IMPAACT 2001 LABORATORY CONSIDERATIONS

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WITH LOA 1-2, CM 1-3

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LPC

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- LPC Version 1.0 is available on the study specific website:
  - http://www.impaactnetwork.org/studies/IMPAACT2001.asp
- LPC Contents
  - Title page
  - Table of Contents
  - Standard LPC Format:
    - Section 1: Protocol-Required Non-standard Reagents and supplies
    - Section 2: Safety and Clinical Laboratory Evaluations
    - Section 3: Specimen Processing Instructions
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OVERVIEW

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   - Prioritization of testing

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I. LABORATORY TESTS

Section 1: Protocol-Required Non-Standard Reagents and Supplies		
Evaluation	**Reagent or supply**	**Order information**
IGRA(QGIT)	QuantiFeron TB Gold(In Tube method) tubes	**Tuberculosis and Control Antigen Blood Collection Tubes** **Catalogue Number: 0590 0301** 1. Nil Control (Grey cap with white ring) 100 x tubes 2. TB Antigen (Red cap with white ring) 100 x tubes 3. Mitogen Control (Purple cap with white ring) 100 x tubes **High Altitude (HA) Blood Collection Tubes *(for use above 3,350 feet)*** **Catalog Number: T0590 0505 (refer Section 5)** 4. Nil Control (Grey cap with yellow ring) 100 x tubes 5. TB Antigen (Red cap with yellow ring) 100 x tubes 6. Mitogen Control (Purple cap with yellow ring) 100 x tubes Tubes are also available in other configurations: 100 x Nil Control, 100 x TB Antigen tubes **(Cat. No. 0590 0201)** 100 x Mitogen Control tubes **(Cat. No. 0593 0201)**
	QuantiFeron TB Gold(In Tube method) ELISA test kit	ELISA Components – **Catalogue Number: 0594 0201**
K3 EDTA vacutainer tubes	BD Vacutainer- **EDTA (Spray-dried) vacutainer tubes**	Whole Blood Tube; Size: 13x75 mm x 2.0 mL K2 EDTA (spray dried) Plastic; Label: Paper; Closure: Hemogard™; Color: Lavender (**Catalog # 367842**) or Lavender (**Catalog # 367841**)

- Required to use 3rd Generation QuantiFeron TB Gold tubes

APPENDIX I-A: MATERNAL SCHEDULE OF EVALUATIONS

NOTE: NIH RECOMMENDATIONS FOR MAXIMUM PEDIATRIC AND ADULT BLOOD DRAW VOLUMES WILL BE FOLLOWED IN THIS STUDY. FOR WOMEN, THE VOLUME OF BLOOD DRAWN SHALL NOT EXCEED 10.5 ML/KG OR 550 ML, WHICHEVER IS SMALLER, OVER ANY EIGHT WEEK PERIOD.

Evaluation	**SCR (*up to 2 weeks prior to entry*)**	**Entry/Intensive PK Sampling (*Days 0-3*)**	**Weekly (7 ± 2 days apart) (*weeks 1-11*)**	**Postpartum Visit (Within 3 days of delivery; if study drug regimen completed prior to delivery only)**	**Monthly (Every 4 weeks ±2 weeks) (*until 24 weeks after delivery*)**	**Study Exit/ Early Discontinuation**
Gene Xpert and sputum microscopy (if needed)	X					
IGRA (3-4mL) (if needed and not available per medical record)	3-4mL					
Complete blood count (CBC)	3mL		3mL (every 4 weeks)	3mL (if clinically indicated)	3mL	
Liver Function tests	3mL		3mL (every 4 weeks)		3mL	
HIV-1 test (confirmatory tests as needed)	6mL					
CD4 count (HIV-1-infected only)		3mL				
Coagulation profile	5mL		5mL (at one visit at >/= 34 weeks gestational age only)	5mL (if clinically indicated)	5mL (if clinically indicated)	
HBsAg, HBsAb, HCV Ab		6mL				
Intensive PK sample collection		22mL				
Sparse PK sample collection			8mL (last dose)			
Plasma sample collection (breast milk PK eligible only)			2-4mL (first and second week postpartum)			
Breast milk PK sample collection (if eligible)			X (first and second week postpartum and last dose)			
TOTAL BLOOD	17-21mL	28-31mL	11-23mL	8mL	6-11mL	

ON STUDY LOCAL LABORATORY TESTS

Maternal Schedule of Evaluations:

- GeneXpert MTB/RIF and sputum microscopy
- IGRA (3-4mL) (if not available per medical record): QuantiFeron Gold In-tube Test (QGIT)
- Complete blood count (CBC): White blood count, Hemoglobin and Platelets
- Liver Function tests: Albumin, AST, ALT, and Total bilirubin
- HIV-1 test (confirmatory tests as needed)
- CD4 count (HIV-1-infected only): CD4 cell counts and percentages
- Coagulation profile: Prothrombin time (PT) only
- HBsAG, HBsAb, HCV Ab: Blood will be collected and stored at Entry visit for later testing of HbsAG, HbsAb, HCV Ab as determined by the protocol team

APPENDIX I-B: INFANT SCHEDULE OF EVALUATIONS

FOR INFANTS, THE VOLUME OF BLOOD DRAWN AT ANY STUDY VISIT SHOULD NOT EXCEED 5 ML/KG IN A SINGLE DAY AND 9.5 ML/KG OVER ANY EIGHT-WEEK PERIOD.

Evaluation	Newborn Visit (Within 3 days of life)	Monthly (Every 4 weeks+/- 2 weeks) (Until 24 weeks after delivery)
Single PK sample collection (if eligible)	2mL	
Coagulation profile (if eligible)	2mL	2mL (if clinically indicated)
Complete blood count (if clinically indicated)		1mL
Liver Function tests (if clinically indicated)		2mL
Cord blood (optional if eligible, during mother's delivery)	X	
TOTAL BLOOD	**2-4mL**	**1-5mL**

*Optional Cord Blood collection does not count against infant total blood volume limits.

ON STUDY LOCAL LABORATORY TESTS

Infant Schedule of Evaluations

- Coagulation profile: if eligible at Newborn visit and as clinically indicated during monthly visits
- Complete blood count (if clinically indicated at monthly visits)
- Liver Function tests (if clinically indicated at monthly visits): includes albumin, AST, ALT, and total bilirubin

SAFETY AND CLINICAL LABORATORY EVALUATIONS

Section 2: Safety/Clinical Laboratory Evaluations

Evaluation	Tube Type	Tests		DMC Test Code	CRF #
Interferon Gamma Release Assay (IGRA)	Nil, TB antigen, Mitogen	QuantiFeron Gold In-tube Test (QGIT)		MTBIGRA	TBW0034
Complete Blood Count (CBC)	EDTA	*White blood count, Hemoglobin and Platelets*		N/A	PE6813
Liver Function Test	SST or NON	*Albumin, AST,ALT, and Total bilirubin*		N/A	PE6818
HIV-1 test (confirmatory test as needed)	SST or NON or EDTA	*As per protocol section 4.1.4*		N/A	
CD4 count (HIV-1 infected only)	EDTA	CD4 cell counts and percentages	Dual platform labs only must also have a WBC and diff.	CD4CD8	LBW0054
Coagulation profile	Sodium citrate	Prothrombin time (PT) and INR if INR is available at the site, as per Sections 8.1.7 and 8.1.8.		N/A	PE6813
HbsAG,HbsAb,HCV Ab	SST or NON	Blood will be collected at Entry visit for later testing of HbsAg, HbsAb, HCV Ab		HEPSER	F3008 & SR0010

PRIORITY OF BLOOD SAMPLES

The priority order of sample collections will be as follows: samples needed for clinical safety assessments/AES will be collected first (specific tests as determined by investigator), followed by samples needed for PK analysis.

Prioritization of sample collection of blood tubes and processing for insufficient draws, by visit		
Visit	**Tube type**	**Purpose**
Screening (up to 2 weeks prior to entry)	3mL EDTA	Complete Blood Count
	3mLRed top or SST	Liver Function Tests
	5mL Blue top Sodium Citrate	Coagulation profile
	3mL QGIT tubes(Nil, Mitogen and TB antigen tube each)	IGRA
	6ml Red top or SST or EDTA	HIV-1 Test (confirmatory test as needed)
Entry/Intensive PK sampling (Days 0-3)	2mL **K2** EDTA (**spray dried)**	Intensive PK
	3mL EDTA	CD4 count
	6ml Redtop or SST	HbsAg,HbsAb,HCV Ab
Weekly Visits (Weeks 1-11),	3mL EDTA	Complete Blood Count
	3mL Red top or SST	Liver Function Tests
	5mL Blue top Sodium Citrate	Coagulation profile
	2mL **K2** EDTA (**spray dried)**	Sparse PK
	2mL **K2** EDTA (**spray dried)**	Plasma (PL1) for PK(1.2 ml minimum blood required)
Postpartum Visit (Within 3 days of delivery)	3mL EDTA	Complete Blood Count
	5mL Blue top Sodium Citrate	Coagulation profile
Monthly Visits (Every 4 weeks, until 24 weeks after delivery)	3mL EDTA	Complete Blood Count
	3mL Red top or SST	Liver Function Tests
	5mL Blue top Sodium citrate	Coagulation profile

II. SPECIMEN COLLECTION, PROCESSING AND STORAGE

SPECIMEN COLLECTION AND PROCESSING

The following tube types and volumes must be available:

- **3mL EDTA** (Complete Blood Count)
- **3mL Red top or SST** (Liver Function Tests)
- **5mL Blue top Sodium Citrate** (Coagulation profile)
- **2mL Blue top Sodium Citrate** (Infant Coagulation profile)
- **3mL QGIT tubes** (Nil, Mitogen and TB antigen tube each)
- **6ml Red top or SST or EDTA** (HIV-1 confirmatory test)
- **2mL K2** EDTA **(spray dried)** (Intensive PK and Sparse PK)
- **3mL EDTA** (CD4 Count)
- **6mL Redtop or SST** (HbsAg, HbsAb, HCV Ab)
- **2mL K2** EDTA (**spray dried)** (Plasma [PL1] for PK; 1.2 mL minimum blood required)

Section 3: Specimen Processing. Refer to Section 4 for tube types and collection volumes					
Evaluation	**Tube Type**	**Special Collection Notes**	**CRF # DMC Test Code**	**Processing**	**Shipping**
Intensive PK sample collection (day 0 and 72 hours following) Collection Time points: prior to first dose(t0), then 0.5, 1, 2, 4, 5, 8, 12, 24, 48, and 72hrs after the dose	**K2** EDTA **(spray dried)** (Polypropylene tube recommended)	Process within one hour of collection. Place plasma aliquots in crushed ice as INH is sensitive to temp. Storage must be within 1 hr at -70^{0}C.	PKW0392 PKINT	*Process within one hour of collection.* Spin blood at 1500 xg for 10min. Remove plasma and prepare 2 equal aliquots. Freeze at approx. -70°C LDMS spec. code: BLD/EDT/PL1 *Min: 2 x 0.2mL*	Ship to University of Cape Town, South Africa upon the team's request.
Sparse PK sample collection at 1, 4, 24, and 48 hrs after the dose	**K2** EDTA **(spray dried)** (Polypropylene tube recommended)	Process within one hour of collection.	PKW0392 PKRAN	*Process within one hour of collection.* *Spin blood at 1500xg for 10min.* Remove plasma and prepare 2 equal aliquots. Freeze at approx.-70°C LDMS spec. code: BLD/EDT/PL1 *Min: 2 x 0.2mL*	Batch ship to University of Cape Town, South Africa at end of the study or may be along with intensive PK samples shipped at that time.
Plasma sample collection (Breast milk PK eligible only) At 1st week after delivery-3 h after drug dose At 2nd week after delivery-6h after drug dose	**K2** EDTA **(spray dried)** (Polypropylene tube recommended)	Process within one hour of collection.	PKW0394 PKRAN	*Spin blood at 1500xg for 10min.* Remove plasma and prepare 2 equal aliquots. Store two plasma aliquots in separate cryoboxes. Freeze at -70°C LDMS spec. code: BLD/DPE/PL1 *Min: 2 x 0.2mL*	Batch ship to to University of Cape Town, South Africa at end of the study
Breast milk PK collection At 1st week after delivery-3 h after drug dose At 2nd week after delivery-6h after drug dose Last dose visit-24h after drug dose	15 mL sterile polypropylene conical tube or sterile plastic container	Place breast milk aliquots in crushed ice as INH is sensitive to temp.Storage must be within 1 hr at -70^{0}C.	PKW0394 PKRAN	Collect 5 mL of breast milk either from one or both breasts. Refer IMPAACT 2001 MOP for details. Prepare 2 x 1.5 mL aliquots or more, stored in cryovials and keep in crushed ice until freeze at -70^{0}C. LDMS spec. code: BMK/NON/BMW	Batch ship to University of Cape Town, South Africa at end of the study.

Section 3: Specimen Processing. Refer to Section 4 for tube types and collection volumes					
Evaluation	**Tube Type**	**Special Collection Notes**	**CRF # DMC Test Code**	**Processing**	**Shipping**
Cord blood PK collection (Optional) Only from women who have received study drug dose within 72 h prior to delivery *Collect 2 mL of Cord Blood if available at sites with capacity (e.g. during normal business hours)*	**K2** EDTA **(spray dried)** (Polypropylene tube recommended)	Process within one hour of collection.Place plasma aliquots in crushed ice as INH is sensitive to temp.Storage must be within 1 hr at -70^{0}C	PKW0393 PKRAN	*Process within one hour of collection.* Spin blood at 1500 xg for 10min. Remove plasma and prepare 2 equal aliquots. Freeze at approx. -70°C LDMS spec. code: CRD/EDT/PL1 Min: 2 x 0.2ML See IMPAACT 2001 MOP for instructions on collection, processing, and storage of Cord Blood.	Batch ship to to University of Cape Town, South Africa at end of the study.
HIV-1 test (confirmatory test as needed)	SST or NON or EDTA	Invert tube 8-10 times gently. Send to processing lab ambient.		Process within 6 hours. For HIV RNA PCR: Spin blood at 800xg for 10 min.Remove plasma. Re-spin plasma at 800xg for 10 min. Freeze 2x 1.8mL aliquots at -70°C or colder. LDMS spec. code: BLD/EDT/PL2	Refer to Protocol Section 4.1.4 **For U.S. sites**: Sample #2 must be tested in a CAP/CLIA approved laboratory. **For Non-U.S.sites**: Sample #2 must be tested in a lab that operates according to GCLP and participates in an EQA program.

CORD BLOOD PK

Cord blood PK collection (Optional)

- Only from women who have received study drug dose within 72 h prior to delivery
- *See IMPAACT 2001 MOP for instructions on collection, processing, and storage of Cord Blood*
- Collect 2 mL of Cord Blood if available at sites with capacity (e.g. during normal business hours)

BREAST MILK AND PLASMA PK SAMPLE COLLECTION

Breast milk PK collection

- At 1st week after delivery-3 h after drug dose
- At 2nd week after delivery-6h after drug dose
- Last dose visit-24h after drug dose

Plasma sample collection (Breast milk PK eligible only)

- At 1st week after delivery-3 h after drug dose
- At 2nd week after delivery-6h after drug dose

COAGULATION PROFILE: PROTHROMBIN TIME SPECIMEN COLLECTION AND PROCESSING CONSIDERATIONS

- **Preferred Specimen:** One full unopened 3.2% sodium citrate (light blue-top) tube.
  - 9:1 ratio of blood to citrate is critical. Do not uncap. Transport at room temperature.
  - **Perform testing within 48 hrs.**
  - Refrigerated or frozen specimen are unacceptable.
- **Alternative Specimen(s):** 1 mL frozen plasma.
  - **If the specimen will not be tested at the local lab and will be delayed longer than 48 hours**, centrifuge specimen for 15 minutes at 2500-3500 rpm. Using a plastic pipette, remove plasma, taking care to avoid the WBC/platelet (buffy) layer and place into a plastic screw-cap vial and freeze at -20° C. Ship on dry ice.
  - Stability if Frozen at -20°C is14 days
  - Stability if Frozen at -70°C is 6 months
- At Screening visit: For laboratories who are not performing coagulation profile testing locally, the maternal specimens collected at screening should be processed and frozen according to the MOP. These specimens should be batch shipped to the testing laboratory *at least every three months* or upon request by the protocol team.

SPECIMEN COLLECTION AND PROCESSING

Training Requirements

- PK Tutorials: Site and/or lab staff that are responsible for collecting and processing the samples, will need to complete the online Clinical Pharmacology Tutorial and keep the documentation in the training and keep the documentation in the training files. The certification is valid for two years.
- DAIDS GCLP Compliant (Non-US labs): training and competency assessments must be performed per site SOPs that are in compliance with DAIDS GCLP Guidelines. All documentation must be available during monitoring and audit visits.
- US labs should follow CAP/CLIA and local guidelines.

SPECIMEN COLLECTION AND PROCESSING

Specimen collection Requirements

- Refer to Manual of Procedures (MOP) Section 9 for detailed information on Specimen Collection and Laboratory Considerations
- Specimen must be collected, processed and stored according to site clinic and/or lab Chain of Custody (Specimen Management) SOP and following GCLP guidelines
- All specimens must be entered into the LDMS, labeled with the LDMS barcodes and stored using LDMS storage system.
- Stored specimens must be shipped in accordance to regulations with LDMS bar coded labels

IV. SHIPPING

SHIPPING: REFER TO LPC, SECTION 5: HELPFUL LINKS AND SHIPPING ADDRESSES

Specimen Repository Facilities:

NIAID Sites

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

NICHD Sites

Fisher Bioservices
625 Lofstrand Lane
Rockville, MD 20850 USA
Attention: Maria Wolff
Phone: (301) 340-1620
Fax: (301) 838-9753
Email: maria.wolff@thermofisher.com
LDMS lab code: 243

SHIPPING:
REFER TO LPC SECTION 5: HELPFUL LINKS AND SHIPPING ADDRESSES

Intensive and Sparse PK, plasma PK, breast milk PK and cord blood PK specimen shipment:

University of Cape Town

Attention: Jennifer Norman/Shameema Witbooi

K50 Division of Clinical Pharmacology; Old Main Building, Groote Schuur Hospital; Observatory; 7925, Cape Town, South Africa.

Telephone: +27 21 404 7695

Email Jennifer.Norman@uct.ac.za, LDMS number 499

IMPORT PERMIT REQUIREMENTS FOR SHIPMENT TO PK TESTING LAB IN CAPE TOWN, SA

- All sites must have an approved import permit in place prior to shipping PK samples.
- "Application for Import Permit for Biological Substances" form now available on the protocol specific webpage: http://www.impaactnetwork.org/studies/IMPAACT2001.asp
- Send form complete with your submitting laboratories information to Jennifer Norman at: jennifer.norman@uct.ac.za
- Requires input of quantity expected to be shipped
  - *Estimate will be based on your sites expected enrollment rate*
- Once submitted, expect 4-6 weeks for approval. Submit ASAP!

IMPAACT 2001
LABORATORY CONSIDERATIONS

What are your questions about the LPC or Lab Evaluations?