Lab Processing Chart
IMPAACT P1078

A Phase IV Randomized Double-blind Placebo-controlled Trial to evaluate the safety of IMMEDIATE (antepartum-initiated) versus DEFERRED (postpartum-initiated) Isoniazid preventative therapy among HIV-infected women in high TB incidence settings

(As per Protocol version 2.0 dated 28 October 2015)

Protocol Version: 2.0, 28 October 2015 **LPC Version:** 2.0, 07 March 2016 **Revised/Updated:** 29-FEB-2016

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Table of Contents

I. Maternal Laboratory Processing Chart (Sections 1-4)…….. 3
II. Infant Laboratory Processing Chart (Sections 1-4)……..29
III. Shipping Instructions (Section 5)……40

I. Maternal Laboratory Processing Chart

Section 1: Schedule of Laboratory Evaluations for All Women (from P1078 Version 2.0 dated 28 October 2015– Appendix I-A: Schedule of Evaluations for All Women

	ANTEPARTUM						POSTPARTUM						
	Screen[1]	Entry	4 weeks after entry (+/- 2 wks)	8 weeks after entry (+/- 2 wks)	Every 4 weeks (+/- 2 wks)	Labor and Delivery[2] (+ 5 days)	4 weeks (+/- 2 wks)	8 weeks (+/- 2 wks)	12 weeks (+/- 2 wks)	16 weeks (+/- 2 wks)	Every 4 weeks (+/- 2 wks)	Suspected Active TB[3]	Early Discontinuation Or last study visit Week 48 (+/- 2 wks)[4]
LABORATORY EVALUATIONS													
Confirmation/Documentation of HIV infection [1]	(6 mL)												
TST assessment						X	X[11]				Wk 44		
CD4/CD8 lymphocyte subsets [13]	3 mL					3 mL							3 mL
Hematology [14]	X					X							
Chemistries [15]	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
HIV RNA RT-PCR [16]		6 mL										6 mL	
Pregnancy test							Urine (5 mL) or serum (1 mL blood in SST or NON tube) test is acceptable. Only collected/performed when pregnancy is suspected or considered clinically indicated by the study site clinician.						
Plasma (Heparin) [17]		15 mL							20 mL		4 or 20 mL (Wk 44)	20 mL	20 mL (Wk 48)
Viable PBMC [18]		from above							from above		from above	from above	from above
Serum (SST or NON) [19]		1 mL											
Hepatitis B Surface Antigen (HBsAg) testing [20]		2 mL											
Interferon Gamma Release Assay (IGRA) and supernatant storage [21]		3 mL				3 mL					**3 mL (**Wk 44)	**3 mL**	
Hair collection				At the visits that coincide with 8 and 28 wks post-entry and Wks 20 & 40 postpartum									
Total Blood Volume (mL) *excluding PK studies*	5 – 11 mL	29 mL	2 mL	2 mL	2 mL	8 mL	2 – 3 mL	2 – 3 mL	2 – 23 mL	2 – 3 mL	2 – 26 mL	31 – 32 mL	5 – 26 mL

	ANTEPARTUM						POSTPARTUM						
	Screen[1]	Entry	4 weeks after entry (+/- 2 wks)	8 weeks after entry (+/- 2 wks)	Every 4 weeks (+/- 2 wks)	Labor and Delivery[2] (+/- 5 days)	4 weeks (+/- 2 wks)	8 weeks (+/- 2 wks)	12 weeks (+/- 2 wks)	16 weeks (+/- 2 wks)	Every 4 weeks (+/- 2 wks)	Suspected Active TB[3]	Early Discontinuation Or last study visit Week 48 (+/- 2 wks)[4]
Pharmacokinetics													
Population PK (all women except those participating in the Intensive PK) [22]			4 mL							4 mL (± 4 wks)			
Intensive PK (0, 1, 2, 4, 6, 8, and 12 hrs post-dose; 4 mL at each time point) [23]			28 mL							28 mL (± 4 wks)			
Blood for DNA and pharmacogenetic variants (all women) [24]				3 mL, at the visit that coincides with 8 wks post-entry									
Total Blood Volume (mL) *for PK substudies*			3 – 31 mL	3 mL	3 mL					4 or 28 mL			

For insufficient blood draws, priorities are as follows:

- At screening: HIV test (if required), hematology, liver enzymes and other chemistries, CD4
- At Entry and follow-up visits:
  1) Safety (liver enzymes, hematology)
  2) TB diagnostics
  3) TB immunology
  4) Pharmacokinetics
  5) Virology

The volume of blood drawn shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

APPENDIX I-A FOOTNOTES

(The footnotes that are applicable to laboratory evaluations are mentioned here with the corresponding protocol footnote numbers)

1. Evaluations should be completed within 30 days prior to study entry. The screening visit may take place on the same day as the entry visit, as long as all screening results are obtained prior to study randomization. If sufficient documentation of HIV status as specified in Section 4.1.1 is not available, HIV diagnostic testing is to be done according to the specified algorithm.
2. The target visit window for the Labor & Delivery visit is up to 5 days postpartum, with an allowable visit window of up to 2 weeks. Every effort should be made to conduct the visit within the target visit window.
3. Suspected Active TB Visits:
   Diagnosis of incident active TB will be made using definitions in Appendix 100. A complete TB evaluation documenting recent exposures/ill contacts, clinical history of illness (i.e., duration of signs/symptoms), and workup performed to make diagnosis will be completed and samples for AFB smear(s) x 2 (induced if necessary), and/or Xpert, and/or culture(s) as well as any other TB diagnostics will be performed on appropriate clinical samples for any enrolled women at this visit. Culture-confirmed *M.tb.* isolates will be stored for drug susceptibility testing. If the woman is seen for a Suspected Active TB visit, the infant should also have a Suspected Active TB visit for evaluation and recommendation for TB prophylaxis care or treatment per Appendix I-B. See P1078 MOP for the suggested work-up for women suspected of having active TB.

4. For women who permanently stop study treatment and refuse any further study follow-up, i.e., off treatment/off study, OR postpartum Week 48. Women who meet a toxicity/intolerance endpoint will be considered off treatment/on study and will follow the usual schedule of study visits but will not undergo study procedures that are specific for women on INH/Placebo for INH (i.e., adherence assessment, pill count, PK samples, hematologies, chemistries) but will come in to clinic for routine evaluation and blood draws as scheduled (CD4, QGIT, stored sample, incident TB, and last study visit). If a toxicity endpoint is reached, follow-up testing needs to be done until resolution of the toxicity (see Section 6.1 for details).

11. Tuberculin skin testing (TST) will be performed using standardized methods (see P1078 MOP) and will be done in all women at the Labor & Delivery and postpartum Week 44 visits, after the blood draw for plasma and IGRA. If TST is not obtained at the Labor & Delivery visit, sites may obtain at the Week 4 visit. Ideally, TST should be read 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. A TST is defined in women in P1078 as positive if ≥ 5 mm.

13. CD4/CD8 lymphocyte subsets must be performed at DAIDS-IQA certified laboratories. Dual platform labs only must have a WBC and differential to complete lymphocyte subset testing.

14. Hematology assessments (complete blood count, cell differential, and platelet count) to be completed from blood collected for CD4/CD8 lymphocyte subsets at indicated visits and as clinically indicated.

15. Liver enzyme tests will be done per the table below:

Study Visit	Chemistry Assessment(s)	Targeted Women
Screen	AST, ALT, total bilirubin (or direct bilirubin, if on atazanavir), glucose, and creatinine	All women
Entry and all other specified study visits	ALT	All women
As needed per Section 6.1	AST, ALT, and total bilirubin (or direct bilirubin, if on atazanavir)	Women with Grade 1 or higher ALT result

16. Every effort should be made to avoid drawing viral load determinations within 14 days of vaccination, systemic infection, or genital HSV outbreak, because of the potential for a transient increase in viral load in response to vaccination or infection. Quantitative HIV-1 RNA PCR must be performed at DAIDS-VQA certified laboratories using a VQA/DCLOT approved method. *It is recommended that the same HIV RNA test be used through all protocol visits.* Note: All participants should have an Entry visit viral load. If HIV viral load is completed at the Screen visit to confirm HIV infection, then that result can be used for both confirmation and Entry assessments.

17. Plasma in Heparin should be stored at the specified visits for future HIV-related and TB-related testing (e.g., baseline vitamin D levels; baseline immune profiles [e.g., immune activation markers] or metabolomic biosignatures among TB cases and non-cases for biomarker discovery; future HIV and TB studies). Blood should ideally be drawn prior to placement of TST.

Study Visit	Volume	Targeted Population
Entry	15 mL	Plasma – All women PBMCs – Approximately 700 women. TB ELISPOT will only be performed in approximately 460 women and the remaining samples will be stored for future testing
Postpartum Week 12	20 mL	Plasma & PBMCs – Approximately 460 women (same women who contributed samples for TB ELISPOT at Entry, if feasible)
Postpartum Week 44	20 mL	Plasma & PBMCs – Approximately 460 women (same women who contributed samples for TB ELISPOT at Entry and Postpartum Week 12, if feasible)
	4 mL	Plasma – Remaining women
Postpartum Week 48	20 mL	Plasma & PBMCs – Approximately 260 women (same women who contributed samples for TB ELISPOT at Entry, Postpartum Week 12, and Postpartum Week 44, if feasible)
Suspected Active TB Visit	20 mL	Plasma & PBMCs – Women who are evaluated for suspected active TB

18. TB ELISPOT will be performed on cryopreserved viable PBMCs (as described in the table above) at the end of the study. Collection of PBMCs will be site restricted.

19. Collect and store as indicated above and in the LPC. This specimen will be stored for future use in TB biomarker, antibody, inflammation, nutrition, or other TB/HIV related studies.

20. Enrollment should not be delayed until the results are available since HBsAg is not part of the inclusion or exclusion criteria. Women with positive HBsAg and their infant(s) should receive best available local standard of care.

21. QGIT: See the P1078 MOP and LPC for information on performing QGIT. Testing should not be performed on participants with suspected or confirmed TB, if prior to the indicated visit. At all indicated visits, QGIT will be performed in a blinded fashion in all women and blood should be drawn prior to TST placement, if applicable. If results are not obtained or are indeterminate, the test should be repeated within 4 weeks. Repeat tests should be minimized as much as possible to avoid interference of the TST with the QGIT results. Refer to Section 3.2, the MOP, and LPC for details regarding QGIT testing.

Study Visit	Assessment(s)	Targeted Women
Entry	Blinded QGIT, Stored QGIT supernatants	All women
Labor & Delivery	Blinded QGIT	All women
	Stored QGIT Supernatants	Subset of 260 women, as in P1078 LPC
Week 44 Postpartum	Blinded QGIT	All women
	Stored QGIT Supernatants	Subset of 260 women, as in P1078 LPC
Suspected TB visit	Blinded QGIT	Women with whom this visit is conducted
	Stored QGIT Supernatants	Women with whom this visit is conducted

22. Collect during the third trimester of pregnancy and then at the Week 16 postpartum visit (± 4 weeks).

23. Collect for women randomized to this collection on HAART, during the third trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH and at the Week 16 postpartum visit (± 4 weeks).

24. DNA and Pharmacogenetic variant samples will be batch shipped.

Section 2: MATERNAL Safety/Clinical Laboratory Evaluations ***Defer to local clinical specimen collection guidelines for tube types and collection volumes whenever discrepancies occur***				
Evaluation	**DMC Test Code**	**Tests**		**CRF #**
Confirmation/ documentation of HIV infection[1]	**N/A**	**Complete HIV testing in accordance with protocol specified requirements as outlined in section 4.1.1**		
TST assessment	**N/A**	Perform in all women at the Labor & Delivery and postpartum Week 44 visits, after the blood draw for plasma and IGRA. If TST is not obtained at the Labor & Delivery visit, sites may obtain at the Week 4 visit. TST should be read 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. Refer p1078 MOP.		TBW0062
Hematology[14]	N/A	Complete blood count, cell differential, platelet count		PE6812
Chemistry[15]	N/A	Screen: AST, ALT, total bilirubin (or direct bilirubin if on atazanavir), glucose and creatinine will be tested on all women. Entry and all other specified study visits: ALT will be done on all women.	If ALT is ≥ Grade 1, AST, ALT, and total bilirubin (or direct bilirubin if on atazanavir) should be done.	PE6817
Pregnancy test	N/A	β HCG (pregnancy test) on urine or serum is acceptable. Only collected or performed when pregnancy is suspected or considered clinically indicated by study site clinician.	Urine test must have a sensitivity of ≤25mIU/mL	F0847
CD4/CD8 Lymphocyte subsets[13]	CD4/CD8	CD4/CD8 cell counts and percentages	CD4/CD8 lymphocyte subsets must be performed at DAIDS-IQA certified laboratories. Dual platform labs only must also have a WBC and diff. to complete lymphocyte subset testing.	LBW0054
Hepatitis B Surface Antigen (HBsAg) testing [20]	N/A	Hep B surface Ag (HBsAg) will be done in real time. Enrollment should not be delayed until results are available.		SRW0026

Section 2: MATERNAL Safety/Clinical Laboratory Evaluations ***Defer to local clinical specimen collection guidelines for tube types and collection volumes whenever discrepancies occur***			
Evaluation	**DMC Test Code**	**Tests**	**CRF #**
Interferon Gamma Release Assay (IGRA) [21]	MTBIGRA	• Testing should not be performed on participants with suspected or confirmed TB, if prior to the indicated visit. • At all indicated visits, QGIT will be performed in a blinded fashion in all women and blood should be drawn prior to TST placement, if applicable. The clinical site must remain blinded to the QGIT result. • If results are not obtained or are indeterminate, the test should be repeated within 4 weeks. Repeat tests should be minimized as much as possible to avoid interference of the TST with the QGIT results. • Complete TBW0060 form and return to DMC via fax or mail.	TBW0060

Section 3: MATERNAL Specimen Processing & Shipping -					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
HIV RNA RT-PCR[16]	**Note***:* It is recommended that the same HIV RNA test be used through all protocol visits.	F3109 F3008	RNAHIV	Must be performed at DAIDS-VQA certified labs using VQA /DCLOT approved method. Send to local DAIDS-VQA certified lab ambient for testing. Spin blood at 800xg for 10 min. Remove plasma; respin plasma at 800xg for 10 min. Store at –70°C or colder until testing. LDMS spec. code: BLD/EDT/PL2	
QGIT Supernatant storage[21] Store supernatant from the TB antigen tube and negative control tube only **No separate blood draw needed.**	**Note:** Do not refrigerate or freeze the blood samples. Deliver to processing laboratory the same day. Processing labs to complete the remainder of TBW0060 and send to FSTRF.	F3008	MTBIGRA	• Collect 1.0ml of blood by venepuncture directly into each of the QuantiFERON®-TB Gold IT blood collection tubes. • Invert tubes 10 times to ensure entire inner surface of tube is coated with blood. Place tubes upright in transport incubator (37ºC + 1ºC) as soon as possible. • The tubes must be transferred to a 37°C incubator as soon as possible, and within 16 hours of collection. • Following incubation, collect plasma according to QGIT package insert instructions. • Divide plasma into 2 aliquots • Use the first aliquot for on-site ELISA testing as per manufacturer's instructions. • The second aliquot may be used for repeat testing if required**.** Site team is not required to request permission from study team to perform the repeat test. Refer to the Laboratory section of the MOP. LDMS spec. code : BLD/~~QFT~~/QTF/PL1/NIL (from Grey cap tube) BLD/~~QFT/~~QTF/PL1/ATG (from Red cap tube)	Store first aliquot of plasma until testing as per manufacturer's instructions. Store remaining aliquot at –70°C or colder until shipping. Batch ship remaining QGIT plasma aliquots to Lab # 564

Section 3: MATERNAL Specimen Processing & Shipping –					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
Plasma (Heparin) (For future HIV related and TB related testing) Blood should be drawn prior to placement of TST **Viable PBMC** TB ELISPOT will be performed on cryopreserved viable PBMCs at the end of study. Collection of PBMCs will be site restricted.	15 ml blood at **E**ntry 20ml blood at PP week 12, 48 and Suspected TB visit and 4 or 20ml blood at week 44 in Sodium heparin tube	F3008	STORMIX	Spin blood at 400xg for 10 min. Remove plasma, respin plasma at 800xg for 10mins. • Entry Visit – Prepare 4-5x1.5mL aliquots • PP Week 44 (if not contributing to PBMC) – Prepare 2x1.0mL aliquots • PP Week 12 and 44, Week 48, and Suspected TB Visits – Prepare 4-6x1.5mL aliquots. Store at –70°C or colder until shipping. LDMS spec. code: BLD/HEP/PL2	Batch ship to BRI, Fisher, or End User Lab as required every 6 months.
				PBMCs: Follow the HANC PBMC Processing SOP. • Entry Visit - Store PBMCs viably in 3 or as many as tubes needed @5x10^6 cells in 0.5 mL aliquots. • PP Week 12 and 44; Week 48; and Suspected TB Visits – Store PBMCs viably in 4 or as many tubes needed @5x10^6 cells in 0.5mL aliquots Please note that the dilution of PBMC in freezing medium is 10x10^6, but only 0.5 mL is placed in each vial. Refer to the Laboratory section of the MOP for additional instructions and examples. LDMS spec. code: BLD/HEP/CEL/DMS	**S**tore the PBMC aliquots in LN2 or in a -150^{o}C freezer. Batch shipped cryopreserved PBMCs ~~on a yearly basis~~ upon request to University of Colorado Denver. LDMS Lab # 174 Shipment is permitted on Monday and Tuesday only.
Serum (For future use in TB/ HIV related studies)	1ml serum in NON or SST tube	F3008	STORMIX	Allow blood to clot upright at least 30 min. Spin at 1100-1300xg for 10 min. (horizontal rotor centrifuge) or 15 min. (fixed angle rotor centrifuge). Freeze 1x0.5mLor as available aliquot at -70°C. LDMS Spec. Code: BLD/SST or NON/SER	Hold the sample at site at -70^o C or lower until further notification from the study team.

Section 3: MATERNAL Specimen Processing & Shipping –					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
Hair collection	20-30 strands folded in foil and stored in a zipper bag	PKW0390		Hair samples should be logged into the LDMS and labeled with LDMS-generated labels. **NOTE:** Also label the bag with the LDMS-generated label. LDMS spec. code: HAR/NON/HAR	Hair samples should be kept at room temperature and in a dark place at each site locally until ship to UCSF, LDMS lab # 607 at end of the study.
Population PK (INH/Placebo for INH random samples) **All women except those participating in the Intensive PK**	4ml EDTA blood Pre-cool vacutainer tubes on wet ice or refrigerator (TEMP 2-8 degrees). Once collected, place tubes on wet ice in a closed, dark container until processed. Process samples within one hour of collection and freeze plasma immediately**.** Collection Timepoints: One sample collected between 2-12 hours post dose.	PKW0287	PKRAN	Centrifuge blood at 1000xg for 10 minutes at 4ºC. Immediately transfer all plasma to pre-labeled 2.0mL cryovials after processing and store at -70°C or lower. LDMS spec. code: BLD/EDT/PL1	Hold the samples at site at -70º C or lower until further notification from the study team.
Intensive PK HAART (12 or 24 hr) + INH (24 hr) **Women randomized to this collection on HAART, during 3rd trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH**	4ml EDTA Blood at each time point Pre-cool vacutainer tubes on wet ice or refrigerator (TEMP 2-8 degrees). Once collected, place tubes on wet ice in a closed, dark container until processed. Process samples within one hour of collection and freeze plasma immediately. Collection Time points: 0, 1, 2, 4, 6, 8, and 12 hrs post-dose.	PKW0286	PKINT	Centrifuge blood at 1000xg for 10 minutes at 4ºC. Immediately prepare 3x0.6 mL aliquots at each time point in pre-labeled cryovials after processing and store at -70^{0}C or lower. LDMS spec. code: BLD/EDT/PL1	Hold the samples at site at -70º C or lower until further notification from the study team.

Section 3: MATERNAL Specimen Processing & Shipping –					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
Non-Viable PBMCs (for DNA and Pharmacogenetic Variants)[24] **All women**	3mL EDTA blood	PKW0344	PKGENO	Non-Viable PBMCs: For cell separation, refer to Cross-Network PBMC Processing SOP, section 16. Save all cells from the 3ml EDTA collection. Count final PBMC preparation and record in LDMS. Centrifuge cell aliquot for 3 minutes in a microfuge at the highest speed (>10,000xg). Aspirate as much supernatant as possible without disturbing the pellet. Store Non-Viable PBMCs non-viably @ -70C or lower. Refer to the Laboratory section of the MOP. LDMS spec. code: BLD/EDT/PEL	Batch ship to BRI, Fisher, or End User Lab as required every 6 months.
AFB smear x 2 (induced if necessary) **AND/OR** **GeneXpert**, **TB Cultures** **MTB isolate storage** (for Drug susceptibility)	3 to 5 ml sputum in sterile container. If possible, oral cavity should be rinsed with clean water that is expectorated prior to providing sputum sample. If unable to produce sufficient sputum, induction by aerosolized inhalation of sterile nebulized saline is acceptable. Record collection method, timing, estimated volume and appearance on CRF. Culture-confirmed *M. tuberculosis* isolates will be stored for drug susceptibility testing locally. Refer to the Laboratory section of the MOP.	TBW0029 TBW0047	AFBZLN CULMTBLI CULMTBMG	Perform AFB smears by carbol fuchsin (ZN stain or equivalent). Perform Gene Xpert testing on collected sputum. Perform cultures by solid LJ medium and liquid culture medium (MGIT) on appropriate clinical samples locally at DAIDS- approved lab if possible, or sent to DAIDS regional or central reference laboratory for testing. Refrigerate within 1 hour after collection if processing will be delayed. Process and culture within 72 hours of collection. Digest/decontaminate sputum prior to smear and culture preparation. LDMS spec. code for non-induced sputum: SPT/NON/SMR/ZLN LDMS spec. code for induced sputum: SPI/NON/SMR/ZLN LDMS spec. code for non-induced sputum culture isolate: SPT/NON/MTB LDMS spec. code for induced sputum culture isolate: SPI/NON/MTB	Store at -70^{0}C locally

Section 4: MATERNAL Evaluations by Visit – Refer to Section 3 for processing specifics					
ANTEPARTUM VISITS (AP) **In LDMS, enter all antepartum visits as the visit number followed by the code "Wk"**					
Screen: Must be completed within 30 days prior to study entry					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Confirmation/ Documentation of HIV Infection	6 mL EDTA Blood		N/A	N/A	*Note: If sufficient documentation of HIV status as specified in Section 4.1.1 is not available, HIV diagnostic testing is to be done according to the specified algorithm.*
Immunology (CD4/CD8 Lymphocyte subsets)	3 ml EDTA blood	LBW0054	N/A	N/A	Must be performed at DAIDS-IQA certified laboratory.
Hematology	No extra blood needed (share with CD4/CD8)	PE6812	N/A	N/A	
Chemistry	2ml NON or SST Blood	PE6817	N/A	N/A	AST, ALT, total bilirubin (or direct bilirubin if on atazanavir) glucose and creatinine

Entry					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry (ALT - All women)	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
HIV RNA RT-PCR	6ml EDTA Blood	F3109 F3008	2x1.5mL	BLD/EDT/PL2	Must be performed at DAIDS-VQA certified laboratories All participants should have an Entry visit viral load. If HIV viral load is completed at the Screen visit to confirm HIV infection, then that result can be used for both confirmation and Entry assessments.
Plasma (All Women)	15ml Sodium heparin Blood	F3008	Plasma: 4-5x1.5mL aliquots.	BLD/HEP/PL2	
Viable PBMC Storage **(Subset of 700 Women -TB ELISPOT on approximately 460 women; and storage for future testing)**	From Above	F3008	Store PBMCs viably in 3 or as many as tubes needed @$5x10^6$ cells in 0.5mL aliquots and store in LN2 or in a -150^oC freezer. Refer to Laboratory section of MOP.	BLD/HEP/CEL/DMS	Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have viable PBMC collected at Entry.
Serum Storage	1 ml SST or NON	F3008	Serum: 1x o.5 mL aliquot.	BLD/SST or NON/SER	
Hepatitis B Surface Antigen (HbsAg) Testing	2ml NON or SST Blood	SRW0026	N/A	N/A	Enrollment should not be delayed until the results are available.

Entry					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
QGIT in real time **All women.**	3 Coated QFT tubes – 1mL each (+ 0.2mL)	TBW0060	N/A	N/A	Will be performed in the blinded fashion (i.e., results will not be disclosed to the clinical site staff). If results are not obtained, the test should be repeated within 4 weeks. QGIT should be done in real-time. If unable to do in real-time, refer to the Laboratory section of the MOP for the batch testing procedure.
QGIT Supernatant storage **All women**	From above Store supernatant from the TB antigen tube and negative control tube only	F3008	Store remaining plasma aliquots Refer to LPC Section 3.	BLD/ ~~QFT/~~QTF/PL1/NIL BLD/ ~~QFT/~~QTF/PL1/ATG	

4 Weeks after Entry (± 2 week)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	
Population PK (INH/ Placebo random samples) **(**All women except those participating in the Intensive PK) **Collect <u>once</u> during the third trimester of pregnancy**	4ml EDTA Blood Time point: 1 sample collected between 2-12hrs post dose	PKW0287	1x1.8ml	BLD/EDT/PL1 LDMS time/unit: ___ / Random Post Dose (RPD)	.
Intensive PK HAART (12 or 24 hr) + INH (24 hr) **Women randomized to this collection on HAART, during 3rd trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH**	4ml EDTA Blood at each time point Time points: 0, 1, 2, 4, 6, 8, and 12 hrs post-dose	PKW0286	3x0.6ml aliquots per time point	BLD/EDT/PL1 LDMS time/unit: 0 pre-dose(Pre) or ____ Hour (Hr) as needed	

8 Weeks after Entry (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Non-Viable PBMCs (For DNA and Pharmacogenetic variants) **Collect <u>once</u> at the visit that coincides with 8 wks post-entry**	3mL EDTA Blood	~~F3008~~ PKW0344		BLD/EDT/PEL	
Hair **(At the visit that coincides with 8 and 28 weeks post-entry, and from the mothers who have provided informed consent)**	20-30 strands folded in foil and stored in a zipper bag	PKW0390	N/A	HAR/NON/HAR	Store at room temperature and in a dark place.
Population PK (INH/ Placebo random samples) **(All women except those participating in the Intensive PK)** **Collect once during the third trimester of pregnancy**	4ml EDTA Blood Time point: 1 sample collected between 2-12hrs post dose	PKW0287	1x1.8ml	BLD/EDT/PL1 LDMS time/unit: ___ / Random Post Dose (RPD)	
Intensive PK HAART (12 or 24 hr) + INH (24 hr) **Women randomized to this collection on HAART, during 3rd trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH**	4ml EDTA Blood at each time point Time points: 0, 1, 2, 4, 6, 8, and 12 hrs post-dose	PKW0286	3x0.6ml aliquots per time point	BLD/EDT/PL1 LDMS time/unit: 0 pre-dose(Pre) or ____ Hour (Hr) as needed	

Repeating Visits Every 4 Weeks (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT and Total Bilirubin(or direct bilirubin, if on atazanavir)
Hair **(At the visit that coincides with 8 and 28 weeks post-entry, and from the mothers who have provided informed consent)**	20-30 strands folded in foil and stored in a zipper bag	PKW0390	N/A	HAR/NON/HAR	Store at room temperature and in a dark place.
Population PK (INH/ Placebo random samples) **(All women except those participating in the Intensive PK)** **Collect once during the third trimester of pregnancy**	4ml EDTA Blood Time point: 1 sample collected between 2-12hrs post dose	PKW0287	1x1.8ml	BLD/EDT/PL1 LDMS time/unit: ___ / Random Post Dose (RPD)	
Intensive PK HAART (12 or 24 hr) + INH (24 hr) **Women randomized to this collection on HAART, during 3rd trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH**	4ml EDTA Blood at each time point Time points: 0, 1, 2, 4, 6, 8, and 12 hrs post-dose	PKW0286	3x0.6ml aliquots per time point	BLD/EDT/PL1 LDMS time/unit: 0 pre-dose(Pre) or ____ Hour (Hr) as needed	
Non-Viable PBMCs (For DNA and Pharmacogenetic variants) **Collect <u>once</u> at the visit that coincides with 8 wks post-entry**	3mL EDTA Blood	~~F3008~~ PKW0344		BLD/EDT/PEL	.

Labor & Delivery (+ 5 days) In LDMS, enter Labor and Delivery visit by the code "L&D"					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
TST assessment **All women**	N/A	TBW0062	N/A	N/A	Perform after the blood draw for plasma and IGRA. (Refer P1078 MOP) If TST is not obtained at the Labor & Delivery visit, sites may obtain at the Week 4 visit.
Immunology (CD4/CD8 Lymphocyte subset)	3ml EDTA Blood	LBW0054	N/A	N/A	
Hematology	No extra blood needed (shared with CD4/CD8)	PE6812	N/A	N/A	
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST,ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
QGIT in real time **All women**	3 Coated QFT tubes – 1mL each (+ 0.2mL)	TBW0060	N/A	N/A	Will be performed in the blinded fashion (i.e., results will not be disclosed to the clinical site staff). QGIT should be done in real-time. If unable to do in real-time, refer to the Laboratory section of the MOP for the batch testing procedure.
QGIT Supernatant storage **On subset of first 260 women**	From above Store supernatant from the TB antigen tube and negative control tube only	F3008	Store remaining plasma aliquots Refer to LPC Section 3.	BLD/ ~~QFT/~~QTF/PL1/NIL BLD/ ~~QFT/~~QTF/PL1/ATG	Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have QGIT supernatant stored at Delivery.

Labor & Delivery (+ 5 days) In LDMS, enter Labor and Delivery visit by the code "L&D"					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hair (At the visit that coincides with 8 and 28 weeks post-entry, and from the mothers who have provided informed consent)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.
Non-Viable PBMCs (For DNA and Pharmacogenetic variants) **Collect <u>once</u> at the visit that coincides with 8 wks post-entry**	3mL EDTA Blood	~~F3008~~ PKW0344		BLD/EDT/PEL	

POSTPARTUM VISITS (PP) In LDMS, enter all postpartum visits as the visit number followed by the code "PPt"					
4 Weeks ± 2 weeks					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
TST assessment **If TST is not obtained at the Labor & Delivery visit, may obtain at the Week 4 visit.**	N/A	TBW0062	N/A	N/A	Perform after the blood draw for plasma and IGRA. (Refer P1078 MOP)
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Hair (At the visit that coincides with 8 and 28 weeks post-entry and wks 20 and 40 postpartum)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.
Non-Viable PBMCs (For DNA and Pharmacogenetic variants) **Collect <u>once</u> at the visit that coincides with 8 wks post-entry**	3mL EDTA Blood	~~F3008~~ PKW0344		BLD/EDT/PEL	

8 Weeks ± 2 weeks					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry (ALT - All women)	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Hair (At the visit that coincides with 8 and 28 weeks post-entry and Wks 20 & 40 postpartum)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.
12 Weeks ± 2 weeks					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry (ALT - All women)	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin (or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected.
Plasma and Viable PBMC Storage **Subset of 460 women ONLY (same women who contributed samples for TB ELISPOT at entry, if feasible)**	20ml Sodium Heparin Blood	F3008	Plasma: 4-6x1.5mL aliquots.	BLD/HEP/PL2	Collect and store plasma only from those women who are assigned to the subset that will have viable PBMC collected at Postpartum Week 12.
			Store PBMCs viably in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL aliquots and store in LN2 or in a -150^{o}C freezer. Refer to Laboratory section of MOP.	BLD/HEP/CEL/DMS	Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have viable PBMC collected at Post-partum Week 12.
Hair (At the visit that coincides with 8 and 28 weeks post-entry and Wks 20 & 40 postpartum)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.

16 Weeks (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Hair (At the visit that coincides with 8 and 28 weeks post-entry and Wks 20 & 40 postpartum)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.
Population PK (INH/Placebo for INH random samples) **Collected on all women at Wk 16 ± 4 weeks (except those participating in the Intensive PK)**	4ml EDTA Blood Time point: 1 sample collected between 2 to 12hrs post dose	PKW0287	1x1.8ml	BLD/EDT/PL1 LDMS time/unit: ___ / Random Post Dose (RPD)	
Intensive PK HAART (12 or 24 hr) + INH (24 hr) **Women randomized to this collection on HAART, during 3rd trimester of pregnancy, and ≥ 2 weeks after start of INH/Placebo for INH**	4ml EDTA Blood at each time point(28mL) Time points: 0, 1, 2, 4, 6, 8, and 12 hrs post-dose	PKW0286	3 x 0.6ml per time point	BLD/EDT/PL1 LDMS time/unit: 0 pre-dose(Pre) or ____ Hour (Hr) as needed	

Repeating Visits --Every 4 Weeks (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
TST assessment **All women**	N/A	TBW0062	N/A	N/A	**At Week 44 visit ONLY.** Perform after the blood draw for plasma and IGRA. (Refer P1078 MOP)
Chemistry **(ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Plasma Storage **Remaining women at Week 44 postpartum ONLY**	4ml Sodium heparin Blood	F3008	Plasma: 2x1.0ml	BLD/HEP/PL2	**Week 44 ONLY**
Plasma and Viable PBMC Storage **Subset of 460 women ONLY (same women who contributed samples for TB ELISPOT at Entry & at Postpartum Week 12,if feasible)**, **at Week 44 postpartum ONLY**	20ml Sodium heparin Blood	F3008	Plasma: 4-6x1.5mL aliquots.	BLD/HEP/PL2	**Week 44 ONLY** Only the women who are assigned to the subset that will have viable PBMC collected at Post- partum Week 44
			Store PBMCs viably in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL aliquots and store in LN2 or in a -150ºC freezer. Refer to Laboratory section of MOP.	BLD/HEP/CEL/DMS	**Week 44 ONLY** Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have viable PBMC collected at Post-partum Week 44.

Repeating Visits --Every 4 Weeks (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
QGIT in real time **All women at Week 44 postpartum ONLY** **Blood to be drawn prior to placement of TST**	3 Coated QFT tubes – 1ml each (+ 0.2mL)	TBW0060	N/A	N/A	**Week 44 ONLY** Will be performed in the blinded fashion (i.e., results will not be disclosed to the clinical site staff). If results are not obtained, the test should be repeated within 4 weeks. QGIT should be done in real-time. If unable to do in real-time, refer to the Laboratory section of the MOP for the batch testing procedure.
QGIT Supernatant storage **On the subset of 260 women at Week 44 ONLY**	From above Store supernatant from the TB antigen tube and negative control tube only	F3008	Store remaining plasma aliquots Refer to LPC Section 3.	BLD/ ~~QFT/~~QTF/PL1/NIL BLD/~~QFT/~~QTF/PL1/ATG	**Week 44 ONLY** Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have QGIT supernatant stored at Post-partum Week 44.
Hair (At the visit that coincides with 8 and 28 weeks post-entry and Wks 20 & 40 postpartum)	20-30 strands folded in foil and stored in a zipper bag	PKW0390		HAR/NON/HAR	Store at room temperature and in a dark place.

Suspected Active TB In LDMS, for Suspected Active TB visit, enter the current week on study followed by the code "SCK"					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Chemistry (ALT - All women)	2ml NON or SST Blood	PE6817	N/A	N/A	
HIV RNA RT-PCR	6ml EDTA Blood	F3109 F3008	2x1.5ml plasma aliquots.		Must be performed at DAIDS-VQA certified laboratories using VQA/DCLOT approved method.
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Plasma and Viable PBMC Storage **Women who are evaluated for suspected active TB**	20ml Sodium heparin Blood	F3008	Plasma: 4-6x1.5mL aliquots	BLD/HEP/PL2	
			Store PBMCs viably in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL and store in LN2 or in a -150^oC freezer. Refer to Laboratory section of MOP.	BLD/HEP/CEL/DMS	
AFB smear and culture **MTB isolate storage** (**for Drug susceptibility)**	Sputum or appropriate clinical samples Store culture- confirmed *M. tuberculosis* isolates at-70^0C locally	TBW0029 TBW0047	N/A	For non-induced sputum: SPT/NON/SMR/ZLN For induced sputum: SPI/NON/SMR/ZLN <u>LDMS code for culture isolates:</u> For non-induced sputum culture isolate: SPT/NON/MTB For induced sputum culture isolate: SPI/NON/MTB	See Section 3 instruction. See section IV of MOP for details

Suspected Active TB					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
QGIT in real time	3 Coated QFT tubes – 1ml each (+ 0.2mL)	TBW0060	N/A	N/A	Will be performed in the blinded fashion (i.e., results will not be disclosed to the clinical site staff). If results are not obtained, the test should be repeated within 4 weeks. QGIT should be done in real-time. If unable to do in real-time, refer to the Laboratory section of the MOP for the batch testing procedure.
QGIT Supernatant storage	From above Store supernatant from the TB antigen tube and negative control tube only	F3008	Store remaining plasma aliquots Refer to LPC Section 3.	BLD/ ~~QFT/~~QTF/PL1/NIL BLD/ ~~QFT/~~QTF/PL1/ATG	

Early Discontinuation OR Last Study Visit 48 Weeks (± 2 weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Immunology (CD4/CD8 Lymphocyte subset)	3ml EDTA Blood	LBW0054	N/A	N/A	
Chemistry (**ALT - All women)**	2ml NON or SST Blood	PE6817	N/A	N/A	Women with Grade 1 or higher ALT result, perform as needed per section 6.1 AST, ALT, and Total Bilirubin(or direct bilirubin, if on atazanavir)
Pregnancy Test	5mL Urine or 1mL SST or NON Blood	F0847	NA	NA	Only perform test if pregnancy suspected or clinically indicated.
Plasma and Viable PBMC Storage **Subset of 260 women ONLY(same women who contributed samples for TB ELISPOT at Entry, Postpartum-Week 12 and Postpartum Week 44,if feasible) at Week 48 postpartum ONLY**	20ml Sodium heparin Blood	F3008	Plasma: 4-6x1.5mL aliquots	BLD/HEP/PL2	**Week 48 ONLY** Collect and store plasma only from those women who are assigned to the subset that will have viable PBMC collected at Post- partum Week 48.
			PBMCs: Store PBMCs viably in 4 or as many as tubes needed @$5x10^6$ cells in 0.5mL aliquots and store in LN2 or in a -150^{o}C freezer. Refer to Laboratory section of MOP.	BLD/HEP/CEL/DMS	**Week 48 ONLY** Refer to the randomization confirmation message to verify if a subject is assigned to the subset that will have viable PBMC collected at Post-partum Week 48.

II. Infant Laboratory Processing Chart

Section 1: Schedule of Laboratory Evaluations (from P1078 Version 2.0 28 October2015)

Appendix I-B: Schedule of Evaluations for Infant Follow-up

Events W=Weeks Visit windows (± W)	Birth[1] (+ 5 days)	4 Weeks (± 2 Weeks)	8 Weeks (± 2 Weeks)	12 Weeks (± 2 Weeks)	24 Weeks (± 4 Weeks)	36 Weeks (± 4 Weeks)	44 Weeks (± 2 Weeks)	Suspected Active TB[2]	Early Discontinuation Or last study visit 48 Week (± 2 Weeks)
LABORATORY EVALUATIONS									
Hematology [5]	1 mL	1 mL		1 mL	1 mL			1 mL	1 mL
ALT	1 mL	1 mL		1 mL	1 mL			1 mL	1 mL
HIV Nucleic Acid Test (NAT) [6]	3 mL							3 mL	3 mL
TST [7]							X	X	
IGRA [8]							3 mL		
Plasma [9]	3 mL			8 mL	3 mL		8 mL	8 mL	
Viable PBMC [9]				from above			from above	from above	
Hair Collection	X				X				
Total Infant Blood Volume	**8 mL**	**2 mL**	**0 mL**	**10 mL**	**5 mL**	**0 mL**	**11 mL**	**13 mL**	**5 mL**

For insufficient blood draws, priorities are as follows:
1) Safety (hematology, chemistries)
2) TB diagnostics
3) TB immunology
4) Virology

No more than 5 mL/kg may be drawn for research purposes in a single day. No more than 9.5 mL/kg may be drawn over any eight-week period.

APPENDIX I-B FOOTNOTES

(The footnotes that are applicable to laboratory evaluations are mentioned here with the corresponding protocol footnote numbers)

2. Suspected Active TB Visits:
   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. Diagnosis of incident active TB will be made using definitions in Appendix 100. A complete TB evaluation documenting recent exposures/ill contacts, clinical history of illness (i.e., duration of signs/symptoms), and TST in infants will be performed using standard algorithms and definitions. If gastric washing is done as part of TB diagnosis work-up, a separate specimen should be collected and stored for future TB testing. Culture-confirmed *M. tb.* isolates will be stored for drug susceptibility testing. See P1078 MOP for the suggested work-up for infants suspected of having TB or exposed to known TB case.

5. Hematology: CBC with differential and platelets.
6. Infant HIV infection status will be assessed by HIV nucleic acid test. HIV DNA PCR is preferred; if not available HIV RNA PCR can be used. Infants with one initial positive HIV NAT test must have a specimen drawn on a different day than the sample that originally tested positive to confirm the results and infection status. Must be performed in a laboratory that operates according to GCLP guidelines and participates in the appropriate external quality assurance (EQA) program. Store any remaining sample for repeat testing.
7. The TST will be performed after the QGIT/ELISPOT assay blood draw. Ideally, TST should be read at 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. A TST is defined as positive as ≥ 10 mm in HIV-negative infants and as ≥ 5 mm in HIV-positive infants. See P1078 MOP for standardized TST method.
8. QGIT: blood will be drawn for QGIT prior to placement of TST (see P1078 MOP) at the Week 44 study visit. If results are not obtained or are indeterminate, the test should be repeated within 4 weeks. Repeat tests should be minimized as much as possible to avoid interference of the TST with the QGIT results. Refer to the Section 3.2, the MOP, and LPC for details regarding QGIT testing. TB ELISPOT will be performed on stored PBMCs as described in footnote 9 below.
9. Plasma and PBMCs will be collected and stored at the specified visits for future HIV-related and TB-related testing (e.g., TB biomarker, antibody, inflammation, or nutrition studies). Blood volumes will be according to age appropriate guidelines. Blood should be drawn prior to placement of TST at the Week 44 and Suspected TB visits. See P1078 MOP and LPC for collection, processing, and shipment instructions.

Study Visit	Targeted Population
Birth	Plasma – All infants
Week 12	Plasma and PBMCs – Infants of the approximately 460 women from whom plasma and PBMCs are collected at postpartum Week 12
Week 24	Plasma – All infants for future HIV diagnostic testing that is not done as part of local standard of care. If HIV DNA PCR or HIV RNA PCR is done as part of local standard of care, the plasma will be used for other HIV or TB related future testing
Week 44	Plasma – All infants PBMCs – Infants of the approximately 460 women from whom PBMCs is collected at postpartum Week 44
Suspected Active TB visit	Plasma and PBMCs – Infants who are evaluated for suspected active TB

Section 2: INFANT Safety/Clinical Laboratory Evaluations
Defer to local clinical specimen collection guidelines for tube types and collection volumes whenever discrepancies occur

Evaluation	DMC Test Code	Tests	CRF #
Hematology	N/A	CBC with differential and platelets. Monitor any grade toxicity until resolved.	PE6812
Chemistry	N/A	ALT Monitor any grade toxicity until resolved.	PE6817
HIV Nucleic Acid Test (NAT)	DNAHIV RNAHIV	HIV NAT – HIV DNA PCR is preferred; if not available HIV RNA PCR can be used. Infants with one initial positive HIV NAT test must have a specimen drawn on different day than the sample that originally tested positive to confirm the results and infection status. **Note:** Must be performed in a laboratory that operates according to GCLP guidelines and participates in the appropriate external quality assurance (EQA) program. Store any remaining sample for repeat testing.	LBW0082
TST		The TST will be performed after the QGIT/ELISPOT assay blood draw. TST should be read at 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. Refer P1078 MOP for standardized TST method.	TBW0062

Section 3: INFANT Specimen Processing & Shipping					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
AFB smear **Culture and** **MTB isolate storage (for drug susceptibility)**	**Note:** If gastric washing is collected as part of TB diagnosis work-up, a separate specimen should be collected and stored for future TB testing. Collect 3-5 mL aspirate in sterile container. Refer to Laboratory section of MOP. Perform AFB smears and cultures by both solid and liquid culture methods locally at DAIDS-approved lab if possible, or sent to DAIDS regional or central reference laboratory for testing. Culture-confirmed *M. tb.* isolates will be stored at-70^{0}C locally for drug susceptibility testing.	F3008 TBW0047 TBW0029	AFBZLN CULMTBLI CULMTBMG	Send to DAIDS approved laboratory on ice or a cold pack for processing/testing and perform locally. Specimen should be processed immediately or neutralized with 100mg of Sodium bicarbonate. Digest/decontaminate aspirate/lavage prior to smear and culture preparation. Refer to Laboratory section of MOP. LDMS specimen code for gastric washing: GAS/NON/ASP/SBC LDMS spec. code for non-induced sputum culture isolate: SPT/NON/MTB LDMS spec. code for induced sputum culture isolate: SPI/NON/MTB	
QGIT in real time	**Note***:* Blood must be drawn for QGIT prior to placement of TST at week 44 If the results are not obtained from for the QGIT, the test should be repeated within 4 weeks. Repeat tests should be minimized to avoid interference of TST with QGIT results.	TBW0060	MTBIGRA	Do not refrigerate or freeze the blood samples. Deliver to processing laboratory the same day. Refer to Laboratory section of MOP.	QGIT should be done in real-time. If unable to do in real-time, refer to the Laboratory section of the MOP for the batch testing procedure.

Section 3: INFANT Specimen Processing & Shipping					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
Plasma & Viable PBMC Storage (For future HIV- related and TB- related testing)	Collect blood in **Sodium heparin** tube **Note:** Blood should be drawn prior to placement of TST at week 44 and Suspected TB visits.	F3008	STORMIX	Plasma: Spin blood at 400xg for 10 min. remove plasma, respin plasma at 800xg for 10mins. Store 4x1.0mL aliquots and any remaining plasma in the 5th vial. Refer to Laboratory section of MOP. LDMS spec. code: BLD/HEP/PL2	Store at –70°C or colder until shipping. Batch ship to BRI, Fisher, or End User Lab as required every 6 months
				PBMCs: Follow the HANC PBMC Processing SOP. Store PBMCs viably in 4 or as many as tubes needed @5x10^6 cells in 0.5 mL aliquots and store in LN2 or in a -150^{o}C freezer. Refer to the Laboratory section of the MOP for additional instructions and examples. LDMS spec. code: BLD/HEP/CEL/DMS	Store in LN2. Batch shipped to University of Colorado Denver ~~on a yearly basis~~ upon request. LDMS Lab # 174 Shipment is permitted on Monday and Tuesday only.
HIV Nucleic Acid Test (NAT) (For HIV DNA or RNA PCR Testing in Real Time)	3mL EDTA Blood	LBW0082 F3109 (for HIV RNA)	HIV DNA PCR DMC Test Code: DNAHIV HIV RNA PCR DMC Test Code: RNAHIV	If performing HIV DNA PCR: Make 2x0.3mL aliquots of whole blood. Use one aliquot for real-time DNA PCR and freeze the remaining aliquot at -70°C, or colder, as a backup. LDMS spec. code: BLD/EDT/WBP If performing HIV RNA PCR: Centrifuge blood at 400xg for 10mins, transfer plasma to a new centrifuge tube and re-spin plasma at 800xg for 10mins. Prepare 1 aliquot with all harvested plasma and store at -70°C, or colder, until testing can be performed. LDMS spec. code: BLD/EDT/PL2	

Section 3: INFANT Specimen Processing & Shipping					
Evaluation	**Special Processing Time Limits**	**CRF #**	**DMC Test Code**	**Processing**	**Shipping**
Hair collection Hair should be collected at Birth and Week 24 of life from infants whose mothers who have provided informed consent.	Collect hair at 20-30 strands folded in foil and stored in a zipper bag	PKW0390	STORPK	Hair samples should be logged into the LDMS and labeled with LDMS-generated labels. **NOTE:** Also label the bag with the LDMS-generated label. LDMS spec. code: HAR/NON/HAR	Hair samples should be kept at room temperature and in a dark place at each site locally until ship to UCSF, LDMS lab # 607 at end of the study.

Section 4: INFANT Evaluations by Visit– Refer to Section 3 processing specifics					
Birth (+5 days)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry-ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
HIV Nucleic Acid Test (NAT)	3ml EDTA Blood	LBW0082	HIV DNA PCR: Make 2x0.3mL aliquots of whole blood	BLD/EDT/WBP	See LPC Section 2 for details Refer protocol v 2.0 Appendix I-B footnote # 6
		F3109 (for HIV RNA)	HIV RNA PCR: Prepare 1 aliquot with all harvested plasma	BLD/EDT/PL2	
Plasma All infants	3ml Sodium heparin Blood	F3008	3x0.5ml	BLD/ HEP/ PL2	
Hair collection	Hair	PKW0390	20-30 strands of hair folded in foil and stored in a zipper bag	HAR/NON/HAR	Store at room temperature and in a dark place.
4 Week (±2 Weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry-ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
12 Week (±2 Weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry-ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
Plasma and Viable PBMC Storage	8ml Sodium heparin Blood	F3008	Plasma: 4x1.0mL.	BLD/ HEP PL2	Collect and store plasma only if the infant's mother is assigned to the subset that will have viable PBMC collected at Postpartum Week 12
(Subset of 460 infants of women from whom plasma and PBMCs are collected at Postpartum Week12)			PBMCs: Prepare viable PBMCs in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL aliquots.	BLD/HEP/CEL/DMS	Refer to the randomization confirmation message to verify if the infant's mother is assigned to the subset that will have viable PBMC collected at Postpartum Week 12.

24 Week (±4 Weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry -ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
Plasma Storage **All infants for future HIV diagnostic testing that is not done as part of local standard of care.**	3mL EDTA Blood	F3008	3x0.5ml	BLD/EDT/PL2	If HIV DNA PCR or HIV RNA PCR is done as part of local standard of care, the plasma will be used for other HIV or TB related future testing
Hair collection	Hair	PKW0390	20-30 strands of hair folded in foil and stored in a zipper bag	HAR/NON/HAR	Store at room temperature and in a dark place.
44 Week (±2 Weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
TST TST will be performed after the QGIT/ELISPOT assay blood draw.	N/A	TBW0062	N/A	N/A	TST should be read at 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. Refer P1078 MOP for standardized TST method.
QGIT in real time **Blood will be drawn for QGIT prior to placement of TST at the Week 44 study visit**.	3 Coated QFT tubes – 1mL each (+ 0.2mL)	TBW0060	N/A	N/A	If results are not obtained or are indeterminate, the test should be repeated within 4 weeks. Repeat tests should be minimized as much as possible to avoid interference of the TST with the QGIT results

44 Week (±2 Weeks)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Plasma Storage **ALL Infants**	8mL Sodium heparin blood	F3008	Plasma: 4x1.0mL.	BLD/ HEP/PL2	Blood should be drawn prior to placement of TST at week 44.
Viable PBMC Storage **Infants of approx. 460 women from whom PBMCs is collected at Postpartum Week 44**	From Above	F3008	PBMCs: Prepare viable PBMCs in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL aliquots.	BLD/ HEP/CEL/DMS	Refer to the randomization confirmation message to verify if the infant's mother is assigned to the subset that will have viable PBMC collected at Post-partum Week 44.

Suspected Active TB					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry-ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
HIV Nucleic Acid Test (NAT)	3ml EDTA Blood	LBW0082 F3109 (for HIV RNA)	HIV DNA PCR: Make 2x0.3ml aliquots of whole blood	BLD/EDT/WBP	See LPC Section 2 for details Refer protocol v 2.0 Appendix I-B footnote # 6
			HIV RNA PCR: Prepare 1 aliquot with all harvested plasma	BLD/EDT/PL2	
TST	N/A	TBW0062	N/A	N/A	TST should be read at 2-3 days after placement. However, it can be read by a trained observer up to 7 days from administration. Refer P1078 MOP for standardized TST method.
AFB smear, Culture and MTB isolate storage (for drug susceptibility)	3-5ml Gastric aspirate for TB work up and storage Culture-confirmed *M. tuberculosis* isolates will be stored for drug susceptibility testing.	TBW0047 TBW0029 F3008	N/A	For gastric washing: GAS/NON/ASP/SBC For non-induced sputum: SPT/NON/SMR/ZLN For induced sputum: SPI/NON/SMR/ZLN <u>LDMS code for culture isolates:</u> For non-induced sputum culture isolate: SPT/NON/MTB For induced sputum culture isolate: SPI/NON/MTB	See Section 3 instructions. Refer to Laboratory section of MOP.
Plasma and Viable PBMCs Storage	8mL Sodium heparin blood	F3008	Plasma: 4x1.0mL.	BLD/HEP/PL2	Blood should be drawn prior to placement of TST at Suspected TB visit.
			PBMCs: Prepare viable PBMCs in 4 or as many as tubes needed @$5x10^6$ cells in 0.5 mL aliquots.	BLD/HEP/CEL/DMS	

Early Discontinuation Or last study visit 48 Week (± 2W)					
Evaluation	**Specimen**	**CRF**	**Aliquots**	**LDMS Code**	**Special Notes**
Hematology	1.0mL EDTA Blood	PE6812	N/A	N/A	
Chemistry-ALT	1.0mL NON or SST Blood	PE6817	N/A	N/A	
HIV Nucleic Acid Test (NAT)	3ml EDTA Blood	LBW0082 F3109 (for HIV RNA)	HIV DNA PCR: Make 2x0.3ml aliquots of whole blood	BLD/EDT/WBP	See LPC Section 2 for details Refer protocol v 2.0 Appendix I-B footnote # 6
			HIV RNA PCR: Prepare 1 aliquot with all harvested plasma	BLD/EDT/PL2	

Clinicians: Specimen Labels must include the following: PID, SID, date of collection, VID, time of collection (if PK sample), and additional additives added.
Lab Techs: Aliquot Labels must include the following: Global Specimen ID, LDMS Specimen number, PID, protocol, date of collection, VID, time of collection (if PK sample), and LDMS Specimen code.

III. Shipping Instructions

Section 5: Helpful Links and Shipping Addresses

ACTG/IMPAACT Laboratory Manual, Shipping Information and other useful information**:**

http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx

If MTA not required OR already executed, please ship directly to the testing laboratories specified below:

ASSAY/STORAGE

For viable PBMCs

Weinberg IMPAACT Immunology Laboratory
Attn: Adriana Tovar-Salazar
12700 E. 19th Ave.
RC2 Room 11480
Aurora, CO 80045
Phone (303)724-4486
Fax (303)724-4485
Email: adriana.tovarsalazar@ucdenver.edu
LDMS lab code: 174

QGIT Supernatant Storage

SUN Immunology Research Group
Tygerberg Campus
Faculty of Medicine & Health Sciences
Francie van Zijl Drive
4th Floor, Teaching Block, Lab 4012
Cape Town 7505, South Africa
Telephone:
Office: +27 (0)21 938 9839
Lab: +27 (0)21 938 9786
Contacts: Belinda Kriel (belindak@sun.ac.za), Back-up contact: Dr Andre Loxton (gl2@sun.ac.za).
LDMS lab code: 564

Hair storage (Dr. Monica Gandhi)

Roy Gerona PhD and Anita Wen
c/o IMPAACT P1078
513 Parnassus Ave
Medical Sciences Building, S-864
San Francisco, CA 94143,USA
Phone: (415) 502-1446
Roy.gerona@ucsf.edu and
Anita.wen@ucsf.edu
LDMS lab code: 607

Pharmacology Lab

Note: For each site intending to ship PK samples, they must complete the application form posted on P1078 PSWP. All applications must come to Jennifer Norman for sign off and to apply for import permit into South Africa for the PK plasma samples. The address on the application form must be as below.

Jennifer Norman/ Shameema Witbooi
K50.30 Division of Clinical Pharmacology
Old Main Building
Groote Schuur Hospital Observatory
7925 CAPE TOWN
Phone: +27 21 404 7695
Fax: +27 86 669 1348
Email: Jennifer.Norman@uct.ac.za Shameema.Witbooi@uct.ac.za
LDMS Lab Code: 499

If no MTA executed with the Testing Labs, ship ALL samples to designated repository

NIAID REPOSITORY SPECIMENS

BRI
John C. Ward
Biomedical Research Institute(BRI)
~~12264 Wilkins Ave., Bay F~~
~~Rockville, MD 20852~~
9410 Key West Avenue, First Floor
Rockville, MD 20850, USA
Phone (301)881-7636
Fax (301)770-9811
Email **brirepository@afbr-bri.com**
LDMS lab code: 999

NICHD REPOSITORY SPECIMENS

Maria Wolff
BIOLOGICAL SERVICES DIVISION, FISHER BIOSERVICES
625 LOFSTRAND LANE
ROCKVILLE,MD 20850 USA
Phone (301) 340-1620
Fax (301) 838-9753
Email Maria.Wolff@thermofisher.com
LDMS lab code: 243

Section 6: Revision History

Protocol Version	LPC Change Date	Page(s)	Description
V1.0	06 Feb 2014	12	Non-Viable PBMCs (For DNA and Pharmacogenetic variants): CRF F3008 is replaced by PKW0344.
		7,11,13,17,19	QGIT Supernatant storage: Replaced 'QFT' by "QTF' in LDMS code.
V1.0		4,6,10	Chemistry (Liver enzymes): Updated footnote 14. Updated as 'AST, ALT, and total bilirubin will be tested at screening. Thereafter ALT will be done.
V1.0		3,4	Documentation of HIV infection: Updated in SOE and added in footnote 1.
V1.0		8,11,14,16,18,20,25,26,27,28	Replaced 'heparin' by 'Sodium heparin' tube.
V1.0		8,16	Plasma storage: Updated for 'Week 44 ONLY'
V1.0		31	Section 5: Addition of Fisher Bioservices address for NICHD Repository specimens.
V1.0		8	Informed to HOLD the INH population and Intensive PK samples at site at -70 C or lower at site until further notification from study team versus batch shipment to BRI
V1.0	31 Oct 2014	25	Updated code for HIV Nucleic Acid Test (NAT) to PE5893in Section 3: INFANT Specimen Processing & Shipping
		7, 33, 36	The LDMS lab number is changed to lab 564. The LDMS address is changed from lab 536 to lab 564.
V1.0	18 June 2015	1,12,15,17,19,21,26	The stored plasma and viable PBMC aliquot quantity is changed.
V 2.0	**29 Feb 2016**	**All relevant pages**	**The LPC is revised as per P1078 protocol v 2.0 dated 28 October 2015**

Instructions for P1078 BRI "Pass Through" Shipments

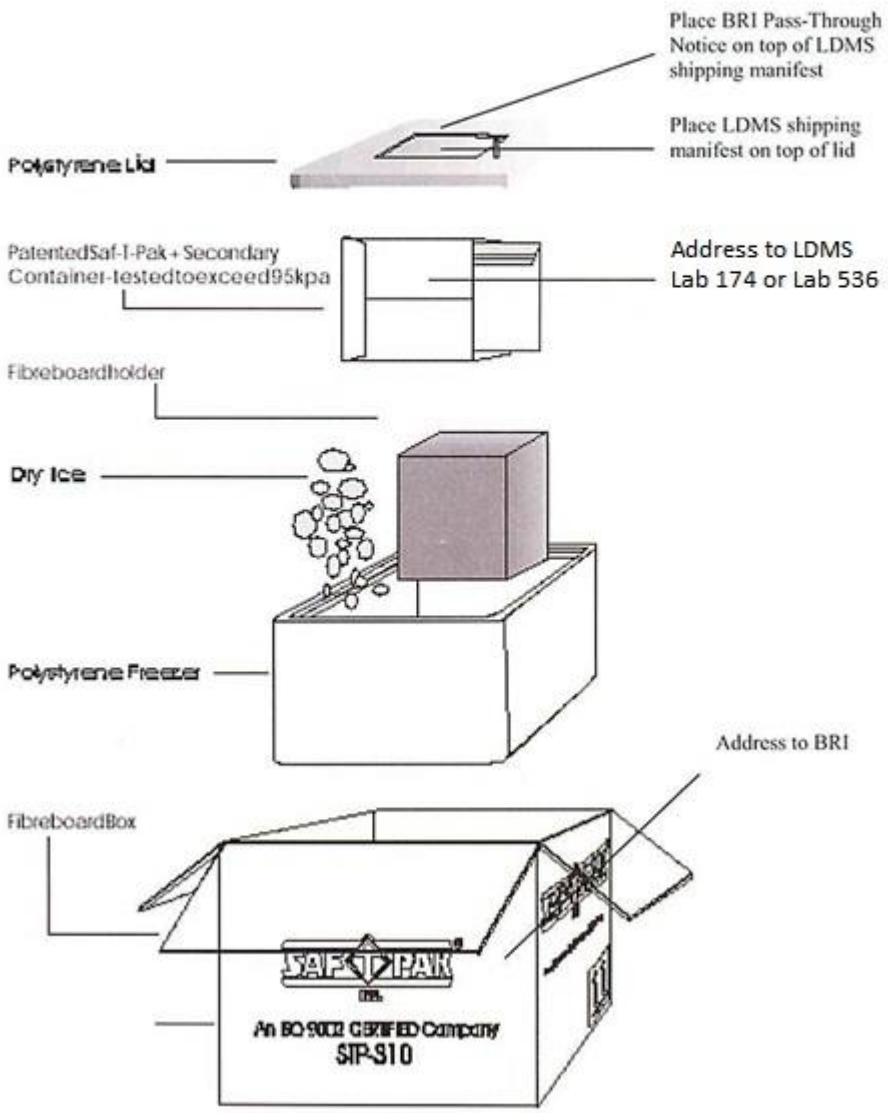
- Note: This process is to be used ONLY if your lab requires a Material Transfer Agreement (MTA) for the shipment of P1078 specimens and you do NOT have a MTA in place with the protocol testing labs (LDMS Lab #174 and 564)

1. When creating the LDMS shipping batch select the appropriate testing Lab 174 and 564 as the shipment destination based on the P1078 LPC
2. When creating the LDMS shipment DO NOT select lab 999 or 996 as the destination.
3. Pack the specimens as you normally would and Address the Safety Pack\World Courier Secondary Packaging to the final shipment destination of LDMS Lab #174 and 564
4. Place this fully packed and addressed shipment within a box and address the outer box to the BRI repository

   Biomedical Research Institute
   c/o John C. Ward, Jr.
   ~~12264 Wilkins Avenue, Bay F~~
   ~~Rockville, MD 20852~~
   9410 Key West Avenue, First Floor
   Rockville, MD 20850, USA
   Phone: (301) 881-7636
   Fax: (301) 770-9811
   Email: **brirepository@afbr-bri.com**
   LDMS lab code: 999

5. Be sure to include the correct "Pass Through" shipping notice inside the box addressed to BRI
6. Be sure to send the LDMS shipping file and any necessary CRFs to the testing lab

Pass-through Shipments: Packing Overview



Helpful Links

- IMPAACT Laboratory Manual: http://www.hanc.info/labs/Pages/ACTGIMPAACTLabManual.aspx
- Cross-Network PBMC Processing SOP: http://www.hanc.info/labs/Pages/PBMCSOP.aspx
- LDMS website: http://www.fstrf.org/ldms/index.html
- Other helpful links found at: http://www.hanc.info/Pages/default.aspx