**Lab Processing Chart for all Protocol Assays**

Protocol # IMPAACT P1058A  
Version 2.2  
*Date of last revision: 03/21/2013*

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*Data Handling* - All specimens must be logged into the specimen management module and all aliquots must be entered into the storage module of the LDMS and exported weekly. Volumes must be accurate and the # of aliquots entered must be adjusted to match the # of aliquots actually prepared.

<table>
<thead>
<tr>
<th>Visit</th>
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For all P1058A related laboratory documents, see the Protocol Specific webpage: [https://impaactgroup.org/](https://impaactgroup.org/).

The protocol-specific web page can be accessed through the member area of the website. Enter the Member/MIS area using your individual username and password. Search for the study number. From the protocol [P1058A] web page you will have the option to click the PSWP tab. The LPC will be located under the section of the PSWP titled “Laboratory”.

**SPECIMENS COLLECTED FOR IMPAACT PROTOCOLS**

NIH Clinical Center guidelines for blood volume in research trials: For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. Investigators should consider further limiting the volume of blood withdrawn for research purposes in patients with significant anemia or compromised cardiac output. In instances of clinical need, it is the responsibility of the patient’s attending physician to determine if phlebotomy in excess of the above limits may be permitted.

For clinical tests, your clinical laboratory instructions and specifications supersede the LPC’s, unless otherwise stated. For IMPAACT/ACTG specific tests and stored samples, LPC instructions must be followed.

Priority of blood samples is: 1) Screening labs (if visits are combined); 2) PK (first priority for PK visit); 3) Viral load; 4) T-cell Subsets; 5) Pharmacogenetics and 6) Plasma storage.

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

[http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx](http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx)

<table>
<thead>
<tr>
<th>Screen/Entry – may be combined</th>
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</table>
| Screen/Entry (May be combined.) | P1058A  
1mL red top tube (no additive) - Send to local clinical lab ambient. Complete CRF # DQW0030 | Chemistry- total bilirubin, AST, ALT, lipase, electrolytes, and glucose. | None |
| Screen/Entry | P1058A  
1mL EDTA tube- Invert 10-15 times gently. Send to local clinical lab ambient. Complete CRF # DQW0030 | Hematology- CBC, Hbg, plt., and diff. | None |
| Screen/Entry  
*Female subjects only of childbearing potential* | P1058A  
1mL SST or red top (no additive) OR urine (1 mL). Send to local lab ambient Complete CRF# F0847. | Beta HCG (pregnancy test) | None |

**Intensive PK visit** - Within 35 days of Screening/Entry; If PK visit cannot be performed within ≤ 35 days from entry visit, contact protocol team.
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<tr>
<td>PK visit – Within 35 days of Screening/Entry</td>
<td>P1058A</td>
<td>1mL red top tube (no additive) - Send to local clinical lab ambient. Complete CRF # DQW0030</td>
<td>Chemistry- total bilirubin, AST, ALT, lipase, electrolytes, and glucose.</td>
<td>None</td>
</tr>
<tr>
<td>PK visit – Within 35 days of Screening/Entry Female subjects only of childbearing potential</td>
<td>P1058A</td>
<td>1mL SST or red top (no additive) OR urine (1 mL) Complete CRF# F0847 Send to local lab ambient.</td>
<td>Beta HCG (pregnancy test)</td>
<td>None</td>
</tr>
<tr>
<td>PK visit – Within 35 days of Screening/Entry Can be drawn anytime during PK visit, 0 hr predose is recommended</td>
<td>P1058A</td>
<td>3 mL EDTA tube- Invert 10-15 times gently. Send to local lab ambient. Submit CRF # F3006 Complete CRF # F3109 Testing to be done in any CLIA-compliant (or equivalent) laboratory.</td>
<td>HIV-1 RNA PCR- Per current IMPAACT and HANC instructions and VQA standards. Test Code: RNAHIV See Appendix I, footnote 6 (<a href="http://www.impaactgroup.org/quality-management">http://www.impaactgroup.org/quality-management</a>).</td>
<td>None</td>
</tr>
<tr>
<td>PK visit – Within 35 days of Screening/Entry Can be drawn anytime during PK visit, 0 hour predose is recommended; in Version 2.0, blood sample may also be drawn at 24 hours post dose.</td>
<td>P1058A</td>
<td>5mL EDTA tube- Invert 10-15 times gently. Send to IMPAACT processing lab ambient to be processed within 30 hours of collection. Submit CRF # F3006</td>
<td>Plasma storage for future Virology studies Test Code: STORVIR</td>
<td>Spin blood at 800xg for 10 mins, remove plasma and re-spin plasma at 800xg for 10mins. Freeze 3-4 x 0.6mL aliquots at -70°C. LDMS spec. code: BLD/EDT/PL2 Ship to repository monthly.</td>
</tr>
<tr>
<td>PK visit –</td>
<td>P1058A</td>
<td>1mL EDTA tube- Invert 10-15 times</td>
<td>CD4 cell counts and</td>
<td>None</td>
</tr>
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| Within 35 days of Screening/Entry  
*Can be drawn anytime during PK visit* |  | gently. Send to local CLIA-compliant (or equivalent) laboratory. Complete CRF # LBW0054  
(If dual platform lab, then a 2nd 1mL EDTA tube must be collected and sent to a local clinical lab for WBC and diff. if CBC is not already being performed.) | percentages  
Test Code: CD4  
Dual platform labs only must also have a WBC and diff. |  |
| PK visit – Within 35 days of Screening/Entry  
*Predose and 1, 2, 4, 6, 8, 12 and 24 hours post-dose* | P1058A | For each time point: 4mL K2EDTA sprayed dried tube- Invert 10-15 times gently. Send to IMPAACT processing lab ambient to be processed within 1 hour of collection. Submit CRF #: PKW0330 | Plasma Pharmacokinetics  
- Intensive  
Test Code: PKINT | For each time point: Process within 1 hour. Spin blood at 800xg for 10 mins. **Remove plasma and aliquot two 0.9 mL volumes to two leak-proof O-ring containing cryovials.** Freeze the aliquots in upright position immediately at -20°C or -70°C until shipment.  
**FOR GROUPS M, N, and O (PKW0330):**  
Ship 1 aliquot for each time point to U. of Alabama (LDMS Lab 191) real-time on dry ice, overnight. One aliquot should be retained at the site as back-up sample.  
**For Group P, ship 1 aliquot to UAB, and 1 aliquot to UCSD PK Lab (LDMS Lab #196) real time for each time point**  
**For Group Q, Ship 1 aliquot for each time point to UCSD PK Lab (LDMS Lab #196) real time. One aliquot should be retained at the site as back-up sample**  
LDMS spec. code: BLD/EDT/PL1 |
| PK visit – Within 35 days of Screening/Entry  
*Can be drawn* | P1058A | 5mL K2EDTA sprayed dried tube- Invert 10-15 times gently. Send to IMPAACT processing lab ambient for processing to be completed within 48 hours of collection. | Pharmacogenetics – Analysis for genetic polymorphisms.  
Test Code: PKGENO | Standard procedures consistent with the Cross-Network Consensus PBMC Processing Guideline should be followed for isolation of PBMC. |
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<tr>
<td>anytime during PK visit. 0 hour predose is recommended</td>
<td></td>
<td>Submit CRF #SPW0350</td>
<td></td>
<td>PBMC should be re-suspended at a concentration of 1-2 X 10^6 cells/mL in PBS per cryovial. Centrifuge vials for 3 minutes at the highest speed in a microfuge (typically &gt;10,000g). Optimally prepare at least 2-4 vials. Aspirate supernatant without disturbing the pellet. Store at -70°C. Store on site until team instructs for shipment to UCSD Pediatric Lab (LDMS Lab. #08)</td>
</tr>
</tbody>
</table>

**Repeat PK visit** – per provider request.

| Repeat PK visit – within 28 days prior to repeat PK | P1058A | 1mL red top tube (no additive) - Send to local clinical lab ambient. Complete CRF # DQW0030 | Chemistry- total bilirubin, AST, ALT, lipase, electrolytes, and glucose. | None |

| Repeat PK visit – within 28 days prior to repeat PK Screen/Entry | P1058A | 1mL SST or red top (no additive) OR urine (1 mL) Complete CRF# F0847 Send to local lab ambient. | Beta HCG (pregnancy test) | None. |

<p>| Repeat PK visit – Predose and 1, 2, 4, 6, 8, 12 and 24 hours post-dose | P1058A | For each time point: 4mL K2EDTA sprayed dried tube- Invert 10-15 times gently. Send to IMPAACT processing lab ambient to be processed within 1 hour of collection. Submit CRF # PKW0330 | Plasma Pharmacokinetics - Intensive Test Code: PKINT | For each time point: Process within 1 hour. Spin blood at 8000g for 10 mins. <strong>Remove plasma and aliquot two 0.9 mL volumes to two leak-proof O-ring containing cryovials.</strong> Freeze the aliquots in upright position immediately at -20°C or -70°C until shipment. **FOR GROUPS M, N, and O (PKW0330): Ship 1 aliquot for each time point to U. of Alabama (LDMS Lab 191) real-time on dry ice, overnight. One aliquot should be retained at the site as back-up sample. |</p>
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<td>For Group P, ship 1 aliquot to UAB, and 1 aliquot to UCSD PK Lab (LDMS Lab #196) real time for each time point</td>
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<td></td>
<td>For Group Q, Ship 1 aliquot for each time point to UCSD PK Lab (LDMS Lab #196) real time. One aliquot should be retained at the site as back-up sample</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>LDMS spec. code: BLD/EDT/PL1</td>
</tr>
</tbody>
</table>

**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: LDMS Specimen number, PID, protocol, date of collection, VID, time of collection (if PK sample), and LDMS Specimen code.

**Shipping Addresses**

**PK SPECIMENS: (ALL Groups EXCEPT Q)**
Kedria Walker  
University of Alabama at Birmingham  
VOLKER HALL RM 270.  
1670 University Blvd.  
Birmingham AL 35294-2182  
Phone (205) 975-2461  
Fax (205) 934-6201  
Email: kedria@uab.edu  
LDMS Lab#: 191

**PK SPECIMENS: (GROUPS P AND Q)**
Pediatric Pharmacology Laboratory  
University of California, San Diego Medical Center  
212 Dickinson Street, CTF B-112  
San Diego, CA 92103  
Phone: 619-543-5293  
FAX: 619-543-5422  
email: ecapparelli@ucsd.edu; respina@ucsd.edu  
LDMS Lab #: 196

**REPOSITORY SPECIMENS:**
John Ward  
Biomedical Research Institute (BRI)  
12264 Wilkins Ave., Bay F  
Rockville, MD 20852  
Phone (301) 881-7636  
Fax (301) 770-9811  
Email: brrepository@aol.com  
LDMS Lab#: 999

Fisher Repository  
Biological Services Division, Fisher Bioservices (243)  
625 Lofstrand Lane  
Rockville, MD 20850  
Phone (301) 340-1620  
Fax (301) 838-9753  
LDMS Lab#: 243

**PHARMACOGENETICS SPECIMENS:**
Attn: Carol Mundy  
Stephen A. Spector, M.D.  
University of California, San Diego Department of Pediatrics  
Division of Infectious Diseases  
Stein Clinical Research Bldg, Room 430  
9500 Gilman Dr., Mail Code 0672  
La Jolla, CA 92037-0672  
Phone: (858) 534-7055  
Fax: (858) 534-7411  
E-mail: saspector@ucsd.edu  
LDMS Lab # : 08