APPENDIX II: SCHEDULE OF EVALUATIONS FOR INFANTS

<table>
<thead>
<tr>
<th>EVALUATION</th>
<th>Birth&lt;sup&gt;8&lt;/sup&gt; (through day 3 of life)</th>
<th>5-9 days of life (infants undergoing washout PK only, see Section 4.4)</th>
<th>16 – 24 weeks of life&lt;sup&gt;10&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLINICAL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Exam&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>HIV Infection Status&lt;sup&gt;2&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>History/Chart abstraction&lt;sup&gt;3&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Audiology assessment&lt;sup&gt;9&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>PHARMACOLOGY</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washout PK Sampling Plasma</td>
<td>2.25 mL&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.75 mL&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dried Blood Spot</td>
<td>0.25 mL&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.25 mL&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL BLOOD VOLUME</strong></td>
<td>0 to 2.5 mL</td>
<td>0 to 1 mL</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX II FOOTNOTES

1. Physical exam should include measurement of length and weight.
2. Record all HIV test results available.
3. At birth, record the following results/events that are available since birth. After the birth visit, record all available results/events since the last visit.
   - Infant feeding status, if available
   - Hematology: All ≥Grade 2 results for CBC with differential and platelets; first and last results at each reportable grade and first normal result afterwards.
   - Chemistries: All ≥Grade 2 results for glucose, AST, ALT for all infants. For HIV-infected infants, also record ≥Grade 2 electrolytes, BUN, creatinine, total and direct bilirubin, lipase, albumin, total protein, triglycerides, cholesterol, CPK, and amylase.
   - Signs/Symptoms: All ≥Grade 1 neurologic events, and all other ≥Grade 2 events.
   - Diagnoses: Any diagnoses as defined in the Diagnoses Appendix (which can be found at www.fstrf.org), including congenital anomalies and mitochondrial disorders.
   - Note that all Grade 3 or 4 events and deaths, and all ≥Grade 1 neurologic events must be further evaluated on the study Event Evaluation Form.
   - Record any concomitant prescription medicines since birth. After the birth visit, record any concomitant prescription medicines taken since the last visit.
4. Collection of PK samples can be omitted, with approval of the protocol team, if there are circumstances that prohibit collection (i.e. delivery at non-study facility). Only infants who meet the requirements for infant washout PK will undergo pharmacology evaluations; see protocol Section 4.4 for infant requirements for washout PK sampling.
5. Blood samples to be collected at 2-10, 18-28 and 36-72 hours after birth. See Laboratory Processing Chart (LPC) for collection, processing and shipping instructions.
6. Single blood sample to be collected between 5 – 9 days of life. See Laboratory Processing Chart (LPC) for collection, processing and shipping instructions.
7. For infants for which informed consent has been obtained for genetic testing: Obtain once, at any collection, at either the Birth (through day 3 of life) Visit OR at the 5 - 9 days of life visit (if washout PK to be collected). See Laboratory Processing Chart (LPC) for collection, processing and shipping instructions.
8. Evaluations can be done from birth through day 3 of life; day of birth = day 0
9. Only for infant of mothers treated with any injectable TB medication. The audiology assessment can be from chart abstraction or otherwise can be performed at any time during follow-up; refer to the LPC for additional information.
10. Abstract data from the participants chart if available within the window for the visit.
## Section 2: Safety/Clinical Laboratory Evaluations

*At birth, record the following results/events that are available since birth. After the birth visit, record all available results/events since the last visit.*

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>DMC Test Code</th>
<th>Tests</th>
<th>CRF #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>N/A</td>
<td>N/A</td>
<td>PE6813</td>
</tr>
<tr>
<td>Chemistry</td>
<td>N/A</td>
<td>N/A</td>
<td>PE6818</td>
</tr>
</tbody>
</table>

For audiology testing information, see the P1026s protocols-specific website at [http://www.impaactnetwork.org/studies/P1026s.asp](http://www.impaactnetwork.org/studies/P1026s.asp).
## Section 3: Specimen Processing & Shipping Instructions

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Tube Type</th>
<th>Special Collection Notes</th>
<th>CRF # DMC Test Code</th>
<th>Processing</th>
<th>Shipping</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Washout PK Sampling Plasma</strong></td>
<td>EDTA or Spray-dried K2 EDTA</td>
<td>N/A</td>
<td>PKW0366</td>
<td>Transfer to laboratory within 1 hour of collection at room temperature.</td>
<td>Send a carbonless copy of the PKW form to the analyses lab(s) with the specimen(s).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PKRAN</td>
<td>Centrifuge blood within one hour of collection at 800xg for 10 mins.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Freeze all plasma in one aliquot at −70°C or lower.</td>
<td></td>
</tr>
</tbody>
</table>

**Blood samples to be collected at 2-10, 18-28 and 36-72 hours after birth, and between 5-9 days of life.**

**ARV PK Samples**
- **All Sites (except African and Thai Sites):** Ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.
- **African Sites:** Only EFV, LPV, NVP, RTV drugs, ship all specimens ‘real-time’ to Lubbe Wiesner, PhD. Laboratory, Cape Town. For other drugs, ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.
- **Thai Sites:** Only EFV, ATV, LPV, NVP, DRV, RTV drugs, ship all specimens ‘real-time’ to Tim Cressey, PhD PHPT Laboratory. For other ARV drugs, ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.

(see shipping instructions at end of LPC)
| Genotyping (Pharmacogenetics) | EDTA or Spray-dried K2 EDTA | Obtain once, at any collection, at either the Birth (through day 3 of life) Visit OR at the 5 - 9 days of life visit.  
**Note:** A 1.0 ml EDTA blood can be drawn by including Washout PK Sample. Blood sample is used for DBS preparation first, remaining blood would be processed for Washout PK plasma aliquot. | F3008 PKGENO | EDTA - Dried Blood Spot (250 microliters of whole blood required to apply 50 microliters to each of the 5 spots on Whatman 903 card).  
Take two Whatman 903 Protein Saver Cards. In one card, take 50 μL of whole blood and apply to the first spot. Skip the second spot and apply in the third spot. Skip the fourth spot and apply in the fifth spot.  
In the second card, take 50 μL of whole blood and apply to the first spot. Skip the second spot and apply in the third spot.  
Label each spot with a different Laboratory Data Management System (LDMS) Global ID.  
Ensure the DBS cards are completely dry before packing. Place both cards in a gas impermeable bag with a desiccant pack and humidity indicator card.  
The sealed bag containing the DBS should be stored in a -20°C freezer until it is shipped. The DBS should not be stored at room temperature.  
During storage the desiccant should be replaced if humidity exceeds 30%.

| **Shipping** | The DBS will be shipped at ambient temperature. Remove the sealed bag containing the DBS from the freezer. Allow the DBS to thoroughly equilibrate to room temperature for a minimum of 30 minutes prior to opening the bag. After equilibrating, the bag should be opened and the desiccant replaced with multiple packs of fresh desiccant prior to shipment.  
All Sites (except Thailand): Batch ship the DBS ambient every 6 months. NIAID Labs ship to BRI and NICHD Labs ship to Fisher repository (see shipping instructions at end of LPC).  
Thai Sites: Ship all DBS ambient to Tim Cressey, PhD PHPT Pharmacology Laboratory every 3 months, with the frozen PK samples if available. Place the DBS in an envelope outside the sealed dry ice shipper (see shipping instructions at end of LPC). |
APPENDIX II

Section 4: Evaluations by Visit - refer to Section 3 for processing instructions

at 2-10, 18-28 and 36-72 hours after birth and between 5 – 9 days of life

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Specimen</th>
<th>CRF</th>
<th>Aliquots</th>
<th>LDMS Code</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washout PK Sampling Plasma</td>
<td>EDTA or Spray-dried K2 EDTA 0.75mL</td>
<td>PKW0337</td>
<td>Freeze all plasma in one aliquot at –70°C or lower.</td>
<td>BLD/EDT/PL1 for EDTA or BLD/DPE/PL1 for spray-dried K2 EDTA</td>
<td>Process and freeze within 1 hour of collection.</td>
</tr>
</tbody>
</table>
### Pharmacogenetics Genotyping

For subjects consenting to pharmacogenetic sampling

Obtain once, at any collection, at either the Birth (through day 3 of life) Visit or at the 5 - 9 days of life Visit.

<table>
<thead>
<tr>
<th>EDTA or Spray-dried K2 EDTA</th>
<th>F3008 PKGENO</th>
<th>BLD/EDT/DBS for EDTA or BLD/DPE/DBS for spray-dried K2 EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25mL EDTA blood can be drawn by including Washout PK Sample. Blood sample is used for DBS preparation first, remaining blood would be processed for Washout PK plasma aliquot.</td>
<td>Two Whatman 903 Protein Saver Cards. In one card, 50 μL of whole blood applied to the first spot, the third spot and the fifth spot. In the second card, 50 μL of whole blood applied to the first spot and the third spot. (See specimen processing in Section 3.)</td>
<td>The sealed bag containing the DBS should be stored in a -20°C freezer until it is shipped. The DBS should not be stored at room temperature. Label each spot with a different Laboratory Data Management System (LDMS) Global ID. Ensure the DBS cards are completely dry before packing. Place both cards in a gas impermeable bag with a desiccant pack and humidity indicator card.</td>
</tr>
</tbody>
</table>

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**APPENDIX II**

### Section 5: Shipping Addresses

ACTG / IMPAACT Laboratory Manual, Shipping Information and other useful information: [http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx](http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx)

Do not ship samples to arrive on Friday/weekend/holiday.
US Sites ship on Monday through Wednesday to arrive the next day. Non-US Sites ship on Monday or Tuesday only.

Ship samples to:
Steven S. Rossi, Ph.D. or Rowena Espina MT
University of California, San Diego
Pediatric Pharmacology and Antiviral Assay Laboratory
9500 Gilman Drive
Medical Teaching Facility (MTF) Building, Room 233
La Jolla, CA 92093
Phone: (858) 246-2806
Phone: (858) 246-2824
E-mail: ssrossi@ucsd.edu or respina@ucsd.edu
LDMS Lab Code: 196

African Sites ship on Monday through Wednesday to arrive the next day.

Ship samples to:
Lubbe Wiesner, PhD
Clinical PK Lab Director
Division of Pharmacology, Univ. of Cape Town, K50.30 Division of Clinical Pharmacology
Old Main Building, Groote Schuur Hospital Observatory, 7925
CAPE TOWN
SOUTH AFRICA
lubbe.wiesner@uct.ac.za
Contact persons: Jennifer Norman and Shameema Witbooi
Jennifer.Norman@uct.ac.za and Shameema.Witbooi@uct.ac.za
Tel: +27 21 404 7695
Fax: +27 (0)86 669 1348
LDMS Lab Code: 499

Thailand Sites ship on Monday through Wednesday to arrive the next day.

Ship samples to:
Tim Cressey, Ph.D
PHPT Laboratory
548 ChiangMai-Lamphun Road, Nong Hoi Muang, Chiang Mai 50000
Thailand
Phone: +66-53-894-431
Fax: +66-53-894-220
Email: tim.cressey@phpt.org or Laddawan@phpt.org
LDMS Lab Code: 251

PHPT will batch ship DBS, Vaginal Swab, Vaginal Secretion specimens every 6 months to BRI.

REPOSITORY SPECIMENS – NIAID Sites ONLY

John Ward
Biomedical Research Institute
9410 Key West Avenue, First Floor
Rockville, MD 20850
Phone (301)881-7636
Fax (301)770-9811
Email brirepository@afbr-bri.com
LDMS lab code: 999

REPOSITORY SPECIMENS – NICHD Sites ONLY

Fisher Bioservices
Attn: Maria Wolff
IMPAACT
625 Lofstrand Lane
Rockville MD 20850
Tel: 301-340-1620
Fax: 301-838-9753
Email: maria.wolff@thermofisher.com
LDMS Lab Code: 243

Revision: 27th March 2017

Page 4 in Section 3: Specimen Processing & Shipping Instructions (ARV PK samples from sites at different locations)

Send a carbonless copy of the PKW form to the analyses lab(s) with the specimen(s).
ARV PK Samples

All Sites (except African and Thai Sites): Ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.

African Sites: Only EFV, LPV, NVP, RTV drugs, ship all specimens ‘real-time’ to Lubbe Wiesner, PhD. Laboratory, Cape Town. For other drugs, ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.

Thai Sites: Ship all specimens ‘real-time’ to Tim Cressey, PhD. PHPT Laboratory.

Page 7 in Section 5: Shipping Addresses

Change addressee and contact persons of Cape Town Lab to
Lubbe Wiesner, PhD
Clinical PK Lab Director, lubbe.wiesner@uct.ac.za

Contact persons: Jennifer Norman and Shameema Witbooi
Jennifer.Norman@uct.ac.za and Shameema.Witbooi@uct.ac.za

Revision: 17th May 2017

Page 4 in Section 3: Specimen Processing & Shipping Instructions (ARV PK samples from sites at different locations)

Thai Sites: Only EFV, ATV, LPV, NVP, DRV, RTV drugs, ship all specimens ‘real-time’ to Tim Cressey, PhD PHPT Laboratory. For other ARV drugs, ship all specimens ‘real-time’ to Steven S. Rossi, PhD. University of California, San Diego.

Revision: 11th September 2017

Page 8 in Section 5: Shipping Addresses

Change Steve S. Rossi Lab address to
9500 Gilman Drive
Medical Teaching Facility (MTF) Building, Room 233
La Jolla, CA 92037
Phone: (858) 246-2806
Phone: (858) 246-2824

Revision: 5th December 2017

There is no change in this App II LPC.

Changes in App I-A and App I-B LPCs: All African Sites ship Cord Blood and Maternal Delivery Samples along with the intensive postpartum PK samples to Lubbe Wiesner, PhD Laboratory, Cape Town.
Revision: 30th August 2018

There is no change in this App II LPC.
Vaginal Secretion Processing in App I-A and AMR LPC was revised.
DLM sample collection and processing were added in App I-B and AMR LPC and App I-C LPC.