the subject return for a supplementary sampling of the required material.

9.2 Form-Specific Instructions

9.2.1 Concomitant Medications

- Complete this form for all medications reported by the subject as taken from 30 days prior to each visit, with the exception of antibiotic/antimicrobials usage which will be collected throughout the entire study period and female contraception, which will be recorded for 12 months prior to each visit.
  - For oral contraceptives, record brand name of oral contraceptive, date initiated (month/year), and whether it is used "continuous dose" (placebo pills not taken, such that menstruation does not occur) or "traditional dose."

- For intrauterine devices, record type of device used (Mirena® versus Paragard® versus other) and date of insertion (month/year).

- For progesterone-based injections and insertion rods, record delivery device (Depo-Provera® versus Implanon™ versus other) and date of insertion or last injection (month/year).

Note: For permanent sterilization methods (tubal ligation and Essure® device), record date of procedure (month/year) on the medical history form under Genital/Reproductive.

- The Medication Name and Medication Start Date fields are required to be completed. Uncommon abbreviations for Medication Name should be avoided if possible. Date should be estimated if unknown.
- Dates entered into AdvantageEDC must be complete to be valid (e.g., "--MAY2003" is not a valid system date). Exact dates are sometimes unknown. If the start date is an estimate, check the box on the source document and check the box on the eCRF labeled "Medication start date estimated". This checkbox is not required and will not be queried if left empty. The actual date format employed should adhere to the date convention described in Section 9.1.1.
- Medications taken on a PRN basis need to be reported only once unless the medication is taken for another reason than already reported.
- It is not required to record the dose and frequency of the medication on the source document; however, it is highly recommended, as that information may be pertinent in evaluating a Serious Adverse Event.
- If a subject terminates early and the medication was ongoing at last point of contact, the field on the eCRF should remain completed as "Ongoing", and a comment should be added stating that the medication was ongoing at last point of contact.
- In AdvantageEDC, the Key Field for the Concomitant Medication form is "Medication Number". Each medication for a subject should be assigned a different number from the drop-down list on the Key Selection screen, so as to uniquely identify the medication. The Medication Number assigned is arbitrary, and will not be used in analysis.

9.2.2 Consent Agreement

- The Consent Agreement screen should be entered in AdvantageEDC once at the time of enrollment for all subjects who are enrolled into the study.
- The "Source Document" corresponding to the Consent Agreement screen is the Informed Consent Form.

- Indicate the date the Informed Consent Form was signed and whether or not the subject consented to the future use of stored specimens. The date entered should be the initial date the subject signed the Informed Consent Form. If the subject was re-consented, this can be noted in the comments.
- The subject's agreement to future use of specimens must be indicated by marking "Yes" on the Consent Agreement eCRF.
- Select the subject's response for use of serum specimens for research unrelated to the Human Microbiome on the Consent Agreement eCRF.
- If a subject changes his/her option for future use of study specimens at any time during the study, update the field on the eCRF to indicate the subject's final wishes for the use of his/her specimens. Additionally, make a note in the comments field that this option was changed by the subject and note the date that it was changed. Any changes to the subject's future use indication should also be updated in GlobalTrace, as appropriate.

9.2.3 Demographics

- Complete all fields as appropriate.
- If a subject refuses to identify his/her race/ethnicity, select "No" or "Unknown" to all categories and note in the comments box that the subject refused or is unable to supply this information.

9.2.4 Eligibility Checklist

- Screening Failures
    - The Eligibility Checklist (Screening) is entered in AdvantageEDC for subjects that fail screening or will otherwise not participate in the study (declined to enroll); it does not require investigator review and signature and should be completed to the extent that data were collected.
- Screening and Provisional Eligibility
    - The Eligibility Checklist (Screening) is completed during the screening visit in conjunction with the Medical History, Targeted Physical and Body Site Specific Screening Examinations. If screening occurs over multiple days, record the date of the first screening visit and ensure that enrollment occurs within 30 days of this date. Subjects that are determined to be provisionally eligible for study enrollment (pending receipt of viral marker results) should have their subsequent baseline sampling visit scheduled at this time. (No information is entered into the data system at screening for

provisionally eligible subjects). Upon staff review of viral marker results, subjects will either proceed with their scheduled baseline sampling visit or will be contacted for site counseling (per site requirements) and subsequent study visit cancelation. (Subjects who are ineligible for study participation due to viral marker results will be entered into the data system as screening failures.)

- Day of Enrollment (First Intervention/Sampling)
    - The Eligibility Verification Checklist (Baseline sampling) is completed and entered in AdvantageEDC in conjunction with the Eligibility Checklist (Screening) for all enrolled subjects on the day of, and prior to, sampling.
    - A physician investigator must sign the Eligibility Verification Checklist that is completed on the day of sampling. The PI may delegate this responsibility to a sub-investigator who is a clinician licensed to make medical diagnoses, i.e., physician, nurse practitioner, physician's assistant, as listed on the site's Investigator of Record form.

Note: Monitoring of viral marker results will be done using the eligibility checklists and the site provided laboratory results source document.

9.2.5 Global Trace Specimen Forms

- For each specimen collection, a GlobalTrace source document will be completed by the clinical laboratory staff, capturing the Volunteer ID, visit number, date of collection, and YES to the question of future use.
- For each specimen that is subsequently processed in the clinical laboratory (e.g. Nucleic acid aliquoting or DNA pooling) the appropriate Global Trace aliquoting or pooling form will be completed.

9.2.6 Medical History

- This document must be completed by study personnel based on information collected during the screening interview with the subject [Visit 00]. The form is completed at screening and reviewed and updated as necessary throughout the study. (Submit in AdvantageEDC for enrolled subjects only).
- Baseline medical history includes all conditions that exist prior to baseline sampling. If conditions are reported by the subject subsequent to the baseline sampling, but existed prior to baseline sampling, update the baseline form/eCRF (visit 00).

- If No is selected for a category header, the subcategories need not be completed. If Yes is selected for a category header, all subcategories MUST be completed and the reason(s) for answering Yes described.
- For females who have undergone permanent sterilization procedures, e.g., tubal ligation and insertion of the Essure® device, record date of procedure (month/year) under Genital/Reproductive.
- Surgical history should be noted in the appropriate body system. If more space is needed, or if the surgery was related to multiple systems, additional information should be entered in the "other significantly relevant medical or surgical history" field.
- A physician investigator must sign the source document prior to study intervention/sampling. The PI may delegate this responsibility to a sub-investigator who is a licensed clinician (i.e., CNP, PA, MD).
- Medical History Return Visit form is completed to document changes in health history.
- **Do not repeat issues noted at baseline unless there is a need to document a change in the status of the system/problem noted.**
- Post screening, this form is only completed and submitted in AdvantageEDC if there is a clinically indicated change (post screening) in Medical History that requires documentation.
- Investigator signature is not required on Medical History Return Visit form unless there is a clinically significant change that would affect the subject's safety or eligibility to continue participating in the trial.

9.2.7 Protocol Deviation

- Complete this document for each deviation that occurs for a subject.
- The Deviation Description and Deviation Date fields are required to be completed. Date should be estimated if unknown.
- If a protocol deviation occurs, it must be reported to NIH, via entry in AdvantageEDC, within 5 days of site awareness and to the site's IRB in accordance with the IRB's policy.
- In AdvantageEDC, the Key Field for the Protocol Deviation form is "Deviation Number". Each deviation for a subject should be assigned a different number from the drop-down list on the Key Selection screen, so

as to uniquely identify the deviation. The Deviation Number assigned is arbitrary, and will not be used in analysis.

- The Deviation Category must be appropriate for the deviation. Refer to the examples provided below:

Circumstance	Deviation Category
Subject did not meet eligibility criteria, but was sampled	Eligibility/enrollment
Specimens collected out-of-window	Specimen collection schedule
• Temperature not taken • Blood not drawn	Protocol procedure/assessment
• Specimen not collected • Insufficient specimens collected	Specimen Collection
Missed or out-of-window visit	Follow-up visit schedule

- Out-of-window visits are reported as protocol deviations. The Deviation Description must include the visit number of the visit that was missed (e.g., Missed Visit 02 or Visit 03 out of window). For out-of-window visits, the Deviation Date is the date the visit actually occurred.
- Non-Subject-Specific Deviations: The non-subject-specific version of the Protocol Deviation form is intended to capture any departures from protocol-specified procedures that are not limited to a specific subject(s).
- Non-subject-specific deviations do not apply to events or data that bear importance to a specific subject's participation in the study, as these data should be included within the subject's case report form via completion of a subject-specific Protocol Deviation form.

9.2.8 Screening and Enrollment Log

- This document is supplied to each site by EMMES and includes the valid Subject IDs to be assigned to subjects.

- As this document is kept with study documents and not in individual subjects' clinical files, it is acceptable to record the subjects' initials on this log.
- Signatures are not required; however, study personnel should initial entries.
- This form will NOT be transmitted to EMMES or NIH after completion.

9.2.9 Serious Adverse Event

- Please see Appendix I for the SAE completion instructions for the CDCC SAE form.
- Events that qualify as Serious Adverse Events, whether solicited or not, must be reported as Serious Adverse Events (refer to the protocol for the definition of a Serious Adverse Event) within 24 hours of site awareness for deaths or life-threatening events, and within 72 hours for all other serious adverse events.
- A physician investigator must sign this source document. The PI may delegate this responsibility to a sub-investigator who is a licensed physician.
- Submit to the IRB, ISM, and NIH (submitted to NIH via CDCC).
- Selected information pertaining to the SAE should also be entered in the Serious Adverse Event eCRF in AdvantageEDC (i.e., reason why the event is an SAE, date of SAE onset, severity, relationship to study intervention, outcome, etc.).

9.2.10 Screening Examination Forms

Screening Examination forms other than the Oral Screening Examination information, completed during the screening visit [Visit 00], will **not** be entered into AdvantageEDC, but will be retained in the subject's study binder. The Oral Screening Examination information [Visit 00] will be entered into AdvantageEDC for enrolled subjects only. If Maxilla and Mandible forms are completed for an enrolled subject, the information from them will be entered into AdvantageEDC.

9.2.11 Study Status Change Form

- This form is visit-based and should be submitted at the last visit (Visit 02 or Visit 03 for subjects who complete the protocol). If a subject completed Visit 02 before the third sampling option was added to the protocol, the

Study Status Change Form submitted after Visit 02 may be deleted, and a new form submitted after Visit 03. If the subject completes Visit 02 and is not interested in providing a third set of specimens, the termination date is the visit date for Visit 02. If a subject completes Visit 02 and is interested in providing a third set of specimens, the study personnel should wait to submit the form until after Visit 03, or after the study closes. At the completion of the study, forms for termination at Visit 02 will be submitted for any subjects who did not provide a third set of samples, and for whom a form was not previously submitted.

- If a subject terminates his/her participation early (i.e., withdraws from the study), the Study Status Change form should be submitted under the visit number of that visit. The termination date is the date of that visit (the last visit to occur). Once submitted in AdvantageEDC, all forms for future visits are automatically un-required.
- If a subject is lost to follow-up, the Study Status Change form is submitted under the visit number that was the first visit missed (i.e., 02). The termination date is the target date of the missed visit, as displayed on the Forms Grid in AdvantageEDC. Once submitted in AdvantageEDC, all forms for future visits are automatically un-required.
- This form must be completed and signed by study personnel within 3 days of the subject completing the study or terminating his/her participation early.

9.2.12 Targeted Physical Exam

- This form is completed at screening [Visit 00] and may be updated as clinically indicated during the sampling visits. If no updates are indicated the form is not submitted in AdvantageEDC.
- If an assessment is repeated (e.g., blood pressure taken again after a nervous subject rests for a period of time), the second measurement should be recorded next to the first and initialed and dated. The value considered to be the accurate assessment should be entered in AdvantageEDC.
- There are three options for assessing each area/system subsequent to sampling: *Normal; Abnormal*; and *Not evaluated*. An assessment of “Abnormal” will require specification to determine clinical significance. An assessment of “Not evaluated” will require justification.

9.2.13 Unanticipated Problem

This form is to be completed upon study staff identification or subject self-reporting of an unanticipated problem. Please refer questions to Wendy Fanaroff, the NIDCR Safety Coordinator:

Wendy Fanaroff RN, MSN
Nurse Consultant
Office of Clinical Trials Operations & Management,
National Institute of Dental and Craniofacial Research (NIDCR), NIH

9.2.14 Visit Documentation

- The visit documentation source documents are tailored to the needs of each visit to record study procedures and events that occurred at that visit. Every visit should be documented, whether clinic or phone contact, scheduled or supplemental. The comments sections of the visit documentation forms may be used to record notes about the sampling procedure or subject characteristics that are not exclusionary but that could affect the sampling process or the study results (e.g., presence of piercings, history of respiratory infection within the last 30 days).
- If a visit is missed, the subject's Visit Documentation for that visit MUST still be completed, with the question "Did the visit occur?" marked "No" and the reason for the missed visit selected. The form is submitted as such in AdvantageEDC. This will remove the requirement for any other visit-based forms scheduled for that visit.
- If Yes is answered to the question regarding a Protocol Deviation, complete the Protocol Deviation form.
- If Yes is answered to the question regarding new or changes to Concomitant Medications, complete a Concomitant Medication form.
- If, at Visits subsequent to screening, the subject discloses a medical condition or medication use that existed at baseline that was not previously reported, this condition/medication does NOT constitute a new event/medication at the subsequent visit, and the question should be answered No on the source document, and in the database.
- For a medical condition that existed at baseline, update the Medical History form completed at Visit 00 with the newly reported information.

- For a medication that was being taken at baseline, submit a Concomitant Medication form. Answer No to the medication question at the current visit (at which the medication was belatedly reported) unless there is another new medication to be reported.
- If a newly disclosed baseline condition or medication would have rendered the subject ineligible, a Protocol Deviation and Subject Status Change form should be submitted.
- If, at visits subsequent to Visit 01, the subject reports a new medication, the start date of which falls prior to the previous visit (but after baseline), make a notation in the comments box of the Visit Documentation form for the current visit that the subject disclosed the medication use at this visit. The previous visit's Visit Documentation data collection form and the database should be updated accordingly by checking Yes to the Medication question at the previous visit. Answer No to the Medication question at the current visit (unless another medication use is reported that would warrant answering the question(s) Yes).

APPENDICES

APPENDIX A:
List of Abbreviations/Acronyms

ACD	Acid Citrate Dextrose anticoagulant solution
ACF_L	Antecubital fossa – Left: Body site-specific label acronym
ACF_R	Antecubital fossa – Right: Body site-specific label acronym
AE	Adverse Event
BOP	Bleeding on probing
BUCC	Buccal mucosa: Body site-specific label acronym
CAL	Clinical Attachment Level
CDCC	Clinical Data Coordinating Center
CEJ	Cementoenamel junction
CFR	Code of Federal Regulations
COR-1	Coriell 1 yellow top blood tube: Body site-specific label acronym
COR-2	Coriell 2 yellow top blood tube: Body site-specific label acronym
CSOC	Clinical Study Oversight Committee
DHHS	Department of Health and Human Services
EDTA	Ethylene diamine tetraacetic acid
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
GING	Keratinized attached gingivae in anterior maxilla: Body site-specific label acronym
GM	Gingival margin
HMP	Human Microbiome Project
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPAL	Hard Palate: Body site-specific label acronym
ICH	International Conference on Harmonisation
IDES	Internet data entry system
INTRO	Vaginal introitus: Body site-specific label acronym
IRB	Institutional Review Board
JAMA	Journal of the American Medical Association
LIMS	Laboratory Information Management System
LMP	Last menstrual period
MIDVAG	Mid-vagina: Body site-specific label acronym
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
N	Number (typically refers to subjects)
NASAL	Anterior nares (right and left): Body site-specific label acronym
NEJM	New England Journal of Medicine
NHGRI	National Human Genome Research Institute
NIDCR	National Institute of Dental and Craniofacial Research

NIH	National Institutes of Health
OCTOM	Office of Clinical Trials Operations and Management
OHRP	Office for Human Research Protections
PI	Principal Investigator
PD	Probing depth
PFORN	Vaginal posterior fornix: Body site-specific label acronym
PTON	Palatine tonsil: Body site-specific label acronym
RAC_L	Retroauricular crease – Left: Body site-specific label acronym
RAC_R	Retroauricular crease – Right: Body site-specific label acronym
SCF	Specimen collection fluid
SAL	Saliva: Body site-specific label acronym
SERUM	Blood specimen for serum: Body site-specific label acronym
SOP	Standard Operating Procedure
SUB	Subgingival plaque: Body site-specific label acronym
SUPRA	Supragingival plaque: Body site-specific label acronym
STOOL	Stool: Body site-specific label acronym
THRO	Throat: Body site-specific label acronym
TONG	Tongue Dorsum: Body site-specific label acronym

APPENDIX B:
Key Roles Contact Information

NIH Representative and Project Team Leader from NIDCR:

Pamela McInnes, DDS, MSc(Dent.)
Director, Division of Extramural Research
National Institute of Dental and Craniofacial Research (NIDCR), NIH

Director, Office of Clinical Trials Operations and Management from NIDCR:

Michelle A. Culp, BSN, MPH
Director, Office of Clinical Trials Operations and Management
National Institute of Dental and Craniofacial Research (NIDCR), NIH

Project Team Leader from NHGRI:

Jane Peterson, PhD
Associate Director, Division of Extramural Research
National Human Genome Research Institute (NHGRI), NIH

Program Director, Ethical, Legal, and Social Implications Program from NHGRI:

Jean E. McEwen, JD, PhD
Program Director, Ethical, Legal, and Social Implications Program
National Human Genome Research Institute (NHGRI), NIH

Medical Monitor:

Holli Hamilton, MD
Senior Medical Officer
Division of Extramural Research
National Institute of Dental and Craniofacial Research (NIDCR), NIH

Safety Coordinator from NIDCR:

Wendy Fanaroff RN, MSN
Nurse Consultant
Office of Clinical trials Operations & Management
National Institute of Dental and Craniofacial Research (NIDCR), NIH

Coordinating Investigator and Study Chair:

James Versalovic, MD, PhD
Professor of Pathology
Baylor College of Medicine

Clinical Study Oversight Committee Chair:

Martin Rosenberg, PhD

Baylor College of Medicine Principal Investigator and Co-Investigators:

Wendy Keitel, MD, Principal Investigator
Professor
Department of Molecular Virology and Microbiology
Baylor College of Medicine

Kjersti Marie Aagaard-Tillery, MD, PhD
Baylor College of Medicine
Department of Obstetrics and Gynecology

James A. Katancik, DDS, PhD
Chairman, Department of Periodontics
The University of Texas Dental Branch at Houston

Baylor College of Medicine Human Microbiome Clinical Laboratory (Laboratory for DNA Extraction):

Joseph Petrosino, PhD
Baylor College of Medicine

Washington University Principal Investigator and Co-Investigators:

Mark Watson, MD, PhD, Principal Investigator
Washington University School of Medicine

M. Nathalia Garcia, DDS
Assistant Clinical Professor
Saint Louis University

Tessa Madden, MD, MPH
Department of Obstetrics and Gynecology
Washington University in St. Louis School of Medicine

D. Douglas Miley, DMD, MSD
Director, Graduate Program in Periodontics
Saint Louis University Center for Advanced Dental Education

Arlyn June Pittler, MSN, BS
Research Instructor in Medicine
Department of Internal Medicine
Washington University in St. Louis School of Medicine

Washington University Co-Investigator (Laboratory for DNA Extraction):

Michael Dunne, PhD
Washington University School of Medicine

Clinical Data Coordinating Center (CDCC):

The EMMES Corporation
401 N. Washington St., Suite 700
Rockville, MD 20850

Media Inquiries:

Geoff Spencer
National Human Genome Research Institute, NIH

J. Craig Venter Institute (Sequencing Center):

J. Craig Venter Institute

Broad Institute (Sequencing Center):

Broad Institute of MIT and Harvard

Human Genome Sequencing Center (Sequencing Center):

Baylor College of Medicine

Genome Sequencing Center (Sequencing Center):

Washington University School of Medicine

Coriell Institute for Medical Research (Specimen Repository):

NHGRI Sample Repository for Human Genetic Research

APPENDIX C:
NIH Human Microbiome Project Subject Information Sheet

Date of scheduled Baseline Sampling Visit: _____

During the indicated time periods before sample collection, you must NOT use the listed medications and cleansing products and you must AVOID the listed activities.

Six months before sample collection visit:

- Any antibiotic, antifungal, antiviral or antiparasitic drugs; by mouth, by injection or intravenous
- Any steroids; oral, intravenous, intramuscular, nasal or inhaled (such as prednisone, Flonase, dexamethasone, Flovent)
- Cytokines or drugs that can stimulate your immune system (such as Interleukin)
- Methotrexate or other agents that suppress your immune system (such as chemotherapy)
- Commercial probiotics in doses greater than or equal to 10^8 colony-forming units (cfu) or organisms per day, including tablets, capsules, lozenges, chewing gum or powders in which probiotic is a primary component. Note that it is acceptable to consume foods such as yogurt and fermented beverages/milks.
- for female subjects, combination hormone vaginal ring for contraception

Four weeks (28 days) before sample collection visit:

- Intranasal influenza vaccine (Note that flu shots are not included in this time restriction.)

Seven (7) days before sample collection visit:

Date to stop using products: _____

- Antibiotics or steroids applied as creams or ointments on the skin of the face, scalp, neck, arms, forearms or hands
- Vaginal or vulvar medications, including antifungals. Permitted vaginal contraceptives may be used until 48 hours before sample collection visit.

48 hours before sample collection visit:

Date and time to stop activities and use of products: _____

- Antimicrobial products including liquid hand soap, bar soap, face washes, hand or mouth washes, toothpaste (such as Softsoap, Dial, Zest, and Clearasil)
- Antiseptic products such as hand or mouth washes, toothpaste, perfumes and sanitizers (such as Listerine mouth wash and Purell hand wash)
- Hair dyes of any kind
- Use of a chlorinated pool or hot tub
- Vaginal, oral or anal sexual activity – This activity could introduce microorganisms to the mouth, the vagina or the lower gastrointestinal tract and could adversely affect the sampling from these sites.
- For women, douching, and use of contraceptive spermicides, diaphragms, cervical caps, contraceptive sponges, suppositories, feminine sprays, and genital wipes
- For women, menstrual blood flow should have stopped at least 48 hours before sampling.

12 hours before scheduled sample collection visit:

Date and time to stop activity:_____

- Showering, bathing, tooth brushing and flossing. Note that hand washing with soap provided in the personal care kit is allowable.

APPENDIX D:
Acceptable Personal Care Products for the 48 Hours Prior to Sampling

Subjects enrolled in the Human Microbiome Project Protocol will be provided with a kit containing personal care products that are acceptable for use during the 48 hours prior to sampling. If you wish to use alternative products, please consult the following list of basic personal care products that do not contain antibacterial agents.

Soap	Camay Bar Soap (Procter & Gamble [P&G]) 100% Castile Soap (various specialty soap manufacturers) Ivory Classic Bar Soap (P&G) Olay Bar Soap (P&G) Tom's of Maine Natural Clear Body Bar Tone Bar Soap with Cocoa Butter (Henkel Corp)
Skin moisturizer	Aquaphor Healing Ointment (Eucerin) Johnson's Baby Oil (Johnson & Johnson [J&J]) Tone Hand and Body Lotion with Cocoa Butter (Henkel Corp) White Petrolatum (Vaseline)
Facial Cleanser	Aveeno Moisturizing Bar for Dry Skin (J&J) Neutrogena Original Formula Facial Cleansing Bar (J&J subsidiary) Oil of Olay Age Defying Series Daily Renewal Cleanser with Gentle Microbeads (P&G)
Shaving Cream	Old Spice Shave Cream (P&G) Gillette Series Gel – Ultra Comfort (P&G) Tom's of Maine Natural Conditioning Shave Cream
After-shave	Nivea for Men After Shave Extra Soothing Balm, Sensitive Skin (Nivea USA) Skintimate Moisturizing After Shave Gel (SCJohnson)
Shampoo	Pantene Pro-V Purity Clarifying Shampoo or Pantene Basic Care Classically Clean Shampoo (P&G) Pert Plus Deep Moisturizing Shampoo Plus Conditioner for Normal Hair (P&G) Walgreen's Pro-Vitamin Shampoo or Walgreen's Shampoo plus Conditioner for All Hair Types
Conditioner	Pantene Pro-V Sheer Volume Conditioner or Pantene Pro-V Daily Moisture Renewal Conditioner (P&G)
Toothpaste	Aquafresh Cavity Protection Toothpaste or Extra Fresh Toothpaste (GlaxoSmithKline) Crest Toothpaste varieties that do not contain Scope (P&G) Tom's of Maine Natural Anticavity Fluoride or Whole Care Toothpaste
Mouthwash	Homemade rinse of ½ tsp salt or ½ tsp baking soda in 8 ounces of water

APPENDIX E:
Personal Care Product Ingredients to Avoid During the 48 Hours Prior to Sampling

During the 48 hours prior to sampling (the "washout" period), subjects should avoid the use of personal products that are labeled "antibacterial", or that contain the following ingredients which could interfere with the study results:

Ingredient Group	**Ingredient**
Anilides	triclocarban
Biguanides	chlorhexidine alexidine polymeric biguanides
Bisphenols	triclosan
Halophenols	PCMX (p-chloro-m-xylenol)
phenols and cresols	phenol cresol thymol
quaternary ammonium compounds	cetrimide benzalkonium chloride cetylpyridinium chloride

APPENDIX F:
Disposition of Study Specimens

Disposition of Screening Blood Specimen

Specimen Type	**Blood** 10 mL (2 tsp) at Screening Visit			
Purpose	**Screening for HIV, HBV and HCV to determine study eligibility**			
Disposition Issue	**Original Specimen**	**Cell Line**	**DNA (genomic)**	**Data from specimens DNA sequence information (genomic)**
Storage – short term	Specimen will be stored in clinical lab and will be destroyed after screening tests are completed.	N/A	N/A	N/A
Banking	N/A	N/A	N/A	N/A
Who has access?	Study personnel and lab personnel will collect and test specimen; subject will receive test results, but they will not be entered in subject's medical record.	N/A	N/A	N/A
Revoked authorization?	Specimen will be destroyed.	N/A	N/A	N/A

Disposition of Blood Specimens, Cell Lines, Human DNA, Human Sequence Data

Specimen Type	**Blood** 30 mL (6 tsp) at Visit 1 Baseline Sampling			
Purpose	**Genome sequencing and development of cell lines** **Storage of serum for future use in human microbiome-related studies (or in unrelated studies, with subject's consent)**			
Disposition Issue	**Original Specimen**	**Cell Line**	**DNA (genomic)**	**Data from specimens DNA sequence information (genomic)**
Storage – short term	Study personnel will code whole blood specimens and ship them to Coriell Institute for temporary storage, DNA extraction, and cell line development. Serum will be coded and stored at sites.	N/A	N/A	N/A
Banking	Coded whole blood specimens will be stored at Coriell Institute until they are processed and lymphocytes are cryo-preserved. Serum will be stored at sites for possible future research.	Coded lymphoblastoid cell lines may be developed and banked indefinitely at Coriell Institute repository.	Extracted DNA will be stored indefinitely as coded samples at Coriell Institute repository.	Genetic sequences will be stored indefinitely.
Who has access?	Serum specimens will be stored in controlled access repositories for future research; specimens will not be available to the subject or the subject's physicians for other testing.	Any cell lines created will be stored in Coriell controlled access repository, and may be distributed to researchers after Coriell, in consultation with NIH (NHGRI), approves research intent; cell lines will not be available to the subject or the subject's physicians.	Coded DNA samples will be stored in a controlled access repository, and may be distributed to researchers after Coriell, in consultation with NIH (NHGRI), approves research intent; DNA samples will not be available to the subject or the subject's physicians for other testing.	Human sequence data will be available to qualified researchers only through a controlled-access internet database; neither the subject nor the subject's physicians will receive sequence information.
Revoked authorization?	Remaining coded blood and serum specimens will be destroyed.	Coded cell lines in repository will be destroyed; attempt will be made to retrieve and destroy any cell lines already distributed to researchers.	Coded DNA samples remaining in repository will be destroyed; attempt will be made to retrieve and destroy any DNA samples already distributed to researchers.	Sequence data will be deleted from future database versions; however, data previously released on the internet database cannot be retracted.

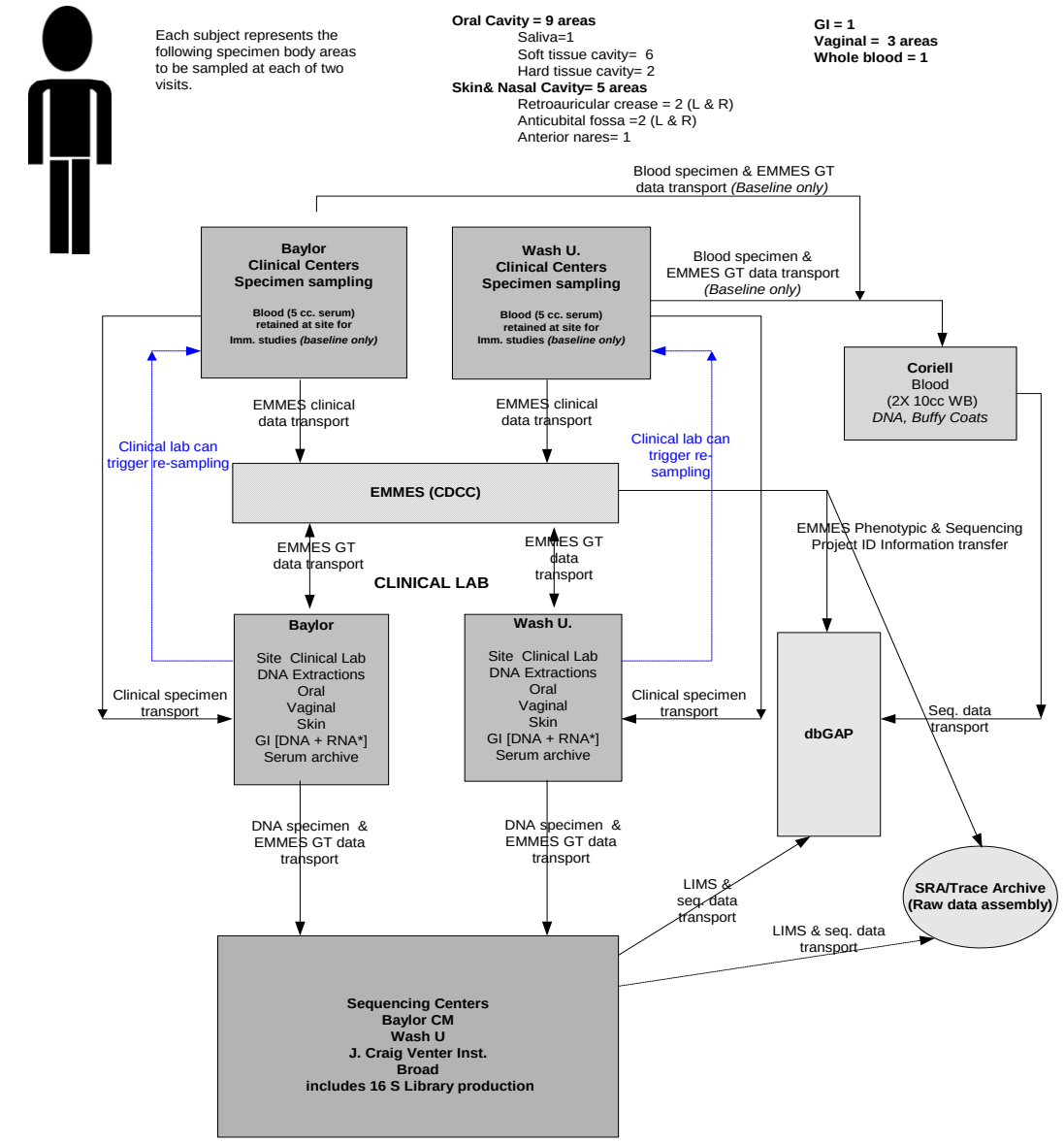
Disposition of Body Site Specimens, Microbial DNA*, Microbial Sequence Data

Specimen Type	**Oral cavity swabs** **Skin specimens** **Nasal swabs** **Stool specimens** **Vaginal swabs**
Purpose	**Metagenome sequencing**

Disposition Issue	**Original Specimen**	**DNA (metagenomic)**	**Data from specimens** **DNA sequence information (metagenomic)**
Storage – short term	Specimens will be stored and processed for DNA extraction at the site clinical lab/tissue procurement bank.	N/A	N/A
Banking	N/A (Any specimen remaining after nucleic acid extraction and sequencing will be destroyed.)	Coded samples of DNA for metagenome sequencing will be stored indefinitely in controlled access repositories at the clinical sites.	Genetic sequences will be stored indefinitely.
Who has access?	Clinical lab/tissue procurement bank personnel will have access to specimen for processing.	Coded DNA samples will be stored in a controlled access repository; they will be provided to sequencing centers for metagenome sequencing and may be distributed to other researchers after the clinical center (Baylor College of Medicine or Washington University) approves research intent; DNA samples will not be available to the subject or the subject's physicians.	Microbial sequence data will be available through an open-access internet database.
Revoked authorization?	If consent is withdrawn prior to DNA extraction, specimen will be destroyed. No specimen will remain after nucleic acid extraction and sequencing.	Coded DNA samples remaining in repository will be destroyed; attempt will be made to retrieve and destroy any DNA samples already distributed to researchers.	Sequence data will be deleted from future database versions; however, data previously released on the internet database cannot be retracted.

***and possibly microbial RNA, depending on specimen availability**

APPENDIX G:
Data Flow Diagram



*RNA to be retained at Baylor & Wash U sites only

APPENDIX H:
Blood Collection, Packaging and Shipment to Coriell: Domestic Sites

When using the SafTPak (STP)-710 (two-bag) secondary pressure vessel system:

1. Subject consent forms must be signed before blood draw begins. A blank copy of the informed consent form must be sent with the sample.

2. Remove the contents from the shipping container with the inner Styrofoam liner and remove the Vacutainers from the bubble pouch. The enclosed yellow top Vacutainer tubes containing ACD Solution A must be used. DO NOT REMOVE THE ABSORBENT MATERIAL FROM THE PACKAGING.

3. Draw two 10-ml tubes of blood from each subject. **KEEP BLOOD AT ROOM TEMPERATURE AT ALL TIMES. DO NOT REFRIGERATE.**

4. Assign a subject identification number to each tube immediately after collection of sample. Write the date the sample was collected on the label of the tube.

5. Using flexible adhesive tape, which may be enclosed or was supplied earlier, wrap the top of each tube and place the tubes into the bubble pouch.

6. Place the bubble pouch into the inner clear plastic bag of the two-bag STP-710 packaging, peel the liner off of the bag and seal according to the instructions printed on the bag. Place this sealed plastic bag into the outer Tyvek bag and seal.

7. Place the sealed STP-710 system and the completed submission forms into the shipping container with the inner Styrofoam liner.

8. ANY 'EMPTY CONTAINER' LABELS FOUND ON THE CONTAINERS MUST BE REMOVED BEFORE SHIPPING SPECIMENS TO THE CORIELL INSTITUTE!

9. Place the Styrofoam lid onto the inner container and seal the top of the outer cardboard container with sealing tape (please do not use the flexible adhesive tape).

10. Attach the following enclosed warning labels to the outside of the container:
    - **DO NOT DELAY BIOLOGICALS**
    - **AVOID HEAT AND COLD**
    - **FRAGILE HANDLE WITH CARE**
    - **UN3373**

11. **DO NOT PLACE DRY ICE OR REFRIGERANT PACKS IN THE CONTAINER; SHIP THE SPECIMENS AT AMBIENT (ROOM) TEMPERATURE!**

12. Attach the airbill pouch to the top of the shipping container and insert the completed air waybill (do not close the airbill pouch by sealing it). PLEASE MAKE SURE THAT THE ADDRESS LABEL USED BY CORIELL TO SHIP TO YOUR LAB IS COMPLETELY COVERED BY THE NEW AIRBILL POUCH AND MAKE SURE THAT NONE OF THE WARNING LABELS ARE COVERED.

13. Contact the carrier (☐ Federal Express, ☐ DHL, ☐ Airborne, or ☐ Other _____) for pickup of the package and ship Priority 1 overnight delivery to the Repository. Contact Barbara Frederick or Brooke Aynbinder by e-mail (bfrederi@coriell.org or bnewman@coriell.org) or by fax [(856)-757-9737] and provide the Carrier's Airbill Package Tracking Number.

14. Provide the date of shipment and number of subjects and tubes enclosed.

For Shipping Records:

The carrier's (☐ Federal Express, ☐ DHL, ☐ Airborne, or ☐ Other _____) Airbill Package Tracking Number was called in to _____on

_____ _____
Date Tracking Number

The shipment was sent on _____ by _____
Date Name

APPENDIX I:
SAE Reporting Instructions

All serious adverse events (SAEs) must be **reported within** one business day of site awareness or as specified by the protocol to the CDCC/ EMMES Corp. by telephone (301-251-1161 x 2810) or facsimile (301-251-1355) of the completed SAE report form.

GENERAL INSTRUCTIONS

1. Elective procedures requiring hospitalization will not be considered SAEs if they were pre-planned prior to signing consent; however, other events may occur during this hospitalization that may be considered serious or non-serious adverse events and will need to be captured according to the protocol.

2. Terms such as **death, hospitalization or a procedural name are not acceptable event terms.**

   - Example: Subject was hospitalized for cholecystectomy due to cholecystitis. The event term would be “Cholecystitis” (not the procedure).
   - Example: “Death” is an outcome not an event term. The investigator should report the primary cause of death as the SAE term.
     - Please submit a copy of the Death Summary and Autopsy Report (if applicable).

3. Protect your subject’s identity**:**

   - Obliterate the subject’s name in the header as well as in the body of the text on any supporting documents. Replace it with the subject’s number **(no initials)** prior to faxing/mailing any data.
   - The individual who obliterates the subject’s identity must date and initial the obliteration.
   - Obliterate the medical record number, account number, social security number, date of birth and any parent or spouse information such as names and address.

4. Review all source documents (examples - hospital discharge summary, hospital notes) as additional SAEs may be detected. If there are any questions as to whether you think an additional SAE may be present, please call EMMES to discuss.

FOLLOW-UP SAE FORM COMPLETION INSTRUCTIONS:

- Before any changes are made on the original SAE form faxed to EMMES, make a copy of the SAE forms.
- On the copy, make any changes, additions or updates. All changes should be neat and legible.
- Initial and date all changes only.
- At the top of the first page indicate the follow-up number the form represents and fax in all three pages even if changes were only made to one or two of the pages.
- Fax to EMMES 301-251-1355
- Retain all fax confirmations with the items faxed.
- For subsequent changes or additional information to the SAE form, always take the last SAE follow-up form sent to EMMES, make a copy and then make all changes on the COPY. Fax this copy and again retain fax confirmation for site records. Repeat the process for all subsequent follow-ups.

DOWNGRADING/DELETION OF A PREVIOUSLY REPORTED SAE:

If the PI determines a previously reported SAE doesn't meet serious criteria, follow the steps below to delete/downgrade the SAE:

- Complete a Follow up SAE report as above.
- Document in the "Event Summary" section a brief explanation of why the PI determined the event to not be serious (i.e., "it was determined that the subject was not hospitalized; therefore, this event does not meet serious criteria" or "the event was determined to be past medical history.")

Event Summary

Complete the event summary to include at a minimum the information in the format below. Add additional pages if needed. A typed summary is acceptable and should be signed, dated and submitted with the SAE forms.

Example**:**

This [age at time of enrollment or at time of event] [week/month/year] old [race and gender] with [indication if appropriate] began trial therapy [details of drug administration, dose, etc. as appropriate] on [date].

On [date] the subject experienced [event as reported]. Include setting of the event, details of severity, duration, treatment, relevant laboratory, radiology or other diagnostic results.

Relate event to appropriate medical history [previous relevant diagnoses].

The investigator considered the event [was, was not] associated to the study intervention because [consistent with animal findings, concomitant med, concurrent disorder, other.] Pending information [follow-up diagnostic testing, discharge date, autopsy findings, etc].

Concomitant Medications

Provide all relevant medications the subject was taking up to 7 days prior to the onset date of the SAE.

- Include the total daily dose and the start and end dates as well as the indication.
- If there are no medications, then mark "None."

Signatures

- **"**Person completing this form": provide the printed name and signature of the individual completing the SAE form and date of form completion.
- "Investigator's Name" – provide the investigator's printed name, signature and date for each report.
    - Do not hold the submission of an SAE if the PI is not available to sign the initial report. The study coordinator and sub-investigator should sign the SAE report if the PI is not available.
    - Any changes must be documented on a SAE form as a follow-up. Indicate follow-up number in sequential order of submission.
- It is MANDATORY that the Principal Investigator (PI) or sub-investigator on the Investigator of Record Form, sign the SAE report if the event is downgraded to an adverse event or deleted as an adverse event.
- Provide dates the initial and follow-up SAE forms were submitted, mailed or faxed to the required areas.

APPENDIX J:
Contents of Oral Examination Kit

It is recommended that at least 3 full kits are available containing the following instruments:

1. Hu Friedy Michigan-O probe with Williams Markings
2. Hu-Friedy College Dressing Pliers
3. Hu-Friedy Micro Mini Five Gracey Curettes (blades are 20% smaller for comfort and ease of insertion and reduce tissue distention)
4. Hu-Friedy single-ended sickle shaped explorer (#54).
5. Hu-Friedy cone socket mouth mirrors # 5- double-sided.

The above instrument kit will be kept in a cassette that is cleaned and autoclaved after each use.

APPENDIX K:
Specimen Sample Chart

Body Site	Specimen	Acronym	Tube type	Solution in tube	Device for obtaining sample	Other materials
Oral Cavity	Saliva	SAL	Falcon 50 mL	None	N/A	Long-handled mouth mirror, dental explorer, surgical scissors, cotton pliers, 2 x 2 gauze pads, cotton rolls, cotton pledgets, wooden tongue depressor, stopwatch, Ziploc bags
	Tongue dorsum	TONG	MoBio 2mL	750 µL MoBio buffer	Catch-All Sample Collection Swabs (7)	
	Hard palate	HPAL	MoBio 2mL			
	Buccal mucosa - right and left	BUCC	MoBio 2mL			
	Attached Gingivae	GING	MoBio 2mL			
	Palatine tonsils	PTON	MoBio 2mL			
	Throat	THRO	MoBio 2mL			
	Supragingival plaque - pooled	SUPRA	MoBio 2mL	750 µL MoBio buffer	Gracey curettes (2)	
	Subgingival plaque - pooled	SUB	MoBio 2mL			
Skin	Retroauricular crease - Right	RAC_R	MoBio 2mL	750 µL MoBio buffer	Catch-All Sample Collection Swabs (4), moistened with SCF-1 solution	Ziploc bags
	Retroauricular crease - Left	RAC_L	MoBio 2mL			
	Antecubital fossa - Right	ACF_R	MoBio 2mL			
	Antecubital fossa – Left	ACF_L	MoBio 2mL			
Nasal Cavity	Anterior nares - right and left	NASAL	MoBio 2mL	750 µL MoBio buffer	Catch-All Sample Collection Swab	Ziploc bags
GI Tract	Stool	STOOL	Plastic tub container	N/A	N/A	spare stool collection container, Ziploc bags. Thermosafe shipping container, 8-10 polar packs for transport, roll of packing tape
Vagina	Introitus	INTRO	MoBio 2mL	750 µL MoBio buffer	Catch-All Sample Collection Swabs (3)	Pederson and Graves speculums, stopwatch, Ziploc bags, Oakton pH Spear
	Posterior fornix	PFORN	MoBio 2mL			
	Midpoint	MIDVAG	MoBio 2mL			
Blood	For DNA isolation and lymphoblastoid cell line generation - 2 x 10 mL blood	COR-1	Yellow-top	ACD	phlebotomy equipment	2 x 2 swabs; band aids; Ziploc bags; rack(?)
		COR-2	Yellow-top			
	Serum - 10 mL blood	SERUM	Site specific Baylor- SST Wash U- EDTA			

Tube/Container Summary per subject enrolled:

16 MoBio tubes (2 mL)

1 Falcon tube (50 mL)

Stool collection kit with extra stool container (tub)

1 Coriell blood kit containing 2 yellow-top tubes

1 red top tube for serum blood sample

Solutions:

MoBio buffer
Sterile SCF-1 solution (Tris-EDTA, 0.5% Tween-20) to wet the Catch-All™ swab for skin specimen collection

Collection devices:

Catch-All™ Sample Collection Swabs [Epicentre Biotechnologies, Madison, WI].

Storage of samples in clinic:

Wet ice for all except for blood - not to exceed 2 hours storage (4 hours for oral specimens)

Blood samples to be kept at room temperature until delivered to lab for processing and/or shipment

Collection time points:

Blood samples collected at Baseline Sampling Visit (Visit 1) only

All other samples collected at Baseline Visit (Visit 1) and at Re-sampling Visits (Visits 2 and 3)

APPENDIX L:
Disinfection of the Oakton pH Spear

Following completion of pH determination of patient:

1. Rinse the tip and shaft of the instrument with running water.

2. Remove excess moisture from the instrument using a Kimwipe®. Gently place Kimwipe® to tip and allow for capillary action to remove excess moisture. This will prevent dilution of the Cidex® solution.

3. Once the instrument has been rinsed, it can be placed in the Cidex® solution. Before using the solution, read the directions for use and hazards located on the bottle label.

4. Immerse the tip and useable area of the instrument's shaft into the Cidex® solution. ****Take care when placing the instrument in the solution to prevent damaging the tip.**

5. Soak the instrument for 30 minutes, which is the minimal time required for disinfection.

6. Remove the instrument from the Cidex® solution and rinse thoroughly with sterile water or tap water.

7. Dry the instrument using a Kimwipe®. Gently place Kimwipe® to tip and allow for capillary action to remove excess moisture.

8. The disinfected instrument may be used immediately or stored in a manner to minimize recontamination.

APPENDIX M:
Topics to Discuss When Pre-Screening Female Subjects

Vaginal pH measurements may be affected by menstrual cycles, by sexual activity, and by the use of various products. In order to minimize the number of female subjects who fail screening due to vaginal pH at the posterior fornix >4.5, the following issues should be discussed with each potential subject when scheduling the screening visit:

- The screening visit must take place at least 48 hours after menstrual flow ends.
- During the 48 hours before screening, the subject must avoid:
    - Vaginal sexual activity
    - Douching
    - Use of spermicides, diaphragms, cervical caps, contraceptive sponges, suppositories, feminine sprays, genital wipes

APPENDIX N:
Example of Clinical Labels (Subject Set)

The original example labels were modified to include Visit 03, for the subset of subjects from whom third specimens will be collected.

01DBA Visit: 01 DT: **COR-1**	01DBA Visit: 01 DT: **COR-2**	01DBA Visit: 01 DT: **SERUM**	**BLOOD**

01DBA Visit: 01 /02 /03 DT: **SAL**	01DBA Visit: 01 /02 /03 DT: **TONG**	01DBA Visit: 01 /02 /03 DT: **HPAL**	**ORAL**
01DBA Visit: 01 /02 /03 DT: **BUCC**	01DBA Visit: 01 /02 /03 DT: **GING**	01DBA Visit: 01 /02 /03 DT: **PTON**	
01DBA Visit: 01 /02 /03 DT: **THRO**	01DBA Visit: 01 /02 /03 DT: **SUPRA**	01DBA Visit: 01 /02 /03 DT: **SUB**	

01DBA Visit: 01 /02 /03 DT: **RAC_R**	01DBA Visit: 01 /02 /03 DT: **RAC_L**	01DBA Visit: 01 /02 /03 DT:	**SKIN & NASAL**
01DBA Visit: 01 /02 /03 DT: **ACF_R**	01DBA Visit: 01 /02 /03 DT: **ACF_L**	01DBA Visit: 01 /02 /03 DT: **NASAL**	

01DBA Visit: 01 /02 /03 DT: **INTRO**	01DBA Visit: 01 /02 /03 DT: **PFORN**	01DBA Visit: 01 /02 /03 DT: **MIDVAG**	**VAGINAL**

01DBA Visit: 01 /02 /03 DT: **STOOL**	01DBA Visit: 01 /02 /03 DT: **STOOL**	01DBA Visit: 01 /02 /03 DT:	**GI**

APPENDIX O:
Aseptic Technique

The collection and processing of all samples will be done utilizing Aseptic Technique. All collection materials will be cleaned and sterile per SOP or confirmed sterile upon purchase and use.

The following are recommendations for Aseptic Technique. All site SOPs for Aseptic Technique will be applied where applicable.

Preparation of Sample Collection Tubes:

1. Sterile supplies to include (but not limited to) tubes, pipets, MoBio buffer and SCF-1.
2. If tubes are filled at the sites, MoBio buffer and SCF-1 will be aliquoted in a laminar flow hood.

Collection of Subject Specimens:

1. Follow all safety guidelines of your local safety committee and/or institutional policies for handling biological specimens. Gloves, safety glasses or face shield, and a lab coat must be worn at all times when aliquoting sera. Observe Universal Precautions guidelines when collecting any biological specimens to include at the least wearing of gloves.
2. When handling the specimen collection tubes during sampling, care should be taken to maintain a cleanly gloved hand, so as to not contaminate the outer or inner tube areas.
3. Clean disposable gloves are recommended for all specimen collections.
    a. When collecting specimens from the skin sites and the nasal cavity, the four skin sites should be sampled before the nares. The investigator collecting the samples may wear a single pair of gloves to sample all of the sites, provided that the hand used to stretch the skin around the sampling areas does not contact the actual sampling areas. If contact occurs, the gloves may be contaminated and should be changed before sampling the next site.
    b. Study personnel may sample the various sites within a subject's oral cavity without changing gloves between collections. Similarly, when collecting the three vaginal specimens from a subject, it is not necessary to change gloves between collections.

Processing of Specimens:

1. Sterile supplies to include (but not limited to) tubes, pipets, and all buffers/reagents.