



**Lab Processing Chart
IMPAACT Protocol P1090**

A Phase I/II Open Label Trial to Evaluate The Safety, Tolerability, Pharmacokinetics and Antiretroviral Activity of Etravirine (ETR) in Antiretroviral (ARV) Treatment –Experienced HIV-1 Infected Infants and Children Aged ≥ 2 Months to < 6 Years.

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**Section 1: Schedule of Laboratory Evaluations
From Appendices 1A through 1C for Cohorts I, II, and III.**

	Study Visits												
	Screen ¹	Entry (Day 0)	Day 14 Intensive PK Visit	Week 4	Week 8	Week 12	Week 16	Week 24	Week 32	Week 40	Week 48	Early Study D/C	Virologic failure ¹⁵
Visit Windows			±4days	±1wk	±1wk	±1wk	±2wk	±2wk	±2wk	±2wk	±2wk		
LABORATORY EVALUATIONS													
Hematology ²	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL
Chemistries ³	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL
Cholesterol/triglycerides ⁴		2mL				2mL		2mL			2mL		
Coagulation assays ⁵	2mL					2mL		2mL					
Urinalysis ⁶	X	X						X			X	X	X
<i>Virology Evaluations</i>													
HIV-1 RNA PCR ⁷	3mL	3mL ¹¹	3mL	3mL	3mL	3mL	3mL	3mL	3mL	3mL	3mL	3mL	3mL
CYP genotyping		X ¹¹											
HIV genotype & phenotype	4mL ¹⁷											4mL	4mL
<i>Immunology Evaluations</i>													
Lymphocyte subset ⁸	2mL	2mL		2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL
Pellets / plasma for storage								2mL			2mL		
<i>Pharmacology Evaluations</i>													
Intensive Pharmacokinetics ⁹			7mL										
Population PKs ¹⁰				1mL ¹²	1mL ¹³	1mL ¹⁴		1mL ¹²			1mL ¹⁴		1mL ¹⁶
TOTAL BLOOD VOLUME	14mL	10mL	13mL	9mL	9mL	13mL	8mL	15mL	8mL	8mL	13mL	12mL	13mL

1. After obtaining Informed Consent, the entry (Day 0) evaluations should be completed, within 60 days following screening.

2. Hematology tests should include complete blood count (CBC), differential and platelets.
3. Electrolytes (sodium, potassium, and HCO_3^-), glucose, creatinine, lipase, phosphorus, and LFTs. LFTs should include total bilirubin, indirect bilirubin, direct bilirubin, alkaline phosphatase, AST, ALT, and albumin. If indirect bilirubin is not reported by the site laboratory, it should be calculated at the site and documented.

The following (listed in order of preference) should be used to determine the upper limit of normal (ULN) values for indirect bilirubin.

- a. "ULN" values reported by the laboratory report for the test, or
- b. "ULN" values routinely used/established by the site, or
- c. "ULN" values as per the Harriet Lane Handbook

Sites must be consistent with the way toxicities are evaluated for all subjects in the study; sites should use the same source throughout the study. Remember to have documentation of calculated indirect bilirubin and source of "ULN", when not reported by your laboratory.

4. If a subject's laboratory results show elevated cholesterol/triglycerides AND if the subject is ≥ 1 year old, the subject should be asked to return to clinic for fasting cholesterol/triglycerides. Children ≥ 1 year to ≤ 2 years of age, should be fasting for 4 hours. Children > 2 years of age, should be fasting overnight.
5. Coagulation assays should include PT, PTT and INR. DAIDS grading table should be used. NOTE: To be performed only at sites with EQA approved labs.
6. A urine dipstick should be performed at each of the visits indicated. A complete microscopic urine assessment is required only if a urine dipstick is abnormal.
7. Virology assays should be performed at an IMPAACT/VQA approved laboratory. The Abbott platform MUST be used for the HIV-1 RNA.
8. Lymphocyte subsets include CD4 and CD8 assays.
9. Subjects should have taken ETR for at least 7 consecutive days prior to the intensive PK, without missing a dose. Intensive PK should be scheduled so that a witnessed dose of ETR is taken approximately 12 hours after the previous dose. PK dosing within a range of 11 to 13 hours after the previous dose is acceptable. The team suggests that a heparin lock be used for the intensive PK. Once the subject has arrived at the clinic, he/she should be offered a breakfast appropriate for age. Within 30 minutes following breakfast, the PK medications should be administered followed by the PK sampling at the specified time points. If the subject vomits within 15 minutes of taking study drug, the dose should be repeated. Subjects may resume food intake 2 hours after their dose of ETR at the intensive PK visit. One (1) mL of blood will be collected at the following time points: pre-dose, 1, 2, 4, 6, 9 and 12 hours post dosing. To allow for some flexibility, the 9-hour sample can be collected with a window of 8 to 10 hours post-dose and the 12 hour sample with a window of 11 to 13 hours. If necessary, the 1-hour post-dose sample and/or the 9-hours post-dose sample can be deleted to reduce the amount of total blood drawn from 7 samples over 12 hours to 5 or 6 samples, over 12 hours. **If a mini or full cohort fails, the failing subjects may have their ETR dose adjusted. In those cases, subjects will be asked to return to clinic to have a truncated intensive PK between Day 7-14 on the new dose with samples collected at the following time points: pre-dose, 2, and between 3-5 hours post dosing. Remember to ship CRFs with PK samples. LDMS Entry of PK samples will be listed in preload as Week 2 PK collections.**
10. 1.0mL of blood will be collected for each of the population pharmacokinetic samples. NOTE: An absolute minimum of 0.5 mL of plasma is necessary for population PK sample. See special shipping instructions for population PKs in Sections 3 and 5. **Remember to ship CRFs with PK samples.**
11. Wherever possible, the CYP genotyping and viral load PCR can be run from the same blood sample. The blood draw should be collected and spun down to isolate pellets for PBMCs (for CYP genotyping) and the plasma can be used for the viral load. Otherwise, a separate blood tube should be drawn.
12. At weeks 4 and 24, one sample should be timed to fall within a window of 1 to 4 hours following a dose of etravirine.
13. At week 8, one sample should be timed to fall within a window of 4 to 8 hours following a dose of etravirine.
14. At weeks 12 and 48, one sample should be timed to fall within a window of 8 to 12 hours following a dose of etravirine. (See Section 9.36 for additional information).
15. If a subject is experiencing virologic failure (as defined in section 6.3), a visit to confirm failure must be conducted between 1 to 4 weeks of the initial suspected failure or rebound.
16. At virologic failure, one sample may be collected at any time following a dose of etravirine.
17. **All** subjects must wait until genotyping and phenotyping results are available BEFORE they can start ETR and OBR.

NOTE: For insufficient blood draws, priorities are as follows:



Hematology (1mL); Chemistry (2mL); Pharmacology (intensive [7mL] or population [1mL]); Virology (HIV-1 RNA PCR – 3mL); Resistance testing (HIV genotype and phenotype – 4mL); Lymphocyte subsets (2mL); CYP genotyping (2mL); Cholesterol / triglycerides (2mL); Coagulation assays (2mL); Plasma/cell pellet for storage (2mL)

Section 2: Safety/Clinical Laboratory Evaluations				
<i>Defer to local clinical specimen collection guidelines for tube types and collection volumes as needed.</i>				
Evaluation	DMC Test Code	Tests		CRF #
Hematology	N/A	CBC, differential and platelets		PE6812
Chemistry with Liver Function Testing	N/A	Collect non-fasting. Electrolytes (sodium, potassium, and HCO ₃), glucose, creatinine, lipase, phosphorus, and LFTs. LFTs should include total bilirubin, indirect bilirubin, direct bilirubin, alkaline phosphatase, AST, ALT, and albumin. If indirect bilirubin is not reported by the site laboratory, it should be calculated at the site and documented.		PE6817
Cholesterol and Triglycerides	Lipid	Collect non-fasting. If a subjects laboratory results show elevated cholesterol/triglycerides AND if the subject is ≥1 year old, the subject should be asked to return to clinic for fasting cholesterol/triglycerides.		PE6817
Coagulation Assays	N/A	Coagulation assays should include PT, PTT and INR. NOTE: Performed only at sites that have EQA approved labs for coagulation testing.		PE6812
Urinalysis	N/A	A urine dipstick should be performed. A complete microscopic urine assessment is required only if a urine dipstick is abnormal.		PE0811
Urine Pregnancy Test	N/A	This test will only be required during long term follow-up testing.	Pregnancy Test must have a <25mIU sensitivity	
CD4+/CD8+	CD4/CD8	CD4/CD8 cell counts and percentages	Dual platform labs only must also have a WBC and diff.	LBW0054



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
Plasma HIV-1 RNA Abbott Realtime HIV-1	EDTA	Invert tube 8-10 times to mix.	F3006 RNAHIV	Spin blood at 800xg for 10 min. Remove plasma; respin plasma at 800xg for 10 min. Prepare 2x0.8mL plasma aliquot and store at -70C° or colder.	Perform at any local VQA certified laboratory using the Abbott platform. If Abbott platform is not available locally at US sites, ship 2x0.8mL plasma aliquot to University of Washington/Seattle real time. Non-U.S. sites should ship RNA sample real-time to the nearest local VQA certified laboratory with the Abbott platform.
CYP Genotyping	EDTA	Packed blood cells from a RNA PCR collection is to be prepared for this assay.	F3006 PKGENO	Save packed cells from RNA PCR plasma collection. Vortex packed blood cells and transfer to a cryovial. Store at -70C° or colder	CYP specimens should be batch shipped upon request to BRI.
Plasma for HIV genotype and phenotype	EDTA	Recommended genotyping labs are listed in Section 5 of the LPC. HIV phenotyping is to be sent to Monogram Biosciences.	F3006 GENOHIV & PHENOHIV	Spin blood at 800xg for 10 min. Remove plasma; respin plasma at 800xg for 10 min. Prepare 2x1.0mL plasma aliquots and store at -70C° or colder until shipped to appropriate laboratory	<i>Shipping is collection and cohort specific as follows:</i> SCREENING (genotyping): Ship 1 aliquot real time to the listed Seattle Lab or to the closest IMPAACT/VQA certified genotyping lab. Phenotyping: <u>U.S. and Brazil</u> – ship 1 aliquot real time to Monogram, <u>All other sites</u> – ship to BRI as a pass through to Monogram. Virologic Failure/Early Study D/C: (genotyping) All sites - ship samples quarterly to the Seattle Lab or the closest IMPAACT/VQA certified genotyping lab. Phenotyping samples: <u>U.S. and Brazil</u> – ship quarterly to Monogram. <u>All other sites</u> – ship quarterly to BRI as a pass through to Monogram.
Stored Plasma	EDTA	NA	SPW0457 STORIMM	Spin blood at 400xg for 10 min. remove plasma, respin plasma at 800xg for 10mins. Prepare 2 x 0.5mL plasma aliquots and store at -70C° or colder.	Ship to appropriate repository quarterly.
Stored PBMC pellets	EDTA	NA	SPW0457 STORIMM	See Cross-Network PBMC Processing SOP for PBMC isolation Prepare 2 (2x10 ⁶ pellets). Store pellets at -70C° or colder	Ship to appropriate repository quarterly.



<p>Intensive PK's</p>	<p>EDTA Spray Dried Only</p>	<p>PK draw times are pre-dose, 1, 2, 4, 6, 9, & 12 hours post dose. Process within 1 hour of collection.</p>	<p>PKW0318 PK-INT</p>	<p>Spin blood at 800g for 10min. remove plasma and prepare a single aliquot for each time point and store at -70C° or colder. LDMS Entry of PK samples will be listed in preload as Week 2 PK collections.</p>	<p><u>U.S.</u> sites ship PKs real time to U. Colorado PK Lab. <u>All other sites</u> - ship real time to BRI as pass through to U. Colorado. See MOP 7.5.1 for pass through instructions. Remember to ship CRFs with PK samples.</p>
<p>Population PK</p>	<p>EDTA Spray Dried Only</p>	<p>Process within 1 hour of collection.</p>	<p>PKW0319 PK-POP</p>	<p>Spin blood at 800xg for 10min. remove plasma. Prepare a single aliquot for each time point and store at -70C° or colder.</p>	<p>All sites should batch ship population PKs as close to real time as possible as follows: <u>U.S. sites</u> will batch ship PKs directly to U. Colorado PK Lab either monthly or with intensive PK shipments, whichever comes first. <u>All other sites</u> ship PK samples to U. Colorado as BRI pass-through samples as often as possible: <ol style="list-style-type: none"> 1.) With intensive PK shipments 2.) With Monogram phenotyping shipments 3.) With Quarterly stored sample shipments Remember to ship CRFs with all PK samples.</p>



Section 4: Evaluations by Visit.					
Screen: Must be completed within 60 days prior to study entry					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Coagulation Studies Conditional Draw. See Section 2	2mL Na Citrate blood	PE6812	N/A	N/A	Make sure that the citrate tube is completely full to have the correct ratio of blood to anticoagulant.
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
HIV Genotype and Phenotype	4mL EDTA blood NOTE: All subjects MUST wait until genotyping and phenotyping results are available before they can start ETR and OBR.	F3006	Freeze 2 x 1.0mL plasma aliquots at -70C° or lower. Ship 1 aliquot real time to closest genotyping lab. Phenotyping: <u>U.S. and Brazil</u> – ship 1 aliquot real time to Monogram, <u>All other sites</u> – ship to BRI as a pass through to Monogram.	BLD/EDT/PL2	<i>It is critical that these samples be sent for testing as soon as possible.</i> See Section 5 for additional shipping instructions.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.
Entry (Week 0):					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	2mL NON or SST blood	PE6817	N/A	N/A	Non-fasting.
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.



Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
CYP Genotyping	Packed cells from RNA sample above	F3006	Mix and transfer packed cell to a single 2mL cryovial	BLD/EDT/WBP	Batch Ship to BRI
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.

Day 14 (±4 days) Intensive PK Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Intensive Pharmacokinetics	1mL EDTA (Spray Dried Only) blood at each time point. Process within 1 hour of collection.	PKW0318 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1 LDMS Entry of PK samples will be listed in preload as Week 2 PK collections.	Subject should have a normal breakfast 30 minutes to drug delivery and start of PK sampling.

Weeks 4 & 8 (± 1wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Population PK	1mL EDTA (Spray Dried Only) blood	PKW0319 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1	Week 4: Time the sample collection 1-4 hours post dose. Week 8: Time the sample collection 4-8 hours post dose
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Week 12 (± 1wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab non-fasting.
Coagulation Studies Conditional Draw. See Section 2	2mL Na Citrate blood	PE6812	N/A	N/A	Make sure that the citrate tube is completely full to have the correct ratio of blood to anticoagulant.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.
Population PK	1mL EDTA (Spray Dried Only) blood	PKW0319 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1	Time the sample collection 8-12 hours post dose if possible.

Week 16 (± 2wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Week 24 (± 2wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab non-fasting.
Coagulation Studies Conditional Draw. See Section 2	2mL Na Citrate blood	PE6812	N/A	N/A	Make sure that the citrate tube is completely full to have the correct ratio of blood to anticoagulant.
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.
Pellets and Plasma for Storage	2mL EDTA blood	SPW0457	Prepare 2 x 0.5mL aliquots of plasma and 2 pellets @ 2 x 10 ⁶ PBMCs.	BLD/EDT/PL2 and BLD/EDT/PEL	This sample may be omitted for younger children if blood volumes are a problem.
Population PK	1mL EDTA (Spray Dried Only) blood	PKW0319 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1	Time the sample collection at 1-4 hours post dose.

Week 32 (± 2wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab



Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.

Week 40 (± 2wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.

Week 48 (± 2wk) Visit:

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab non-fasting.
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Pellets and Plasma for Storage	2mL EDTA blood	SPW0457	Prepare 2 x 0.5 aliquots of plasma and 2 pellets @ 2 x 10 ⁶ PBMCs.	BLD/EDT/PL2 and BLD/EDT/PEL	This sample may be omitted for younger children if blood volumes are a problem.
Population PK	1mL EDTA (Spray Dried Only) blood	PKW0319 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1	Week 48: Time the sample collection 8-12 hours post dose

Early Study D/C Visit:

Every attempt should be made to collect the following samples for patients that discontinue the study early.

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
HIV Genotype and Phenotype	4mL EDTA blood	F3006	Freeze 2 x 1.2mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	See Section 3 for detailed shipping instructions
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Follow-Up Visit for Subjects that Discontinue Etravirine but Remain on Study: Week 4 post ETR Discontinuation from Appendix ID.

NOTE: Subjects who are withdrawn from study drug within the first 48 weeks of study will not enter long term follow-up, but will return to clinic 4 weeks after ETR discontinuation, as per Appendix IE. Subjects with AEs will be followed until satisfactory clinical resolution (i.e. value returns back to subjects baseline value) or stabilization (to be agreed upon with the sponsor). All grade 3 and grade 4 laboratory abnormalities as well as any laboratory abnormalities resulting in an increase of 2 DAIDS Grades from baseline, will be followed until return to baseline or within 1 Grade from baseline. If an AE resolves within 4 weeks of discontinuation of ETR, the subject should still return for the final 4 week study visit.

For insufficient blood draws, priorities are as follows:

Hematology (1mL); Chemistry (2mL); Virology (HIV-1 RNA PCR – 3mL); Lymphocyte subsets (2mL); Cholesterol / triglycerides (2mL)

Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	3mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	1mL NON or SST blood	PE6817	N/A	N/A	Send to local lab non-fasting.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Virologic Failure Confirmation					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
HIV Genotype and Phenotype	4mL EDTA blood	F3006	Freeze 2 x 1.2mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	See Section 3 for detailed shipping instructions
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.
Population PK	1mL EDTA (Spray Dried Only) blood	PKW0319 Remember to ship CRFs with PK samples.	Transfer all the plasma to a single cryovial after centrifugation and freeze as soon as possible	BLD/DPE/PL1	Collect this sample at any time post etravirine dosing.



Long Term Safety Follow-up for Subjects Receiving Study-Provided Etravirine

SCHEDULE OF EVALUATIONS - Long-term Safety Follow-up for Subjects Receiving Study-Provided Etravirine (From Appendix IE)				
For insufficient blood draws, priorities are as follows: Chemistry (2mL); Virology (HIV-1 RNA PCR – 3mL); Lymphocyte subsets (2mL); HIV genotype/phenotype (4mL); Cholesterol / triglycerides (2mL)				
	Every 12 weeks (±2 weeks)	Every 24 weeks (±2 weeks)	Early Discontinuation	14 Days Post Therapy (±1 week)
LABORATORY EVALUATIONS				
Chemistries ²	X ⁹	2mL	2mL	3mL
Hematology ³	X ⁹	2mL	2mL	2mL
Cholesterol/triglycerides ⁴	X ⁹	2mL		
Urinalysis ⁵		X	X	X
Urine pregnancy test (if applicable) ⁶	X	X	X	X
Virology				
HIV-1 RNA PCR ⁷	X ⁹	3mL	3mL	3mL
HIV genotype & phenotype			4mL	
Immunology				
Lymphocyte subset ⁸	X ⁹	2mL	2mL	
TOTAL BLOOD VOLUME	None	11mL	13mL	8mL

NOTE: Visits during long term follow up are every 12 weeks with specific lab tests being collected every 24 weeks (for additional details, see footnote 9 below).

Footnotes

2. Electrolytes (sodium, potassium, and HCO₃), glucose, creatinine, lipase, phosphorus, and LFTs. LFTs should include total bilirubin, indirect bilirubin, direct bilirubin, alkaline phosphatase, AST, ALT, and albumin. If indirect bilirubin is not reported by the site laboratory, it should be calculated at the site and documented.

The following (listed in order of preference) should be used to determine the upper limit of normal (ULN) values for indirect bilirubin.

- a. "ULN" values reported by the laboratory report for the test, or
- b. "ULN" values routinely used/established by the site, or
- c. "ULN" values as per the Harriet Lane Handbook

Sites must be consistent with the way toxicities are evaluated for all subjects in the study; sites should use the same source throughout the study. Remember to have documentation of calculated indirect bilirubin and source of "ULN", when not reported by your laboratory.

3. Hematology tests should include complete blood count (CBC), differential and platelets.

4. If a subjects laboratory results show elevated cholesterol/triglycerides AND if the subject is ≥1 year old, the subject should be asked to return to clinic for fasting cholesterol/triglycerides. Children ≥1 year to ≤2 years of age, should be fasting for 4 hours. Children >2 years of age, should be fasting overnight.

5. A urine dipstick should be performed at each of the visits indicated. A complete microscopic urine assessment is required only if a urine dipstick is abnormal.

6. A urine pregnancy test should be performed at every visit on any female subject who is capable of becoming pregnant.



7. Virology assays should be performed at an IMPAACT/VQA approved laboratory. For protocol required plasma RNA testing, the Abbott platform MUST be used. During the long term follow up, any standard of care HIV-1 RNA results (i.e. those obtained at non-protocol required time points) available within ± 4 weeks of the study visit should also be reported on the study CRF, even if done using non-Abbott assays.
8. Lymphocyte subsets should include CD4 and CD8.
9. If these tests are performed as part of standard of care for the subject within ± 4 weeks of the visit, they should be recorded on the study CRFs. Otherwise; these tests should not be done for the study at this time point.

Repeat Visits Every 12 weeks (± 2 weeks)					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Urine Pregnancy	1mL Urine		NA	NA	To be performed on female subjects capable of becoming pregnant.
Repeat Visits Every 24 weeks (± 2 weeks)					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Cholesterol/Triglycerides	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab non-fasting.
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Urine Pregnancy	Urine (from UA above)		NA	NA	To be performed on female subjects capable of becoming pregnant.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70°C or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.



Early Discontinuation Visit					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Urine Pregnancy	Urine (from UA above)		NA	NA	To be performed on female subjects capable of becoming pregnant.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.
HIV Genotype and Phenotype	4mL EDTA blood	F3006	Freeze 2 x 1.2mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	See Section 3 for detailed shipping instructions
Lymphocyte Subsets	2mL EDTA (Spray Dried Only) blood	LBW0054	None	BLD/DPE/BLD (if entered into the LDMS)	Send to local IQA certified lab at ambient temps.
14 Days Post Therapy (± 1 week)					
Evaluation	Specimen	CRF	Aliquots	LDMS Code	Special Notes
Hematology	1mL EDTA blood	PE6812	N/A	N/A	Send to local lab
Chemistries	2mL NON or SST blood	PE6817	N/A	N/A	Send to local lab
Urinalysis	5-10mL clean catch urine if possible.	PE0811	N/A	N/A	Send to testing lab as soon as possible. Refrigerate if there is a delay in testing.
Urine Pregnancy	Urine (from UA above)		NA	NA	To be performed on female subjects capable of becoming pregnant.
Abbott RealTime HIV-1 RNA PCR	3mL EDTA blood	F3006 and F3109 if results are not reported in the LDMS	Freeze 2 x 0.8mL plasma aliquots at -70C° or lower.	BLD/EDT/PL2	Can be performed at any VQA certified local laboratory.



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>

Special Shipping Instructions for Population PKs.

All sites should batch ship population PKs as close to real time as possible as follows: **U.S. sites** will batch ship PKs directly to U. Colorado PK Lab either monthly or with intensive PK shipments, whichever comes first.

All other sites ship PK samples to U. Colorado as BRI pass-through samples as often as possible:

- 4.) With intensive PK shipments
- 5.) With Monogram phenotyping shipments
- 6.) With Quarterly stored sample shipments

Remember to ship CRFs with all PK samples.

<p>Pharmacology Specimens Remember to ship CRFs with PK samples. ATTN; Lane Bushman Colorado Antiviral Pharmacology Laboratory University of Colorado Denver Skaggs School of Pharmacy Room 4410, V20-C238 12850 E Montview Blvd. Aurora, CO 80045 Phone: (303)-724-6132 Fax : (303)-724-6135 lane.bushman@ucdenver.edu LDMS Code : 533</p>	<p>REPOSITORY SPECIMENS – NIAID Sites ONLY John Ward Biomedical Research Institute(BRI) 12264 Wilkins Ave., Bay F Rockville, MD 20852 Phone (301)881-7636 Fax (301)770-9811 Email brirepository@aol.com LDMS lab code: 999</p>	<p>REPOSITORY SPECIMENS – NICHD Sites ONLY Fisher Bioservices Attn: Maria Wolff PACTG (IMPAACT) 625 Lofstrand Lane Rockville MD 20850 Tel: 301-340-1620 Fax: 301-838-9753 Email: maria.wolff@thermofisher.com LDMS lab code: 243</p>	<p>University of Washington for domestic (RNA & genotyping only) Dr. Lisa Frenkel Attn: Dr. Ingrid Beck University of Washington – Children’s Hospital of Seattle 1900 Ninth Ave. Seattle WA 98101 Phone: 206-884-3440 Fax:206-884-7311 Frenkelabshipments@seattlechildrens.org Please complete attached testing form for genotyping samples sent to Lab 238 LDMS Lab #238</p>
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<p>Phenotyping Specimens: Add P1090 to the Monogram test request & make sure that Tim Persyn is notified.</p> <p>Monogram Biosciences Inc. Attn: Tim Persyn 345 Oyster Point Blvd. South San Francisco, CA 94080-1913 Phone: (650) 866-7482 Fax: (650) 624-4457 Email: Persynt@labcorp.com & receiving@MonogramBio.com</p> <p>LDMS Lab Code : 903</p>	<p>Africa Resistance Testing (Genotyping only) Johannesburg CLS Laboratory Dr. Wendy Stevens Spencer Lister Building, 4th Floor, NHLS Complex Cnr De Korte and Hospital Street Braamfontein Johannesburg, 2000 South Africa Phone: 011-27 11 489-9765 Fax: 011-27-11-489-8554 Email: wendy.stevens@nhis.ac.za</p> <p>LDMS Code: 350</p>	<p>India Resistance Testing (Genotyping only)</p> <p>To Be Determined</p>	<p>South America Resistance Testing (Genotyping only) Fiocruz, Brazil Mariza G. Morgado or Deise Luci Rufino dos Santo Pavilhao Leonidas Deane, 4th Floor Avenida Brazil, 4365 Manguinhos, RJ 21045-900 Brazil Phone: 011-55-21-25984583 Fax: 011-55-21-22801589 Email: mmorgado@ioc.fiocruz.br Or : camposmello@hotmail.com</p> <p>LDMS Code: 319</p>
<p>Thailand Resistance Testing (Genotyping only) PHPT Laboratory Laddawan Laomanit 548 ChiangMai-Lamphun Road, Nong Hoi Muang, Chiang Mai 50000 Thailand Phone: +66-53-894-431 Fax: =66-53-894-220 Email: laddawan@phpt.org</p> <p>LDMS Code: 251</p>			

Revision History : (Created 11/30/2011), 2 /12/2012 : Minor edits and formatting changes for final version 1.0. 5/7/2012: CRF numbers for stored samples and for pharmacology collections have been updated. 5/24/2012: "Remember to ship CRFs with PK samples." added to PK entries and virologic failure and early DC genotype/phenotype shipping is now quarterly. 6/1/2012: Changes in PK shipments for Brazilian sites as pass-through BRI were made. 10.23/2012: Version 2.0 edits including title change, removal of cohorts IIB and IIIB, changes for coagulation testing at select sites, Appendices renaming, and address change for the Pharmacology Group. 11/3 2012: Removed IIIB note from page 6 Day 7-10 Intensive PK Visit. 12/6/2012: Added statement "LDMS Entry of PK samples will be listed in preload as Week 2 PK collections" to footnote 9 of SOE, Section 3 Intensive PK information and Section 4, Day 7-12 entry. 12/13/2012: Revised footnote 17 on page 2 to read: "All subjects must wait until genotyping and phenotyping results are available BEFORE they can start ETR and OBR." Cohort III was removed and phenotyping was added to the previous iteration of the footnote. 12/24/2012: Revised page 5 Genotyping entry note to read. "NOTE: All subjects MUST wait until genotyping and phenotyping results are available before they can start ETR and OBR", replacing Cohort III with All and adding phenotyping. 2/28/2013: LPC 2.4 Section 5 shipping logs were updated for UNC (Contact person changed from Melissa to Nicole) and Monogram (Tim Persyn added to the email address). Section 3 and Section 4 screening geno/pheno collections clarified to indicate that one aliquot go to Monogram and 1 aliquot to local testing lab at screening. 11/7/2013: Added Version3 changes. 12/7/2013: updated intensive PK language to state 14 +/- 4 days from Days 7-12. 2/10/2014: Updated shipping instructions for Population PKs in sections 3 & 5 and in the SOE footnote 10. 8/10-14/2014: Updated shipping instructions. Changed UNC to University of Washington because UNC no longer doing sequencing. Removed references to UNC from Section 3. 10/8/2014: Added testing form for Frenkel lab. Updated section 3 genotyping shipping language. 11/20/2014: Corrected week 48 Random PK window from 4-8 hours to 8-12 hours post dose. 12/10/2014: Added VQA to SOE footnotes for HIV RNA testing. LPC updated to Version 4.0 to reflect protocol version. Footnote 9 (p3) updated to reflect failed PK repeat change. 01/15/2015: Corrected p.6 header Screen from 45 day to correct 60 days.

