P1078
A Phase IV Randomized Double-Blind Placebo-Controlled Trial to Evaluate the Safety of Immediate (Antepartum-Initiated) Versus Deferred (Postpartum-Initiated) Isoniazid Preventive Therapy Among HIV-Infected Women In High TB Incidence Settings

Manual of Procedures

Version 2.0
29 March 2016
## Overview of Section Contents and Identification of Current Section Versions

<table>
<thead>
<tr>
<th>Section</th>
<th>Current Version</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 1</td>
<td>Version 2.0</td>
<td>• Updated communication guidance for study-related communications</td>
</tr>
<tr>
<td>Study Resources</td>
<td>29 March 2016</td>
<td>• Added instructions for contacting the CMC for clinical and toxicity</td>
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<td></td>
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<td>Section 4</td>
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<td>Laboratory Procedures</td>
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<td>Section 5</td>
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<td>• Added template participant handout on signs and symptoms of hepatotoxicity</td>
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# GLOSSARY OF TERMS

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ADA</td>
<td>Adenosine Deaminase Activity</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral Therapy</td>
</tr>
<tr>
<td>ARV</td>
<td>Antiretroviral</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette-Guérin</td>
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<tr>
<td>BPNS</td>
<td>Brief Peripheral Neuropathy Screen</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CRPMC</td>
<td>Clinical Research Products Management Center</td>
</tr>
<tr>
<td>CRS</td>
<td>Clinical Research Site</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CXR</td>
<td>Chest X-ray</td>
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<tr>
<td>DAERS</td>
<td>DAIDS Adverse Experience Reporting System</td>
</tr>
<tr>
<td>DAIDS</td>
<td>Division of AIDS</td>
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<tr>
<td>DMC</td>
<td>Data Management Center</td>
</tr>
<tr>
<td>DPRS</td>
<td>DAIDS Protocol Registration System</td>
</tr>
<tr>
<td>EAE</td>
<td>Expedited Adverse Event</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ELISPOT</td>
<td>Enzyme-linked Immunosorbent Spot Assay</td>
</tr>
<tr>
<td>EPR</td>
<td>Electronic Protocol Registration</td>
</tr>
<tr>
<td>FSTRF</td>
<td>Frontier Science and Technology Research Foundation</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly Active Antiretroviral Therapy</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HSP</td>
<td>Human Participants Protection</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IMPAACT</td>
<td>International Maternal Pediatric Adolescent AIDS Clinical Trials Group</td>
</tr>
<tr>
<td>INH</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>IPT</td>
<td>Immediate Prophylaxis</td>
</tr>
<tr>
<td>LDMS</td>
<td>Laboratory Data Management System</td>
</tr>
<tr>
<td>LPC</td>
<td>Laboratory Processing Chart</td>
</tr>
<tr>
<td>MGIT</td>
<td>Growth in Middlebrook Broth</td>
</tr>
<tr>
<td>MMR</td>
<td>Measles, Mumps, and Rubella</td>
</tr>
<tr>
<td>MTB</td>
<td><em>Mycobacterium Tuberculosis</em></td>
</tr>
<tr>
<td>MTCT</td>
<td>Mother-to-Child Transmission of HIV</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid-Upper Arm Circumference</td>
</tr>
<tr>
<td>OPCRO</td>
<td>Office for Policy in Clinical Research Operations</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cell</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PID</td>
<td>Patient Identification Number</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of Mother-to-Child Transmission of HIV</td>
</tr>
<tr>
<td>PN</td>
<td>Peripheral Neuropathy</td>
</tr>
<tr>
<td>PPD</td>
<td>Purified Protein Derivative</td>
</tr>
<tr>
<td>PRO</td>
<td>Protocol Registration Office</td>
</tr>
<tr>
<td>PSWP</td>
<td>Protocol Specific Web Page</td>
</tr>
<tr>
<td>RMP</td>
<td>Rifampin</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>RSC</td>
<td>Regulatory Support Center</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QFT</td>
<td>QuantiFERON-TB® Gold</td>
</tr>
<tr>
<td>QFT-GIT / QGIT</td>
<td>QuantiFERON-TB® Gold In-Tube Test</td>
</tr>
<tr>
<td>SID</td>
<td>Site Identification Number</td>
</tr>
<tr>
<td>SPN</td>
<td>Symptomatic Peripheral Neuropathy</td>
</tr>
<tr>
<td>TST</td>
<td>Tuberculin Skin Test</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
1.0 Study Resources

This section specifies the resources available to P1078 study site staff, including contact information, an overview of Protocol Registration and the Data Management Center International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) Portal and informational resources and guidelines.

1.1 Study-Related Information and Communications

All IMPAACT P1078 visits and procedures must be conducted in accordance with the study protocol. The purpose of this Manual of Procedures is to supplement the protocol, not to replace or substitute for it. In the event that this manual is inconsistent with the protocol, the specifications of the protocol take precedence. Please notify the IMPAACT Operations Center of any such inconsistencies.

The Protocol Team has developed study-specific contacts for various types of issues and questions, as summarized below, with further details provided in Figure 1-1. For issues and questions directed to the study team, a response from the appropriate team member can generally be expected within 24 hours.

- **General questions**: Questions related to protocol interpretation or study implementation, including administrative, ethical, regulatory, counseling, data, and laboratory operations should be emailed to the P1078 Protocol Team as listed in Figure 1-1. Any questions that are not answered by the protocol or this document should also be emailed to the P1078 Protocol Team.

- **Clinical and toxicity management questions and notifications**: Questions concerning clinical management of study participants and adverse experiences should be emailed to the Clinical Management Committee (CMC) as listed in Figure 1-1. Additional detail is listed in Figures 1-2 and 1-3.

- **Study implementation questions**: Questions related to participant eligibility, co-enrollment, potential enrollment of an ineligible participant, and/or deviation from other protocol requirements for screening and enrollment should also be directed to the P1078 Core Team as listed in Figure 1-1.

- **Other types of questions** should be managed as listed in Figure 1-1.
**Figure 1-1**
Contact Information for Study-Related Questions

<table>
<thead>
<tr>
<th>Topic</th>
<th>Contact for Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adding site staff to protocol email group <em>(<a href="mailto:impaact.protp1078@fstrf.org">impaact.protp1078@fstrf.org</a>)</em></td>
<td><a href="mailto:usersprt@fstrf.org">usersprt@fstrf.org</a> (include the protocol number in the participant line of your email message)</td>
</tr>
<tr>
<td>Any aspect of protocol interpretation or study implementation not listed below</td>
<td><a href="mailto:impaact.teamp1078@fstrf.org">impaact.teamp1078@fstrf.org</a> for triage to other team members as needed</td>
</tr>
<tr>
<td>Participant eligibility, potential enrollment of an ineligible participant, and/or deviation from protocol requirements for screening and enrollment</td>
<td><a href="mailto:impaact.corep1078@fstrf.org">impaact.corep1078@fstrf.org</a></td>
</tr>
<tr>
<td>Clinical and toxicity management issues</td>
<td><a href="mailto:impaact.p1078cmc@fstrf.org">impaact.p1078cmc@fstrf.org</a> (see Figures 2, 3, and 4)</td>
</tr>
<tr>
<td>Co-enrollment</td>
<td><a href="mailto:impaact.corep1078@fstrf.org">impaact.corep1078@fstrf.org</a></td>
</tr>
<tr>
<td>Data management computer and screen problems</td>
<td><a href="mailto:usersprt@fstrf.org">usersprt@fstrf.org</a> or by phone: +716-834-0900 x7302</td>
</tr>
<tr>
<td>Participant Enrollment System</td>
<td><a href="mailto:rando.support@fstrf.org">rando.support@fstrf.org</a> or by phone: +716-834-0900 x7301</td>
</tr>
<tr>
<td>Study drugs (other than study drug orders)</td>
<td><a href="mailto:Kashin@niaid.nih.gov">Kashin@niaid.nih.gov</a> or by phone: +240-627-3047</td>
</tr>
<tr>
<td>Study drug orders</td>
<td><a href="mailto:BIO.CRPMC.Ph@Thermofisher.com">BIO.CRPMC.Ph@Thermofisher.com</a> or by phone: +301-294-0741</td>
</tr>
<tr>
<td>Expedited Adverse Event (EAE) Reporting</td>
<td><a href="mailto:RSCSafetyOffice@tech-res.com">RSCSafetyOffice@tech-res.com</a> or by phone: 800-537-9979 or +301-897-1709 or by fax: 800-275-7619 or +301-8977-1710</td>
</tr>
<tr>
<td>DAIDS Adverse Experience Reporting System (DAERS)</td>
<td><a href="mailto:DAIDS-ESSupport@niaid.nih.gov">DAIDS-ESSupport@niaid.nih.gov</a> (questions also may be submitted from within the DAERS application)</td>
</tr>
</tbody>
</table>

**Clinical and Toxicity Management Communications**

The IMPAACT P1078 Clinical Management Committee (CMC) is composed of study team members who have been designated to receive and reply to clinical management questions and notifications. When submitting clinical and toxicity management questions to the P1078 CMC, please address each of the points listed in Figure 1-2, to help ensure that CMC members have adequate information to respond in a timely manner. The responding CMC member will reply to your question or notification by return email. All persons copied on the original question or notification will be copied on the reply.

Replies can generally be expected within 24 hours. When it may not be possible to provide a complete response within 24 hours, the person who submitted the question or notification will be provided with an interim response and informed that more time is needed to provide a complete response.

Print and file a copy of the email exchange in the participant’s study chart.
Questions for P1078 CMC: Please copy and paste this listing into the body of your email message to impaact.p1078cmc@fstrf.org to help ensure that all required information is included. Include “Question for P1078 CMC” and PID in the participant line of your email.

1. Site name and number:
2. Name of person submitting query:
3. Participant type:
   - Mother
   - Infant (note that infant management is the responsibility of the clinical care provider at the site)
   - Both mother and infant
4. PID(s):
5. Reason for query (choose one). Specify severity grade and relationship assessment to INH/Placebo for INH when applicable:
   - General AE
   - Peripheral neuropathy
   - Asymptomatic elevation in liver function tests
   - Symptomatic hepatitis
   - Other (specify in case description)
6. Sex and age of participant:
7. Is the mother currently pregnant or has she delivered her infant?
   - Currently pregnant (include gestational age in case description)
   - Has delivered (include date of delivery or infant age in case description)
8. Current week on study:
9. Case description and question or notification for CMC:

Print and file a copy of the email exchange in the participant’s study chart.

The P1078 protocol also details the circumstances in which Investigators of Record (IoRs) must consult with the study’s CMC. All protocol requirements must be followed. For ease of reference, a summary of issues requiring consultation with the CMC, and those for which consultation is available but not required, is provided below in Figure 1-3. IoRs are also encouraged to contact the CMC with any other issues, questions, or concerns related to study drug regimens for mothers and infants.
## Requirements for Consultation with the P1078 Clinical Management Committee

### Issues Requiring Consultation with the CMC

#### General Adverse Events
- Maternal deaths while on study, regardless of attribution to INH/Placebo for INH, as soon as possible and within 3 days of site awareness
- Management of mothers that experience a confirmed ≥ Grade 3 AE that is possibly, probably or definitely related to INH/Placebo for INH
- Management of mothers that experience a confirmed ≥ Grade 4 AE, regardless of attribution to INH/Placebo for INH

#### Peripheral Neuropathy
- Management of mothers with confirmed ≥ Grade 3 peripheral neuropathy

#### Asymptomatic Elevations in Liver Function Tests
- Management of participants with confirmed ≥ Grade 3 or 4 asymptomatic elevations in liver enzymes (ALT, AST, and total bilirubin, or direct bilirubin if the participant is on atazanavir)
- Restarting INH/Placebo for INH in participants following hepatotoxicity adverse events that have resolved to ≤ Grade 1, that are considered to be related to a confounding cause
- Management of INH/Placebo for INH and frequency of repeat toxicity assessments for reasons other than the reasons listed in protocol Section 6.1.4

#### Symptomatic Hepatitis
- Management of participants with confirmed ≥ Grade 3 signs or symptoms of hepatitis, including new or worsening nausea, vomiting, unexplained loss of appetite; yellowing of the skin or eyes; increased weakness or fatigue; pain in the upper abdomen (liver tenderness or hepatomegaly); pale or clay-colored stools; and/or unexplained weight loss

### Issues for which Consultation with the CMC is Available But Not Required

#### General Adverse Events
- Management of mothers that experience a Grade 3 AE that is probably or definitely not related to INH/Placebo for INH
- Management of vitamin B₆, prenatal multivitamins, or concomitant medications, including ARVs
1.2 Investigator Responsibilities

At each site, this study must be conducted in accordance with the United States (US) Code of Federal Regulations (CFR) and the International Conference on Harmonization (ICH) Consolidated Guidance for Good Clinical Practice (GCP). The Division of AIDS (DAIDS) policies on Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials and Requirements for Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials are useful for interpreting and operationalizing the regulations and guidelines in accordance with DAIDS expectations. These policies are available at the following web site and must be followed throughout implementation of P1092:

http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Pages/ClinicalSite.aspx

This study also must be conducted in accordance with all site-specific regulations, policies, and guidelines applicable to human participants research in general and/or the conduct of study procedures in particular. Copies of all of the above-listed regulations, policies, and guidelines should be maintained in on-site essential document files.

The Investigator of Record (IoR) at each site must sign a DAIDS Investigator of Record Form to formally indicate his or her agreement to conduct the study in accordance with the protocol and all applicable regulations, policies, and guidelines. The obligations and responsibilities assumed by the IoR when signing this form are listed on the form, which is available on the DAIDS Regulatory Support Center (RSC) website:

http://rsc.tech-res.com/protocolregistration/

IoRs may delegate their obligations and responsibilities for conducting this study to other study staff; however, delegation does not relieve the IoR of his or her ultimate responsibility for all study procedures performed and all study data collected. Delegation of IoR responsibilities must be formally documented throughout the period of study implementation.

Consistent with the regulations, guidelines, and policies cited above, the IoR at each site must obtain all applicable drug regulatory and ethical review approvals prior to study initiation; the IoR must also maintain these approvals in good standing throughout the period of study implementation. With regard to drug regulatory authorities (DRAs), the IoR must complete initial and continuing submissions in accordance with DRA policies. With regard to institutional review boards and ethics committees (IRBs/ECs), further guidance on initial and continuing review requirements is available in 45 CFR 46 and the ICH GCP guidance, as well as on the web site of the US Office for Human Research Protections (OHRP):

http://www.hhs.gov/ohrp/

All sites are encouraged to request an acknowledgement of receipt for all documents submitted to their DRAs and IRBs/ECs and to request that DRAs and IRBs/ECs note the effective and expiry dates of all approvals. Because P1092 involves pediatric participants, IRBs/ECs must consider the potential benefits, risks, and discomforts of the study to children and assess the justification for their inclusion in the study. As part of this assessment, IRB/ECs must assess the level of risk to children in the following categories:
§46.404  Research not involving greater than minimal risk

§46.405  Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual participants

§46.406  Research involving greater than minimal risk and no prospect of direct benefit to individual participants, but likely to yield generalizable knowledge about the participant’s disorder or condition

§46.407  Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children

The risk category assessed by the IRBs/ECs then determines the informed consent requirements for participation of children in the study. Specifically, per 45 CFR 46.408 (b), “the IRB may find that the permission of one parent is sufficient for research to be conducted under §46.404 or §46.405. Where research is covered by §46.406 and §46.407 and permission is to be obtained from parents, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.”

IRBs/ECs should document their risk determination and study sites should adapt the signature pages of their informed consent forms as needed to accommodate the parental consent requirements associated with the IRBs/ECs determination. In the absence of a clearly documented determination from the IRBs/ECs, the most conservative approach specified in the regulations should be followed.

Complete documentation of all correspondence to and from all responsible DRAs and IRBs/ECs (i.e., complete copies of all submissions, responses, and approvals) must be maintained in on-site essential document files. All submission letters should list the date of the submission, the contents of the submission, and the version number and/or version date of each document.

1.3 Protocol Registration

This section was applicable to the initial study protocol registration process that all sites completed under protocol Version 1.0. Please refer to prior versions of this section for more information about the process.

1.4 Data Management Center IMPAACT Portal

The IMPAACT Portal of the DMC website provides information, documents and tools to assist site staff with the data management aspect of conducting IMPAACT protocols. The documents and tools that can be found in the Portal are Case Report Forms (CRFs), Annotated CRFs, CRF appendices, data collection forms schedules, Drug Code Lookup, Forms Manual, calculator utilities, quality assurance (QA) tools, Participant Calendar and study-specific messages. The Participant Enrollment System, Order Entry System, and Forms Management Utility can also be accessed on the Portal.
Site staff members apply for access to the Portal by submitting a registration form located on the Frontier Science and Technology Research Foundation (FSTRF) home page. All requests for Portal access are participant to review and verification by User Support before processing. The site leader or site coordinator will be contacted by the DMC to ensure legitimate affiliation of the applicant. To request for DMC IMPAACT Portal access complete the form located at https://www.fstrf.org/apps/cfmx/apps/common/register/index.cfm. Confirmation of registration will be sent via email from User Support.

The portal can be accessed from the FSTRF home page at https://www.fstrf.org/. Click on the IMPAACT project link to enter the project website. A log-in screen appears. Enter your username (format: lastname.firstname) and the password that you set up when you registered for DMC web access.

For randomization and clinical user support, send an email message to impaact.support@fstrf.org or call +1 (716) 834-0900 x 7302 (x7200 if outside US hours). If you experience problems, or have questions about the IMPAACT portal in the FSTRF website, please contact the Webmaster at webmaster@fstrf.org and include a detailed description of your question or the problem you encountered.

1.4.1 CRF Completion and Data Entry

The DMC has developed a Forms Manual to assist site staff in the accurate completion of CRFs used for DAIDS-sponsored Clinical Trials. The Forms Manual is located in the DMC IMPAACT Portal under the Case Report Forms heading.

The manual outlines standards and guidelines which when followed, will result in fewer queries, shorter delinquency lists, and most important, straightforward and timely analyses. The manual includes sections that cover topics such as the CRF notebook, reporting data, understanding forms, forms components and conventions, submitting data, data collection formats and participant status categories.

For reporting TB diagnosis, sites should refer to CRF Appendix 100 for the TB diagnosis definitions. For all other diagnoses, CRF Appendix 100 (or the most current version of the CRF appendix) should be used. To obtain the most current version of the CRF appendix, please refer to the IMPAACT Portal of the DMC website:

https://www.fstrf.org/apps/cfmx/apps/common/Portal/index.cfm

1.4.2 Obtaining CRFs and other related materials

CRFs and other related materials can be ordered through the Order Entry program located in the DMC IMPAACT Portal under the Systems heading. A DMC Web site password and level 2 data access are required to use the Order Entry program. If you are unable to use the Order Entry program, requests can be emailed to orders@fstrf.org. Include your name, site mailing address (for UPS deliveries), the items to be ordered and the quantity.

1.4.3 Participant Calendar

The Participant Calendar utilities help track when a study participant should be scheduled for tests or visits. They are located in the Utilities heading of the DMC IMPAACT Portal.
To generate a calendar for mothers, choose the Fixed Week Interval Calendar utility. Please note that two calendars should be generated. The first calendar is generated at entry following the fixed week visits in the antepartum period. The antepartum calendar is used until the labor and delivery visit occurs. After labor and delivery, a new calendar should be generated following the fixed week visits in the postpartum period. The Calendar start date should be the date of randomization. The Start of treatment date should be the date of the first dose of study drug, which must be within the protocol mandated window if it does not occur on the day of randomization. Visit windows can be included in the calendar by choosing the protocol directed visit window from the “week window” drop-down menu.

To generate a calendar for infants, choose the Variable Week/Day Interval Calendar utility. Start of treatment and Calendar start should be the same date, which is the Date of Birth. Enter the visit week number in the data fields in the week column, using one line per visit. The data fields in the day column can be left blank since all the visits in the schedule of evaluations are in weeks. Visit windows can also be included in the calendar by choosing the protocol directed visit window from the “window” drop-down menu. If the site prefers, two calendars can be generated for the two different visit windows as shown in the schedule of evaluations.

1.5 Study Web Page

A variety of P1078 study-related materials and information can be found on the P1078 study of the IMPAACT website: [www.impaactnetwork.org/studies/P1078.asp](http://www.impaactnetwork.org/studies/P1078.asp).

Resources available on this site include:
- Current version of the protocol
- Current study implementation materials, including the Laboratory Processing Chart
- Study training materials

1.6 Documentation Requirements

1.6.1 Source Documentation and Essential Documents

All sites must comply with the DAIDS policy on Requirements for Source Documentation and Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials. Refer to the detailed operational guidance provided in Appendix I of this policy. Both the policy and the appendix are located in the Site Implementation and Operations section of the DAIDS Clinical Research Policies and Standard Procedures Documents website:


1.7 Clinical Resources

1.7.1 World Health Organization (WHO) Guidelines and Recommendations

Treatment of Tuberculosis

- Treatment of Tuberculosis: guidelines for national programs
- Guidance for national tuberculosis programs on the management of tuberculosis in children
HAART Initiation in pregnant women

- **Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants** *(Recommendations for a public health approach, 2010 version)*
  

- **Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants** *(Programmatic update, April 2012)*
  

Breastfeeding

- **Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV Infection** *(June 2013)*
  

- **Breastfeeding and Maternal Tuberculosis**
  

1.7.2 Other Guidelines

- **Guidelines for the Management of a Newborn Infant in Contact with TB** are provided in appendix I.

- **Patient Health Questionnaire (PHQ):** A nine-item assessment tool (PHQ-9) that has been internationally validated will be used as a tool to capture depression in pregnancy and postpartum in P1078. Further information about the PHQ, including available translations, is available at [http://www.phqscreeners.com](http://www.phqscreeners.com)

2.0 Data Management Procedures

2.1 Case Report Form (CRF) Completion Guidelines

2.1.1 Screening Failure

A Screening Failure and Non-Enrollment Results (SCR0023) case report form (CRF) must be completed for each woman for whom informed consent is obtained who does not enroll in the study for any reason. Study sites are asked to complete and key enter these CRFs as soon as possible after ineligibility is determined so that reasons for non-enrollment can be carefully tracked by the Protocol Team. Mothers identified as ineligible for the study should be referred to non-study care and treatment as needed.

2.1.2 Signs and Symptoms

Regardless of severity grade, all signs and symptoms occurring ≤ 30 days before study entry must be recorded on the CRFs.
Post-entry, the following guidelines apply for all mothers:

a. All signs and symptoms Grade 1 or higher that are related to Peripheral Neuropathy or Hepatotoxicity must be recorded on the appropriate CRFs.
   - The Peripheral Neuropathy Grading Table (Protocol Appendix II) and Grading of Symptomatic Peripheral Neuropathy in the Peripheral Neuropathy section under Clinical Procedures below should be used for grading symptomatic peripheral neuropathy.
   - The Modified Grading Table for Hepatotoxicity (Protocol Appendix III) should be used for grading ALT, AST, and total bilirubin. Signs or symptoms of hepatotoxicity should be graded per DAIDS grading criteria.
   - All signs and symptoms Grade 2 or higher that are related to Peripheral Neuropathy or Hepatotoxicity must be evaluated as per Protocol Sections 6.1.3 and 6.1.4, respectively.

All participants should be counseled on the signs and symptoms of hepatotoxicity. Please see Appendix II for a template participant handout on the signs and symptoms of hepatotoxicity.

b. All signs and symptoms Grade 2 or higher that are NOT related to Peripheral Neuropathy or Hepatotoxicity must be recorded on the CRFs.
   - Signs and symptoms Grade 3 or higher must be further evaluated and may require additional supporting information to assess the relationship to study drugs. The additional evaluations must be recorded on the CRF.
   - Relationship to treatment does not need to be assessed for signs and symptoms Grade 2 or lower, unless treatment is modified due to the toxicity.

For infants, all ≥ Grade 2 signs and symptoms must be recorded on the appropriate CRFs.

### 2.1.3 Laboratory Evaluations

At screening, entry and post-entry, all laboratory values and grading for all mothers must be recorded on the CRFs.

a. All Grade 3 or higher laboratory values not related to hepatotoxicity and laboratory values of any grade that leads to a change in treatment must be further evaluated and may require additional supporting information to assess the relationship to study drugs. Refer to the Toxicity Management section for mothers in the protocol (Sections 6.1.1 and 6.1.2) for required evaluations of laboratory values of any Grade that are NOT related to Hepatotoxicity, symptomatic hepatitis, or peripheral neuropathy.

b. All Grade 2 or higher laboratory values for ALT (SGPT), AST (SGOT) and total bilirubin (or direct bilirubin, if on Atazanavir) must be evaluated. Refer to the Hepatotoxicity management section of the protocol (Section 6.1.4) for required evaluations of laboratory values of any Grade that are related to Hepatotoxicity.

For infants, refer to the Toxicity Management section for Infants in the protocol (Section 6.2) for guidance on management of adverse events and toxicities.

### 2.1.4 Diagnoses

a. For mothers:
   - At entry, all diagnoses identified by the Pediatric/Maternal Diagnoses criteria during the current pregnancy are to be recorded.
• After entry, all diagnoses identified since the last study visit are to be recorded on the CRFs.
• All Grade 3 or higher diagnoses must be evaluated.

b. For Infants:
• All diagnoses identified by the Pediatric/Maternal Diagnoses criteria are to be recorded on the CRFs.

For reporting TB diagnosis, sites should refer to Appendix 100 (available in the CRF Appendix Codes section of the Frontier Science IMPAACT Portal) for the TB diagnosis definitions. For all other diagnoses, the most current version of the CRF appendix should be used. To obtain the most current version of the CRF appendix, please refer to the IMPAACT Portal of the DMC website.

2.2 Appendix 100 and Cantwell Criteria

2.2.1 Appendix 100

Appendix 100, the Diagnoses Appendix Criteria for Clinical and Other Events, Version 1.0 dated 07/01/2011, will be the reference for diagnosing TB, except for congenital TB. The appendix can be obtained from the Frontier Science IMPAACT Portal at: https://www.fstrf.org/IMPAACT/.

2.2.2 Cantwell Criteria

The Cantwell Criteria will be used for diagnosing congenital tuberculosis. Congenital TB is thought to be a rare condition as the placenta usually forms a protective barrier against the invasion of the fetus by the tuberculosis organisms. The Cantwell Criteria [5] for the diagnosis of congenital TB are:

Proven tuberculosis lesions in the infant plus at least one of the following:
• Lesions occurring in the first week of life;
• A primary hepatic complex or caseating hepatic granulomas;
• Maternal genital tract or placental tuberculosis, OR
• Exclusion of postnatal transmission by thorough investigation of contacts, including infant’s hospital attendants

3.0 Clinical Procedures

This section details the procedures for clinical evaluations specific to P1078.

3.1 Anthropometric Procedures for Mothers

3.1.1 General Information

Whenever possible, measurements should be taken by a team of two measurers. One measurer takes the measurements while the other measurer records. The measurer taking the measurements calls out the results to the recorder. The recorder repeats the results and then calls out the name of the next measurement. The measurer keeps the measuring instrument in place until the recorder repeats the number. The recorder checks the participant's position during the procedure. The participant's cooperation is extremely important for obtaining accurate measurements.
Circumferences are made once before repeating them a second time in the same sequence by the same observer. Document measurement conditions (type of equipment use, participant’s behavior during procedure, etc.). Always take two measurements in each category. A third measurement will be needed when the second measurement differs from the first one by more than 0.5 cm.

All measurements should be taken on the right side of the participant being measured. If measurement on the right side is contraindicated or otherwise not possible, the measurement should be taken on the left side and the reason for this should be recorded in source documents and on relevant case report forms. If a participant’s first measurement is taken on the left side, for consistency, all subsequent measurements should also be taken on the left side.

3.1.2 Mid-Upper Arm Circumference

*Equipment:* Fiberglass or paper measuring tapes specific to 1.0 mm
Grease marking pencil or washable felt-tip marker

Circumferences should be recorded with the zero end of the tape held by the left hand above the remaining part of the tape held by the right hand. The plane of the tape around the body part should be perpendicular to the long axis of the body part being measured.

To locate the midpoint, the participant’s elbow is flexed to 90° with the palm facing superiorly. The measurer stands above or behind the participant and locates the lateral tip of the acromion by palpating laterally along the superior surface of the spinous process of the scapula as shown in Figure 3-1. The tape is placed from the acromion process to the tip of the olecranon and the midpoint is marked with a horizontal mark at the midpoint on the posterior surface of the arm (see Figure 3-2). The arm is now repositioned to hang loosely at the side with the palm facing the thigh.

The tape is passed around the arm from left to right, and the free and fixed ends are transferred. Ensuring that the tape is at the same level as the mid-upper-arm mark, the measurer tightens the tape so that it touches the skin all around the circumference but does not compress the tissue or alter the contour of the arm. The circumference is then read. Because the arm in cross-section is not an exact circle but rather oval, some difficulty may be met in ensuring that the tape actually touches the skin on the medial side of the arm. If necessary, the middle finger of the left hand can be used to gently press the tape to the skin.
3.1.3 Height

This measurement should be carefully performed using a stadiometer, a measuring rod that is attached to many physicians' scales, or other device that is carefully mounted and maintained throughout the study. If no designated equipment is available for measuring height, a tape measure or series of yardsticks could be carefully attached to a wall, with the zero end just touching the floor.

*Reminder: Shoes should be removed before height is measured.*

For wall-mounted measuring devices, the participant should stand with their back to the device. The participant should be aligned so that the device runs up the middle of the body and standing with heels together and heels, buttocks, and shoulders touching the wall. The participant should tuck his/her chin down into the chest and stand as tall as possible. If the measuring device has a horizontal bar to assist with the measurement, the bar should be raised above the participant’s head and lowered until it just touches the head (the skull; not just the hair). It is important to make certain that the bar is completely horizontal. If the bar is at an angle greater or less than 90° to the wall, the measurement of height will be inaccurate.

If the measuring device does not have a horizontal bar, some surrogate should be constructed to make certain that height is recorded from the point on the measuring device that is exactly horizontal to the top of the center of the head. A plastic right triangle or two pieces of wood attached at a 90° angle could serve this purpose.

Height should be reported in the units provided by your measuring device (inches or centimeters). Repeat height measurement two more times and record each measurement taken on the CRF.

3.1.4 Weight

- The same scale should be used for all measurements performed for this protocol. The scale should be calibrated monthly.
- Please refer to the general guidelines listed above for conditions under which participants should be weighed.
Before the participant is weighed, make certain that the scale is in balance if it is a beam-balance scale or reads zero if it is an electronic scale.

Instruct the participant to stand with both feet centered on the scale with arms at the sides. The participant should not move or hold onto anything during the measurement.

Allow the scale to stabilize and record the weight in the units provided by the scale (lbs. or kg).

### 3.2 Peripheral Neuropathy

#### 3.2.1 Definition of Peripheral Neuropathy

The case definition for peripheral neuropathy (PN) is based on the presence of distally predominant and symmetrical neuropathy sensory symptoms.

Symptomatic PN (SPN) is defined by evidence of neuropathy symptoms and usually at least one neuropathy sign (decreased tendon reflexes, diminished vibratory sensation). Neuropathy symptoms may be described as positive (pain, paraesthesias) or negative (numbness) sensory symptoms. To be considered significant, neuropathy symptoms should be present for a minimum of two weeks.

Neuropathy diagnosis documented at screening and/or entry visits will be defined as entry HIV-associated peripheral neuropathy. Subsequent to entry, peripheral neuropathy will be defined by either incident PN diagnosis or PN diagnosis coupled with worsening of neuropathy symptom grade.

#### 3.2.2 General Instructions

The neuropathy exam will be conducted by site staff. Site staff are strongly encouraged to consult with the site investigator for any questions relating to the neuropathy examination.

The focused neuropathy assessment will include the following:

- Grading of sensory symptoms
- Vibration sensibility using a 128-Hz tuning fork
- Tendon reflex testing

#### 3.2.3 Training

Neuropathy exams will be performed by the site investigator, who must complete the P1078 specific training to perform this examination. It is recommended that the same site staff person or investigator evaluate a particular participant to reduce inter-examiner variability. Appropriate training is defined as participation in an in-person, video- or web-conference training session. Training materials, including an instructional video on how to perform the peripheral neuropathy exam, are available on the P1078 study-specific webpage available at: [www.impaactnetwork.org/studies/P1078.asp](http://www.impaactnetwork.org/studies/P1078.asp)
3.2.4 Materials

The focused neuropathy exam will assess distal lower extremity vibratory sensation and lower extremity reflexes. The exam is to be conducted with the following materials:

- 128-Hz tuning fork
- Reflex hammer

3.2.5 Grading of Symptomatic Peripheral Neuropathy

SPN severity will be graded on the basis of symptom severity, recorded with symptom scores from the Brief Peripheral Neuropathy Screen (BPNS). Grades of PN severity will be determined by the single highest symptom severity score, assuming symptom severity scores for the right and left legs are within 2 points of each other. Should symptom severity vary by greater than 2 points between the two legs, causes of neuropathic symptoms other than PN should be considered (e.g., lumbosacral radiculopathy) as clinically indicated. It is particularly important that, when grading symptom severity, attention be directed specifically to positive and negative neuropathic sensory symptoms, avoiding confounding non-neuropathic pain syndromes such as lower extremity edema, hip pain and the like. Severity grades will be defined as defined in the protocol Appendix II.

3.3 WHO TB Symptom Screen

In P1078, we refer to the WHO recommended TB symptom screen as current cough, fever, weight loss or night sweats. We will also include additional symptoms of failure of weight gain and duration of fever, cough, as well as targeted physical exam looking for new or increasing cervical or axillary adenopathy. However, patients who have a positive WHO symptom screen (any current cough, fever, weight loss or night sweats) will be excluded and can only be reconsidered for entry if TB is convincingly ruled out.

This is taken from the most recent 2010 WHO guidelines document entitled “Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings”, Department of HIV/AIDS, Stop TB Department, World Health Organization, Geneva, Switzerland.


Strong recommendation, moderate quality of evidence

Adults and adolescents living with HIV should be screened for TB with a clinical algorithm and those who do not report any one of the symptoms of current cough, fever, weight loss or night sweats are unlikely to have active TB and should be offered immediate prophylaxis (IPT). As part of the guidelines development process, a comprehensive systematic primary patient data meta-analysis, including 12 observational studies involving over 8000 people living with HIV, was used to develop the best screening rule to identify adults and adolescents living with HIV who are unlikely to have active TB disease. The analysis found that the absence of all the symptoms of current cough, night sweats, and fever or weight loss can identify a subset of people living with HIV who have a very low probability of having TB disease. This best screening rule has a sensitivity of 79% and a specificity of 50%. At 5% TB prevalence among people living with HIV, the negative predictive value was 97.7% (95% CI: 97.4–98.0). This high negative predictive value ensures that those who are negative on screening are unlikely to have TB and
hence can reliably start IPT. Therefore, the Guidelines Group recommended that adults and adolescents living with HIV should be screened for TB using a clinical algorithm at every visit to a health facility or contact with a health worker. Those who do not have current cough, fever, weight loss or night sweats are unlikely to have active TB and should be offered IPT.

**NOTE:** Persons with very advanced HIV (CD4 < 100) may require more intensive TB screening as TB disease may present more atypically. If such persons can expectorate (irrespective of symptoms), examining a sputum for GeneXpert (or AFB smear if GeneXpert not readily available) is advised. A shielded chest radiograph can also be considered but is left to the discretion of the site investigator and local/national TB screening guidance.

### 3.4 Assessment of the BCG Scar in Infants

The BCG scar will be at the site of inoculation, typically the upper arm in the sulcus at the insertion of the deltoid muscle. The reaction is usually visible between two and six weeks after vaccination but may in some individuals be absent.

The following will be evaluated and recorded:
- Presence / absence of reaction
- Appearance: Scar/crust/secretion
- Size: record two dimensions, in mm
- Record presence / absence of axillary adenitis (> 10 mm, fistula, suppuration)

### 3.5 Tuberculin Skin Test (TST)

**NOTE:** Blood for QGIT must be drawn before the TST is applied if both are being done at the same visit.

The TST will be performed after the QGIT/ELISPOT assay blood draw, and read ideally at 48-72 hours (but can be read up to one week) by a trained observer.

Regardless of TB exposure, a positive TST is defined as follows:
- **Infants:** if ≥ 10 mm in HIV-negative infants and if ≥ 5 mm in HIV-positive infants
- **Mothers:** if ≥ 5 mm

See detailed directions for administering and reading TST below.

#### 3.5.1 Administering the TST

A Mantoux TST of purified protein derivative (PPD) will be administered.

A Centers for Disease Control and Prevention (CDC) podcast, which includes sections in administering and reading the TST, can be viewed at:

[www2c.cdc.gov/podcasts/browse.asp?exactMatch=1&topic=TB+Skin+Test&formsButton=Go%21](http://www2c.cdc.gov/podcasts/browse.asp?exactMatch=1&topic=TB+Skin+Test&formsButton=Go%21)
Site should follow locally applicable procedures and guidelines for administration of TST. If local guidelines are not available, sites may follow procedures as described in the Clinical Policies and Protocols of the Bureau of Tuberculosis Control of the New York City Department of Health and Mental Hygiene:


3.5.2 Reading and Interpreting the TST

The test result should be read only by a trained site investigator. Participants should never be allowed to read their own reaction.

The following procedure should be used to read the reaction:

- Read the result ideally 2-3 days after placement, but it can be read by a trained observer up to 7 days from administration.
- Inspect the injection site for raised areas.
- Palpate the arm for a hard, raised area known as an induration. Feel the edges of the induration with the index finger.
- Mark the 2 edges of the induration with a dot, using a black, watermark pen, if available.
- Measure the induration (not redness) at its widest point transversely, from 1 marked edge to the other, using a flexible TST ruler. If the reading is between 2 points, the lower value should be used. Swollen areas, if they feel hard, (but not red areas) should be palpated and included in the measurement.
- Record the size in millimeters and not simply as “positive” or “negative”. If there is no induration, record the result as “00 mm.”
- Interpret the reaction as positive or negative based on the size of the induration being as described in protocol Section 8.2.2.6 and MOP Section 3.5, above.
- Explain the meaning of a positive or negative reaction to the individual and refer for follow-up evaluation, if needed.

**NOTE:** If the participant fails to return for the scheduled reading but returns up to a week (7 days) after the test, examine the test site and measure any induration present; if it is large enough to be classified as positive, record the result. No further testing is needed. If there is no reaction, there is no need to repeat the test. If the induration is too small to be classified as positive, record the diameter and repeat the test, if feasible. A repeat test can be given immediately.

3.6 Suggested Work-up for Mothers and Infants Suspected of Having Tuberculosis Disease

Mothers and infants in P1078 who are suspected of having active TB disease will be evaluated following local standard of care and per the procedures included in the Schedule of Evaluations. In addition, to establish a minimum standard of diagnosis across all sites participating in P1078, guidelines are provided below for the diagnosis work-up of suspected TB in mothers and infants.

The evaluations including laboratory and ancillary tests used to confirm the diagnosis of TB should be reported in the appropriate CRF. The results of clinical evaluations should be reported in the CRF. The date of specimen collection and results of laboratory and ancillary tests should also be reported in the CRF.
3.6.1 Mothers

Minimum evaluations:
- Documented close exposure to someone with TB in the past 2 years
- Symptoms on presentation:
  - Cough including duration
  - Unusual/excessive fatigue
  - Participative weight loss
  - Fever
  - Night sweats
  - Hemoptyysis
  - Any sign suggestive of extra-pulmonary TB (e.g., unilateral dullness on percussion, cervical lymphadenitis)
- Sputum for Rapid TB diagnostic GeneXpert or AFB smear if GeneXpert not readily available (expectorated or induced, if required, see guidance in Section 12)
- Shielded CXR (e.g., pleural effusion, disseminated/miliary disease, parenchymal cavitation)
- Smear from any other specimen collected

Additional evaluations if available:
- Sputum culture
- Culture from any other specimen collected
- Line probe assay
- Histopathology
- Radiology (e.g., CT scan of chest)
- Drug susceptibility profile (at least INH and RMP)
- Any other relevant bacteriological tests (e.g., PCR)
- Any other relevant fluid/serological tests (e.g., ADA, CSF cell count and chemistry)

3.6.2 Infants

If the infant is suspected to have active TB, if the mother is seen for a Suspected Active TB visit, or if the infant is exposed to a TB source case, the infant should have a Suspected Active TB visit following the procedures per protocol Appendix I-B.

Minimum evaluations:
- Documented close exposure to someone with TB
- Symptoms on presentation:
  - Presence of BCG scar
  - Cough including duration
  - Unusual/excessive fatigue or reduced playfulness
  - Objective weight loss or failure to thrive
  - Fever
  - Any sign suggestive of congenital TB (e.g., enlarged liver, abdominal distension)
  - Any sign suggestive of extra-pulmonary TB (e.g., cervical adenopathy)
- CXR (e.g., disseminated/miliary disease, lymph node enlargement)
- TST
- Lumbar puncture if any signs suggestive of TBM, congenital TB or disseminated disease (CSF cell count and chemistry)
Additional evaluations if available

- Gastric aspirate smear and/or culture
- Smear or culture from any other specimen collected
- Any other radiology (e.g. CT scan of head)
- Drug susceptibility profile (at least INH and RMP)
- Any other relevant bacteriological tests (e.g. PCR, GeneXpert)
- Any other relevant fluid/serological tests (e.g. ADA, CSF cell count and chemistry)

3.6.2.1 Guidance for Gastric Aspirate from Children

The instructions are provided as a guide only and are not meant to replace established site-specific procedures. As with the TB diagnostic tests, sites should follow their site-specific procedures and best locally available method.

The following procedures are provided as a guide for collecting the gastric aspirate from the infant. However sites should follow their site-specific procedures if available.

a. The parent/legal guardian will be instructed regarding overnight fasting of at least 4 hours before early morning gastric aspirate. The procedure is performed early in the morning when the child comes for a study visit.

b. Use an assistant (counselor) to help as this procedure requires two people.

c. Prepare all equipment for the procedure.

d. Position the child on the back or the side with the help of an assistant.

e. GA will be obtained according to a standard technique: a nasogastric tube is passed from the nose into the stomach in a child that is awake to aspirate gastric contents.

f. Measure the distance of the nasogastric tube to the stomach (from nose to stomach) to estimate distance that will be required to insert the tube.

g. Attach a syringe (10 or 20 mL) to the nasogastric tube (size 8-12 French, depending on the size of the child).

h. Gently insert the nasogastric tube through the nose and advance it into the stomach

i. Withdraw (aspirate) gastric contents using the syringe attached to the nasogastric tube (2-5 mL)

j. To check that the position is correct, use litmus paper: blue litmus turns red (acidic stomach contents). Alternatively, check by pushing some air from the syringe into the stomach (e.g. 3-5 mL), and listening with a stethoscope over the stomach).

k. Normal saline 20 mL will be inserted down the tube, left for three minutes, and then aspirated. An additional 5-10 mL of normal saline will be inserted and aspirated until a minimum of 10-20 mL aspirate is obtained.

l. If no fluid is aspirated, insert 5-10 milliliters of sterile water or normal saline and attempt to aspirate again. If still unsuccessful, attempt this again. Do not repeat more than 3 times

m. Transfer 5-10 cc gastric fluid from syringe into a sterile container (screw-top sputum collection cup).

n. Add an equal volume of sodium bicarbonate to the specimen (in order to neutralize the acidic gastric contents and so prevent destruction of tubercle bacilli).

*NOTE: DO NOT use bacteriostatic sodium bicarbonate as it will kill the bacilli.*

A sample of the instructions for collecting sputum that can be given to participants can be found in Appendix III.
3.6.2.2 Guidance for Induced Sputum collection from children

The instructions are provided as a guide only and are not meant to replace established site-specific procedures. As with the TB diagnostic tests, sites should follow their site-specific procedures and best locally available method.

Sputum induction is typically used in patients who are unable to produce sputum spontaneously. The patient inhales nebulized hypertonic saline solution, which liquefies airway secretions, promotes coughing and allows expectoration of respiratory secretions. In young children, nasopharyngeal aspiration is usually required for sputum collection.

The following procedures are provided as a guide for collecting induced from children. However sites should follow their site-specific procedures if available.

Contraindications/precautions
• As hypertonic saline causes broncho-constriction, the procedure should only be performed after pre-medication with salbutamol and under medical supervision in patients with asthma or severely impaired lung function.
• As the procedure induces coughing, it should not be performed in patients in whom severe coughing may be harmful, including patients with:
  o Hemoptysis
  o Acute respiratory distress
  o Unstable cardiovascular status
  o Pertussis-syndrome
  o Hypoxia (sats <92% in room air)
  o Pneumothorax
  o Chest trauma
  o Recent eye surgery

Infection control
The minimum requirement for sputum induction is a single room with door closed and air exhausted to the outside of the building without recirculation. Ideally, the room should be fitted with an air extractor allowing for generation of negative pressure in the procedure room. A “no-entry” sign should be fitted outside the door for the duration of the procedure.

Staff performing procedure must wear the recommended TB respiratory protection (particulate respirators) and disposable gloves when handling sputum specimen.

Equipment required
• Spacer with mask
• Salbutamol (100µg/puff)
• Suction apparatus
• Pulse oximeter
• Nebulizer with tubing and face mask
• Hypertonic (3-5%) saline solution
• Disposable gloves and P2 respirator masks
• Paper towels
• Kidney dish
• Sputum specimen container (screw-top)
• Laboratory forms
• Laboratory bags
• 5ml and 10ml syringes
• 19G needle
• Sharps container
• Sterile 6/7G mucus extractor or nasogastric catheter

Procedure
1. Sputum induction is performed by a research nurse trained in this technique, and is undertaken after 2 to 3 hours of fasting.
2. Clinical evaluation form is completed before procedure, documenting general observations and chest auscultation. Detection of severe respiratory distress is a contra-indication for the procedure.
3. Oxygen saturation and pulse rate must be monitored throughout the procedure. Stop the procedure in event of a fall in saturation <90% and a pulse rate >180 or <100 bpm.
4. Child is pretreated with 200µg salbutamol via metered dose inhaler with attached spacer to prevent bronchoconstriction. This is done by placing the assembled metered dose inhaler/spacer/mask onto child’s mouth and nose. Child is allowed to settle until breathing freely. One puff is activated, keeping the mask in the same position, and the child is allowed to breathe 4 to 5 times. Mask is removed. After one minute, another puff is given in the same way.
5. The child’s nose is suctioned to remove nasal mucus prior to sputum induction. A soft catheter size F6/7 is used for suctioning and is discarded immediately afterwards.
6. A jet nebulizer attached to oxygen at a flow rate of 5 L per minute delivers 5 mL of 5% sterile saline for 15 minutes or until child starts to cough.
7. Once the child starts to cough, sputum is obtained by suctioning through the nasopharynx with a sterile mucus extractor of catheter size 6 or 7. Once mucus is obtained, catheter is withdrawn from the nose. If mucus is to be obtained from the oral cavity as well, the mouth should be rinsed/wiped with anti-bacterial, non-alcohol-based mouth wash prior to the entire procedure, in order to avoid contamination.
8. If the child does not cough after nebulization, chest percussion is done over the anterior and posterior chest wall. Mucus is then extracted as (7) above.
9. Sputum induction is best done approximately 6 hours after the early morning gastric lavage.
10. Specimens will be transported directly to the laboratory for processing.
11. Spacers and nebulizer equipment will be gas sterilized after use in every patient.
12. Complete clinical evaluation form after procedure. Any new signs of respiratory distress not settling after 5-10 minutes of supplemental oxygen via face mask must be reported immediately to attending clinician. SAE form must then be completed.

Following completion of the procedures (both gastric aspiration and sputum induction):
1. Wipe the specimen jar with alcohol/chlorhexidine to prevent cross-infection and ensure that the specimen jar is labeled.
2. Complete the lab forms, as needed.
3. Offer the child a snack after the procedure.
4. Specimens will be stored and transported out of direct sunlight.

Bacteriological specimens will be transported to the microbiology laboratory.
1. Transport the specimen (in a cool box) to the lab for processing as soon as possible (within 4 hours).
2. If a delay of longer than 4 hours is anticipated, place specimens in the fridge and store at 4 to 8°C until transport, which may occur on the same day, or on the next day.

3.7 Contraceptive Counselling Guidelines

Contraceptive counseling for mothers will be provided according to local standards of care. The FDA and WHO provide guidance on acceptable contraception methods for FDA category C drugs and contraceptive counseling, respectively.

INH is considered a Category C drug and risk to the fetus cannot be definitively ruled out. Category C means that adequate, well-controlled human studies are lacking, and animal studies have shown a risk to the fetus or studies in women and animals are not available. However INH has been used in pregnancy for treatment of active TB when the risk of untreated active TB far outweighs any potential risk to the fetus from INH. There is a chance of fetal harm if the drug is administered during pregnancy; but data to date suggest that the benefits from the use of the drug in pregnant women are likely to be acceptable despite its potential risks.

Mothers of reproductive potential and who have not undergone surgical sterilization (e.g., hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy) should have a pregnancy test administered when pregnancy is suspected or considered clinically indicated during postpartum study follow-up. If a mother becomes pregnant during the study (i.e., experiences a repeat pregnancy during study follow-up), the mother should be discontinued from INH/Placebo for INH.

If participating in sexual activity that could lead to pregnancy, mothers should be counseled and strongly encouraged to use a form of contraception as listed in the FDA guidance for Category C medications (see section on FDA guidance below) while receiving INH/Placebo for INH and for 2 weeks after stopping the INH/Placebo for INH.

If a mother undergoes postpartum surgical sterilization (e.g., hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy) she does not require the use of a contraceptive method to prevent pregnancy. However if she is in a HIV discordant relationship or is a non-monogamous relationship, she should be counseled to encourage her male partner to use condoms to prevent acquisition/transmission of sexually transmitted diseases.

Additional references to FDA and WHO guidance on contraception, particularly in relation to FDA category C medications, are linked below.

FDA Guidelines

http://www.fda.gov/ForConsumers/ByAudience/ForWomen/ucm118465.htm

WHO Guidelines:


NOTE: Postpartum Tubal Ligation is considered a form of sterilization for Category C drugs. Participant self-report is an acceptable form of documentation of tubal ligation.
4.0 Laboratory Procedures

4.1 QuantiFERON®-TB Gold In-Tube Test (QFT-GIT or QGIT)

The QuantiFERON®-TB Gold In-Tube test (QFT-GIT or QGIT) measures IFN-γ production by immune cells following stimulation with mycobacterial peptides. Tubes are pre-coated by the manufacturer with peptide antigens from certain mycobacterial proteins (ESAT-6, CFP-10, and TB7.7) or controls. Following incubation for 18 to 24 hours, an aliquot of plasma is removed for testing for IFN-γ by ELISA. The test result is determined by comparing the result obtained from the tube containing the mycobacterial stimulation compared with the nil control. A positive mitogen control tube is also used to determine that a valid response is obtainable with the patient’s sample and that the assay is performed correctly.

Detailed QuantiFERON®-TB Gold (QFT) information and resources are available at:


A reference library for QFT related information is also available through www.gnowee.net. (Please go to www.gnowee.net to sign up for your personal Gnowee access.)

QFT instructional videos are available at the following links:

Blood draw and incubation

http://www.youtube.com/watch?v=TOXF6CzPJYA&list=UUSBDJPFhVjOID7Bj44-olQ&index=1&feature=plcp

http://www.youtube.com/watch?v=mpj_q6PDjnk&list=UUSBDJPFhVjOID7Bj44-olQ&index=2&feature=plcp

Reagents and reconstituting

http://www.youtube.com/watch?v=eFl2KiU6e4q&list=UUSBDJPFhVjOID7Bj44-olQ&index=3&feature=plcp

The most current package insert (in English and various language translations) for QGIT can be found at:


4.1.1 QGIT Collection and Testing in P1078

QGIT testing should either be done in real-time or appropriately collected and stored for batch testing as described below. Follow the procedure for Incubation of Blood and Harvesting of Plasma in the QGIT package insert.

If unable to run real-time, batch testing will be done, following the guidelines below.

- Follow the procedure for Incubation of Blood and Harvesting of Plasma in the QGIT package insert.
- Harvested plasma (or plasma stored in the blood collection tubes after centrifugation) can be stored at 2°C to 8°C for up to 28 days or below -20°C for extended periods.
- Refer to the QGIT manufacturer’s instructions for the details for batch testing within 90 days of collection.
The clinical site must remain blinded to the QGIT result. **Do NOT** report results of QGIT testing to the clinical site staff. Submit all completed CRFs and results to zimmer@fstrf.org or fax to (716) 834-8432.

4.1.2 QGIT Supernatant (plasma) Storage

QGIT supernatant will be stored and will be used for a variety of immune-related studies and TB biomarker discovery on a subset of mothers, who are selected at enrollment. Selection of these participants is indicated in the confirmatory message from the FSRTF Subject Enrollment System.

QGIT supernatant can be used for multiplex cytokine platforms. There are also micro RNAs and proteomic approaches that are forthcoming and it is anticipated that the supernatant can also be used for biomarker studies using these platforms.

Refer to the LPC for processing, storage and shipping instructions for the QGIT supernatant samples.

4.2 PBMC Processing and Cryopreservation

Peripheral Blood Mononuclear Cell (PBMC) processing and cryopreservation will be done among a subset of mothers and infants, who are selected at enrollment. Selection of these participants is indicated in the confirmatory message from the FSRTF Subject Enrollment System.

PBMC processing should be completed as soon as possible but within 8 hours of specimen collection; thus transfer from the clinic to the processing laboratory should also occur as soon as possible. Blood should be transported to the processing laboratory at room temperature. Samples must be accompanied to the processing laboratory with a completed F3006 tracking form.

**NOTE:** PBMCs will be collected at a limited number of pre-approved sites.

For the comprehensive procedures, refer to the IMPAACT procedures as described in the Cross-Network PBMC Processing Standard Operating Procedure. See HANC Cross-Network PBMC Processing Guide:

http://www.hanc.info/labs/labresources/procedures/Pages/pbmcSop.aspx

Refer to the LPC for processing, storage and shipping instructions for the PBMCs.

4.3 Mycobacteriological Studies

Sites should follow their site-specific SOP and use the best locally available method for performing the TB diagnostic tests in adults and infants.

4.3.1 Sputum Sample Storage/Transport Guidelines

Please refer to the HANC Cross-Network Mycobacteriology Sputum Sample Storage and Transportation guidelines:
4.3.2 Storage of Culture-Confirmed TB Isolates

For each participant, isolates from any positive culture (either liquid or solid or both) demonstrated to be positive for mycobacterium tuberculosis (MTB) must be stored frozen for further evaluation. All specimen labels should be generated via the LDMS using the mandated barcode label format. See the LPC for specific LDMS specimen codes.

Once the isolates are prepared, incubated, and examined for purity, the original culture plates or slants may be discarded per local procedures and policies.

Organisms preserved for future study must be maintained in a viable state free of contamination and without changes in genotypic or phenotypic characteristics. Laboratories that do not perform drug susceptibility testing of MTB cultures may be required to ship the organisms to another laboratory. The microbial viability of TB strains depends on the storage temperature and the suspending medium.

a. Isolate types

Mycobacterial culture isolates on Lowenstein-Jensen, 7H10, or other comparable solid AFB media or in a liquid culture broth from systems such as BACTEC 12B or BACTEC MGIT 960.

Failure to submit “pure” cultures will delay and/or adversely affect the drug susceptibility process.

b. Storage Cryovial

Add 2 to 5 ml of 5% concentration skim milk or brucella broth with 5% glycerol (cryoprotectant) to plastic (polypropylene) or glass (borosilicate) tubes with screw tops and silicone washers that can withstand very low temperature and maintain a seal.

c. Growth in Middlebrook broth (MGIT)

Grow isolate in Middlebrook broth; specimens are centrifuged at 3000 to 3500 g to create a pellet of organisms. The pellet is withdrawn and re-suspended in 2 to 5 mL of broth with the cryoprotectant added.

d. Growth on Solid Media

A sample of broth containing cryoprotectant is placed on the surface of the agar with growth. The surface is scraped with a pipette or sterile loop to suspend organisms. The entire growth should be scraped and suspended. The broth mixture is pipetted directly into the freezer vials.

Alternative (solid media only): The agar surface can be scraped with a sterile loop then transferred directly into the storage vial of cryoprotectant broth and emulsified into a final dense suspension.

e. Growth in BACTEC 12B Media
Growth must be sub-cultured to LJ slants, and then follow directions above for growth on solid media.

f. Storage

Freeze at temperatures of -70°C to -196°C for prolonged periods. TB isolates are to be stored locally until further instructions from the P1078 protocol team.

If stored in liquid nitrogen, store in the vapor phase and use certified cryovials for liquid nitrogen temperatures only. Disinfect storage vials with an approved laboratory disinfectant prior to storage.

4.3.3 Gastric Aspirate Sample Processing and Storage

Step 1: NALC-NaOH Processing
1. Work in a biological safety cabinet.
2. Take a sterile aerosol-free 50 ml centrifuge tube with screw cap, add equal amounts of specimen and activated NALC-NaOH solution.
3. (BD: BBL Mycoprep Kit REF 240862 – kit also contains sachets for phosphate buffer, which must be sterilized after reconstitution)
4. Cap the centrifuge tube and mix well in a shaker for 15 minutes. If there is no shaker available vortex well and let stand for 15 minutes with occasional gentle shaking. Do not treat for longer than 15 minutes.
5. Add the prepared phosphate buffer to the 50 ml mark and mix. Centrifuge for 15 minutes at 3000 X g.
6. Carefully decant the supernatant fluid.
7. Add a small amount of phosphate buffer of pH 6.8 and re-suspend the sediment.
8. (Now you can either inoculate your culture tube – e.g. MGIT tube or store the specimen by freezing [See below].)

Step 2: Freezing Method – Glycerol peptone water with beads
1. Glycerol Peptone Water Medium
   • Difco Protease peptone no. 3: 2.5g
   • Glycerol: 20 ml
   • Reversed osmosis water: 230 ml
   • Boil to dissolve and adjust pH to 7.3
   • Aliquot in small bottles (20 to 30 ml)
   • Sterilize at 121°C for 20 min

2. Method for Preparing the Tubes with Glycerol Peptone Water
   • Put 10 to 15 glass beads (LASEC - 4 mm Glass Beads #GlasB6M4) in 2 ml Nunc vials.
   • Autoclave for 20 min at 121°C (the caps must not completely tighten).
   • After cooling, add 0.1 ml of the sterilized glycerol-peptone medium to each vial, aseptically.
   • Add the pellet that has been re-suspended in Phosphate buffer from the processed sputum or gastric fluid.
   • Mix on a vortex for 30 seconds.
   • Store the vial at -70°C (-20°C if -70°C is unavailable).
Step 3: Retrieval for PCR
1. Remove one to two glass beads under sterile conditions
2. Ensure that the remaining beads do not thaw.
3. Put bead/s in distilled water or saline – about 300 to 500 microliters, and boil for 20 to 30 minutes in a 2 ml eppendorf tube – send it for PCR.

4.4 Hair Collection

Hair is to be collected from all mothers who have consented at the visits that coincide with 8 and 28 weeks post-entry and Weeks 20 and 40 postpartum and from all infants for whom consent has been given at Birth and Week 24 of life.

4.4.1 Materials Required
- Small pair of scissors
- Alcohol wipes
- Aluminum/Tin foil. Cut into squares approximately 5cm x 5cm and fold into quarters.
- Zipper bags
- Labels (2) with PID; visit; collection date and time; and collector’s initials
- Desiccant pellets
- Hair Sample Collection CRF PKW0390

As shown in the above picture, the sites should prepare the hair collection bags with all supplies needed for hair collection prior to a participant visit.
4.4.2 Collection Procedure

a. Clean the blades of a pair of scissors with an alcohol pad and allow blades to completely dry prior to use.

The pair of scissors used to collect the hair samples should be cleaned prior to using on each participant. Reclean the blades of the scissors with an alcohol pad and allow blades to completely dry prior to reuse.

b. Unfold the piece of tin foil before cutting the hair.
c. Lift up the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (~20-30 strands) from underneath this top layer of hair (can use a hair clip to keep the top layer of hair away). **From Infants:** Isolate a small thatch (~20-30 strands) from the back of infant’s head.

d. Cut the small hair sample (20-30 strands) off the patient’s head as close to the scalp as possible, keeping fingers firmly on part farthest from scalp (distal end).
e. For short hair, collect straight into foil; no need to label distal end (1 cm = 1 month growth)
f. For braided hair, collect from short strands between braids.

g. Place the cut thatch of hair inside the piece of foil with fingers over the distal end.
h. Place a small label with the participant PID over the distal end of the hair thatch (the side furthest away from the scalp).

![Label with PID; visit; collection date and time; and collector's initials]

i. Refold the foil over to completely enclose the hair. Place a Label with PID; visit; collection date and time; and collector’s initials on the top of the foil. Place the folded piece of foil inside the plastic zipper bag. Place a desiccant in each bag and seal the bag.

![Plastic zipper bag with folded foil and label]

j. Complete CRF PKW0390.
4.4.3 Storage of Hair

Hair samples should be logged into the LDMS and labeled with LDMS-generated labels. Label the bag with the LDMS generated label.

Hair samples should be kept at room temperature and in a dark place at each site until instructed to ship.

4.4.4 Hair Collection Supplies

- **Aluminum Foil**: Quill Diagnostics, product name Handy Foil or Standard Aluminum Foil, catalogue number 035-11205: 12 inches x 100 feet. Alternatively, the foil can be purchased locally, if sites can find a better price.
- **Desiccants**: Order from U-Line. Phone: 1-800-295-5510; fax: 1-800-295-5571. The product is called 1/2 g Silica Gel Desiccants, catalogue number S-8032: 1 pail (6000 bags/container), $133.00.
- Scissors, and Hair clips can be purchased locally.
- Zipper bags should be small and can be purchased locally.
5.0 Resources

Appendix I: Guidelines for the Management of a Newborn Infant in Contact with a TB Source Case

This algorithm is an example from the South African sites. It is included as an example only. Sites should refer to their local/national guidelines if available.

Important principles:
At antenatal visits and during labour, always ask the mother about TB disease or exposure:
1. Symptoms suggestive of TB (cough ≥14 days, fever, night sweats, loss of weight, chest pain, haemoptysis)
2. Current TB treatment in the mother
3. Current contact with a household TB source case (or suspected source case)

Action:
1. Document findings on the antenatal clinic card
2. Immediately refer mother (or other source case) to the TB clinic for investigation if suggestive symptoms present
3. Always check maternal HIV status, as this will affect the infant’s management

What represents close TB contact in an infant?
A mother with recently diagnosed TB (irrespective of type of TB or smear/culture result):
Who has received less than 2 months of TB treatment at the time of delivery or
Whose sputum smear or culture has not yet converted to negative or is unknown at the time of birth
Close contact with any infectious (usually adult) TB source case – see bullets above

*If contact is with a drug-resistant source case, immediately refer to specialist clinic for management
Management of the infant

**Well infant**
- No symptoms or signs of any disease

**Unwell infant: any of following**
- Respiratory rate $\geq 60$/min or difficulty with breathing
- Feeding problems
- Abdominal distension or masses, prolonged jaundice

**Do not give BCG vaccine**
- Record TB contact and action taken on RTHC
- Refer to TB clinic for Isoniazid Preventive Therapy (IPT)

**Establish HIV exposure status of infant & refer HIV-exposed infant to primary health care clinic for HIV PCR testing / PMTCT follow-up (see infant HIV testing algorithm)**

**At delivery (MOU or hospital)**

**At TB clinic**

**Isoniazid Preventive Therapy (IPT)**

**Well infant**
- Monthly follow-up for symptoms / signs of TB disease
- Adjust INH dose for current weight of infant (refer to dosing chart below)
- Establish HIV status of infant & refer HIV-infected infants for ART

**Unwell infant**
- Hospital referral

**No TB disease suspected**
- Complete 6 months IPT

**Establish HIV status of infant**

**HIV PCR negative**
- Give BCG vaccine
  - Or refer to MOU / hospital for BCG vaccination

**HIV PCR positive**
- Do not give BCG vaccine (contra-indication)
  - Ensure HIV-infected infant referred for ART

**TB disease suspected**
- Investigate and initiate TB treatment if necessary
- Do not give BCG vaccine after TB treatment completion

**Note:** the breastfed HIV-exposed infant on PMTCT or infant whose mother is on HAART and who tests HIV negative after completion of IPT: give BCG even if continuing to breastfeed.
IMPORTANT PRINCIPLES TO REMEMBER

1. Early diagnosis and treatment of TB in a pregnant woman will prevent TB exposure in the infant.
2. Women with suspected TB during pregnancy should be urgently referred to the TB clinic for a sputum smear and culture and to be assessed by the TB doctor. (Refer to PALSA plus guidelines)
3. Most TB drugs are safe during pregnancy and should be continued if a woman becomes pregnant, with the exception of streptomycin, kanamycin and amikacin which are ototoxic to the fetus.
4. A pregnant women who develops side effects of treatment should be evaluated by the TB doctor.
5. Immediate prophylaxis (IPT) to infants in contact with a TB source case(s) is extremely important, as infants have a very high risk of progressing to TB disease if they do not get prophylaxis.
6. Mothers should be counseled regarding the risk to their infants if they are exposed to TB through other individuals in the household.
7. A mother on TB treatment may breastfeed as long as the infant receives prophylaxis.

### Dosing for Isoniazid Preventive Therapy (IPT) in Children

<table>
<thead>
<tr>
<th>Body Weight (kg)</th>
<th>Daily isoniazid (INH) 100mg tablet</th>
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<tbody>
<tr>
<td>2 – 2.4</td>
<td>¼</td>
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<td>2.5 – 4.9</td>
<td>½</td>
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<td>5 – 7.4</td>
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<td>7.5 – 9.9</td>
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<tr>
<td>10 – 19.9</td>
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<tr>
<td>20 – 24.9</td>
<td>2 ½</td>
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<td>≥ 25</td>
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### TB Drug Dosing Chart for Children < 8 years of age (2008)

<table>
<thead>
<tr>
<th>Body Weight (kg)</th>
<th>Uncomplicated TB disease</th>
<th>Complicated TB disease (excluding TB meningitis &amp; miliary TB)</th>
<th>Body Weight (kg)</th>
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<tbody>
<tr>
<td></td>
<td>Intensive phase 2 months</td>
<td>Continuation phase 4 months</td>
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<tr>
<td></td>
<td>RHZ dissolvable tablets 60/30/150mg (scored)</td>
<td>RH dissolvable tablets 60/30mg (scored)</td>
<td>RHZ dissolvable tablets 60/30/150mg (scored)</td>
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<td>2 - 2.9</td>
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<td>Use Eto ¾</td>
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<td>5 - 6.9</td>
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Compiled by the Paediatric HIV/TB Policy Reference Group, Western Cape Department of Health, 2010
Adapted from South African National Tuberculosis Management Guidelines, 2008

H = Isoniazid, R = Rifampicin, Z = Pyrazinamide, E = Ethambutol, Eto = Ethionamide

**Uncomplicated TB disease in children** = new smear negative pulmonary TB, or mild forms of extrapulmonary TB e.g. lymphadenitis, pleural effusion;

**Complicated TB disease in children** = new smear positive pulmonary TB, or extensive parenchymal/cavitating lung disease, or extrapulmonary TB (excl. TB meningitis or miliary TB), or patients with severe immunosuppression from HIV disease

Children with suspected **TB meningitis or miliary TB** should be referred to hospital and discussed with an expert for advice on investigation & management.
Appendix II: Template Participant Handout on Signs and Symptoms of Hepatotoxicity in IMPAACT P1078

You may be receiving a medication in this study called isoniazid (INH). You may experience some symptoms of liver disease if your body is not doing well with the medication. These are shown below. Some people may not have any signs or symptoms but may still have liver disease. This is why we test your blood. In rare cases, liver damage from INH can be very serious or even cause death. **If you have any of these symptoms, please immediately stop taking your medication and call your doctor and the site investigator as soon as possible.** If the site contacts you because your liver tests are abnormal, please return to the site as soon as possible, even if you do not have any signs or symptoms. [Sites: insert here the emergency contact number]

Nausea, vomiting, or unexplained loss of appetite

Pain in the upper abdomen

Yellow eyes or yellow skin

Increased weakness or fatigue

Other signs or symptoms include

- unexplained weight loss
- changes in the color of urine (may get darker, like Coca Cola)
- or pale or clay-colored stools


You may also have skin rash, loose or watery stools, or fever. If any of these symptoms occur with those pictured, immediately stop taking your medication. Call your doctor and the site investigator as soon as possible.
Appendix III: Participant Instructions for Collecting Sputum from Mothers

You may be asked to give sputum samples during the study.

1. You will get special cups for the collection. The cups will be labeled for you and they may have the numbers 1 and 2. Please use them in order. For instance, for the first specimen, use the cup labeled #1 and so on. Please do not open the cup until you are ready to use it. It is very clean inside.
   a. Write the date and time on the label just before you use it.

2. The doctor/nurse will tell you when they want the sample. It may be in the clinic or at home.

3. Before you give the sample, take a deep breath and hold it. Slowly breathe out. Take another deep breath and then cough hard from the lungs until sputum comes into your mouth. Cough as deeply as you can to bring up the sputum.

4. Spit the sputum into the cup. Do not touch the inside of the cup.

5. Screw the top on very tightly. Wipe off the outside of the container.

6. Put the container into a bag.

7. If you collect the sample in the clinic, give it to the doctor/nurse. They will tell you if there is enough sputum.

8. If you collect the sample at home, collect it first thing in the morning before you eat or drink. Rinse your mouth with clean water.
   a. Collect the sample outside and away from others.
   b. Please try to collect a morning sputum sample, even if you cannot come to the clinic until late in the day.
   c. Please bring the sample back to the clinic as soon as possible. It must be within 3 days of collection. If you cannot bring the sample in on the same day you collect it, store it in the refrigerator. If you do not have a refrigerator, please do not collect the sample until the morning of your clinic visit.
   d. If you cannot produce any sputum, you might try drinking a couple glasses of warm water first.
   e. If you are unable to produce a proper sputum sample after drinking the warm water, please come to the clinic and they will try other methods like asking you to breathe in a saltwater, steam-like mist through a tube or a mask.

Please be sure to give whatever sample you produce at all times as requested.

Thank you!