HPTN 035

Laboratory Overview

SSP Laboratory Procedures

- As the transmission of HIV can occur through contact with contaminated needles, blood, blood product, and vaginal secretions, appropriate blood and body fluid precautions will be employed by all personnel in the drawing of blood and handling of all specimens for this study in a clinical or laboratory setting.

SSP Laboratory Procedures

- A copy of the CDC's guidelines entitled "Universal Precautions For Prevention of Transmission of HIV And Other Bloodborne Infections" can be found at: http://www.cdc.gov/ncidod/hip/Blood/UNIVERSA.HTM. In addition, information about "Universal Precautions, Including Injection Safety" put out by the World Health Organization (WHO) can be downloaded at: http://www.who.int/hiv/topics/precautions/universal/en/

SSP Laboratory Procedures

- Additional laboratory reference information can be found the Section 13 of the HPTN Manual of Operations and the HPTN Laboratory Standard Operating Procedures Manual.

SSP Laboratory Procedures

- Questions about specimen collection should be raised with your site investigator or the HPTN Central Lab Manager Estelle Piwowar-Manning (410.614.6736 or epiwowa@jhmi.edu). Questions about LDMS and specimen shipping should also be raised with Estelle Piwowar-Manning. For problems or technical questions about LDMS only, contact the LDMS User Support (716.834.0900 x311 or hptn.ldms.usersupport@fstrf.org.

- In all settings, laboratory procedures will be performed according to study site standard operating procedures (SOPs) that have been approved by the HPTN Central Lab and test kit package inserts (where applicable).

Local Laboratory Specimens

- Blood for HIV, syphilis serology, Hematology, Coagulation, and Chemistry.
- Urine and genital swabs for STD testing.
- Urine for pregnancy testing.
- Blood for storage and HSV-2 testing

Possible Clinic Tests

- HIV
- Pregnancy testing
- Dipstick Urinalysis
- Wet Mount

REMEMBER

RESPONSIBILITY

FALLS

ON THE LAB!

- The same documentation and (QC) practices required in the laboratory are required in the clinic.
- Laboratory Manager (or designee) must review at least once per month.
- In the event that proper QC procedures are not followed in the clinic, or that adequate QC is not maintained, the Laboratory Manager is responsible for ensuring that corrective action is taken and documented.

- Regardless of whether tests are performed in clinic or laboratory settings, study staff who perform the tests must be trained in proper testing and associated QC procedures prior to performing the tests for study purposes; training documentation should be available for inspection at any time

Local Laboratory Specimens

Urine for Pregnancy

Urine will be collected at certain visits (see Appendix II and III) for pregnancy testing. The laboratory and/or the clinic will report test results directly to SCHARP via DataFax.

Urine for Pregnancy

- Participant should not have urinated within one hour prior to collection. First morning specimens generally contain the highest concentration of hCG and are recommended for early detection of pregnancy.
- Provide participant with a labeled [participant ID label] screw-top urine collection cup. Write in the date.
- Instruct women to not clean the labia prior to collecting the urine specimen.
- Instruct participants to collect 20 mL only of first portion of urine stream (i.e., first void/ dirty catch urine).
- Instruct participant to screw lid on tightly.

- At visits when pregnancy testing and/or dipstick urinalysis is required, aliquot 5 mL for these tests and store the remaining urine at 2-8° C for subsequent chlamydia and gonorrhea testing.

Local Laboratory Specimens

Urine for Pregnancy

Pregnancy– On Site

- Conduct pregnancy test using the **Quick Vue Pregnancy** Test kit.

- Record results on the applicable Lab Results Form

- Pregnancy status is a critical participant safety consideration in HPTN 035.

The Quidel QuickVue One-Step test

was selected for use in this study because of its ease of use and the validity of test results in the presence of the HPTN 035 study gels.

All sites must maintain an adequate inventory of pregnancy test kits at all times. Inventory should be monitored closely and re-supply orders placed

at least 8-12 weeks in advance

of actual need (or longer if needed per site procurement policies and procedures).

- All sites will be required to report their kit inventories, including kit lot numbers, to the HPTN Central Lab on a monthly basis. Notify the Central Lab **immediately** if any kit inventory or quality control problems are identified, so that appropriate action can be taken.

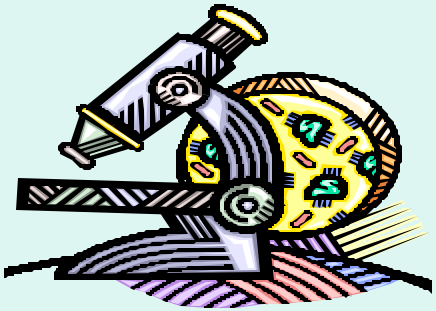
Urinalysis

- At visits when dipstick urinalysis — for leukocytes and nitrites — is required to test for possible urinary tract infections, dip the urinalysis test strip into a 5 mL aliquot of urine. At visits when both pregnancy testing and dipstick urinalysis are required, the same aliquot should be used for both tests, but the urinalysis should be performed after urine has been pipetted from the aliquot for the pregnancy test.

- The QuickVue UrinChek 10+ SG urine test strips must be used at all sites.

Local Laboratory Specimens

Vaginal Wet Mount, pH



- Vaginal wet mount and pH will be conducted to test for *Trichomonas vaginalis*, *Candida albicans* and bacterial vaginosis (BV)

Local Laboratory Specimens Gram stain

Dried Smears for Gram Stain

Prepared locally, shipped to Central Lab

- Dried smears for gram stain assessment of BV are collected in duplicate for all participants at certain visits (See Appendix II and III).
- Shipment – in development
- Send one/store one

Blood Testing

- The blood tests performed at each study visit vary depending on the timepoint of the visit and the clinical presentation of the participant. At most visits in which blood testing is required, 5-10 mL of blood will be collected, however additional blood may be collected if clinically indicated.

- Prior to specimen collection, label all required tubes with a SCHARP-provided PTID label.
- After collection, centrifuge red top tubes per site SOPs to yield serum for syphilis, liver function, and renal function testing.

- Lavender top (EDTA) and blue top (sodium citrate) tubes require no additional processing prior to testing, but these tubes should be gently inverted at least eight times after specimen collection to prevent clotting.

- At time points when rapid HIV testing is performed, pipette blood from the EDTA tube for the rapid test (s) and then deliver the remainder of the blood in the tube to the local laboratory for testing per protocol

Local Laboratory Specimens
Syphilis

- All participants will have blood collected according to local procedures during screening, annually, study exit and if clinically indicated for syphilis at the site's local lab. (refer to Appendix II and III)
- Blood will be collected according to local procedures. All tubes will be properly labeled with participant ID label and hand written date.

- Syphilis testing will be performed using a rapid plasma reagin (RPR) screening test followed by a confirmatory microhemagglutinin assay for treponema pallidum (MHA-TP) or treponema palliidum haemagglutination assay (TPHA).

- Any RPR, MHA-TP, and/TPHA test may be used at each study site, however titres must be obtained and reported for all positive RPR tests.
- RPR tests may be performed on either serum or plasma. MHA-TP and TPHA tests must be performed on serum.

Clinical Management:

- re-test the titre quarterly.

- If the titre has not returned to baseline at that time, re-treat.

Local Laboratory Specimens HIV

- All participants will have blood collected during screening, at quarterly follow up visits and study exit as well as when clinically indicated to test for HIV.
- All tubes will be properly labeled with participant ID label and hand written date.

REMINDER

- The site's laboratory is responsible for reviewing the results and quality assuring the results.

- Anticoagulated blood will be tested for evidence of HIV infection using tests **that have been validated at the study site**. These same tests will be used to test plasma specimens archived at enrollment for participants who test HIV-positive at their first HIV testing timepoint during follow-up.

SCREENING

- What happens at this visit?

Follow Up

- What happens at follow up?

Local Laboratory Specimens

Plasma Archive

- All participants will have blood collected at enrollment and study exit, and when clinically indicated for plasma archive.
- Plasma archived at enrollment and study exit will be tested for HSV-2

Local Laboratory Specimens

Plasma Archive

- Blood will be collected according to local procedures. All tubes will be properly labeled with participant ID label and handwritten date.
- Log samples into the LDMS and generate LDMS labels.
- Process blood for plasma samples within 24 hours according to local procedures.

- Make at least five (5) 1mL plasma aliquots as possible using labeled [LDMS generated label] cryovials according to local procedures.
- Store plasma aliquots in a -20 to –70° freezer at the local repository.

HSV

- Herpes Simplex Virus 2 (HSV-2) testing will be performed in batches during the final year of study implementation, and thereafter, using the Focus Technologies HerpesSelect-2 ELISA will be performed and documented according to site SOPs and the package insert, with the exception that testing will be performed on archived plasma specimens, rather than sera.

Hematology

- Complete blood counts with either three- or five-part differentials will be performed at all sites.

Liver and Renal Function

- The following tests will be performed to evaluate liver and renal function:
- Liver Function

  Alkaline phosphatase (Alk Phos)

  Alanine transaminase (ALT)

  Aspartate aminotransferase (AST)

  Gammaglutamyl transaminase (GGT)

  Total bilirubin

Renal Function

Blood urea nitrogen (BUN)

Creatinine

- The following coagulation tests will be performed on anticoagulated (sodium citrate) blood:
- Activated partial prothrombin time (aPTT)
- Prothrombin time (PT)
- INR

Central Laboratory Specimens
Swab for Multiplex PCR (for HSV, H. ducreyi and T. pallidum)

- All participants with clinical evidence of genital ulcer disease at any visit will have a swab taken for multiplex PCR.
- No transport media is required

Central Laboratory Specimens

Swab for Multiplex PCR (for HSV, H. ducreyi and T. pallidum

- The base of the ulcer should be swabbed using a plastic shaft Dacron swab.
- The swab should be placed immediately in a 1.8ml nalgene screw top transport tube and the end should be broken off to facilitate closure. Label with participant ID label and hand written date.

- After securely fastening the lid, log sample into the LDMS and label tube with LDMS label. Place tube into plastic ziplock biohazard bag. Place the bag immediately in a cooler with ice pack.
- Note: Swab of ulcer may be stored refrigerated up to 24 hours before freezing at -20 to –70C.

LDMS Laboratory Specimen Processing

- LDMS is used to track the collection, storage, and shipment of laboratory specimens tested at remote laboratories (the HPTN Central Laboratory or a regional laboratory) or stored for future analysis.

- All containers into which specimens are initially collected (e.g., urine collection cups, blood collection tubes) will be labeled with SCHARP-provided Participant ID (PTID) labels. Microscope slides used for evaluation of vaginal fluids also will be labeled with SCHARP-provided PTID labels. PTIDs are pre-printed on these labels; however study staff must write the specimen collection date each label.

LDMS Laboratory Specimen Processing

- A copy of the current LMDS manual can be obtained at http://www.fstrf.org/ldms/ldms.html. Questions about LDMS and specimen shipping should also be raised with Estelle Piwowar-Manning. Please contact Missy or Corey at SCHARP with questions about Scharp labels and specimen tracking sheets. For problems or technical questions about LDMS only, contact the LDMS User Support (716-834-0900 x311 or hptn.ldms.usersupport@fstrf.org).

- When specimens are tested at the local lab, any additional labeling required for on-site specimen management and chain of custody will be performed in accordance with site SOPs.

- Stored plasma specimens will be entered into the Laboratory Data Management System (LDMS) and labeled with LDMS-generated cryovial labels.

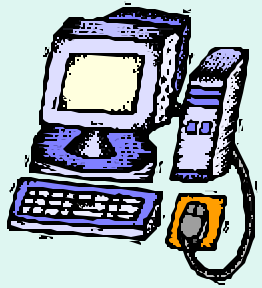
- BLD/EDT/PL1 or 2

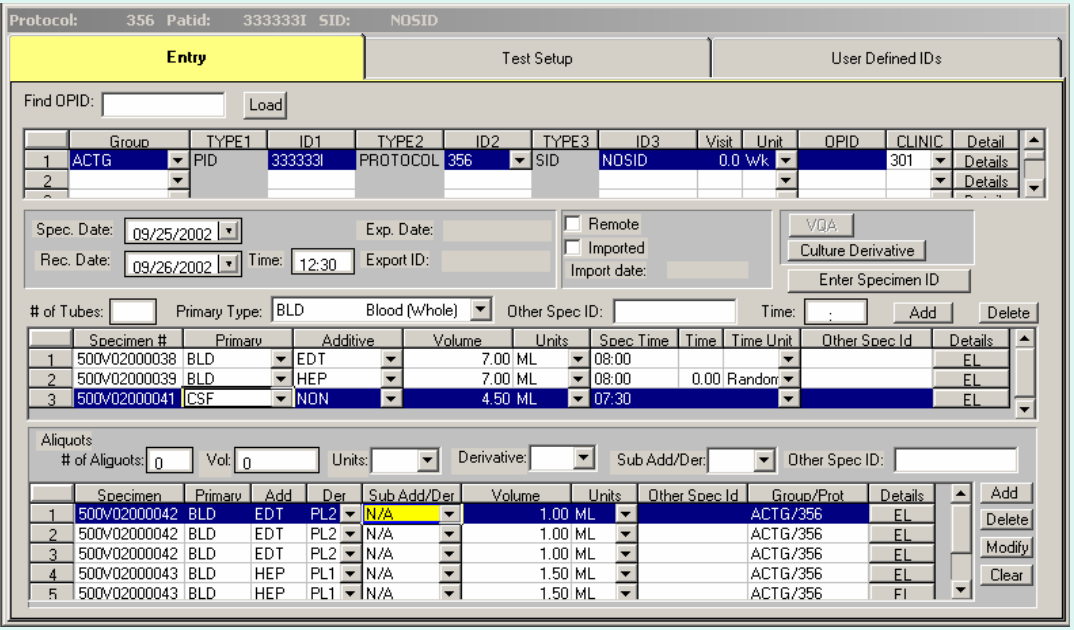
- Genital ulcer swabs collected for Multiplex PCR testing at the HPTN Central Lab also will be entered into LDMS and labeled with LDMS-generated cryovial labels.

- GLU/NON/SWB

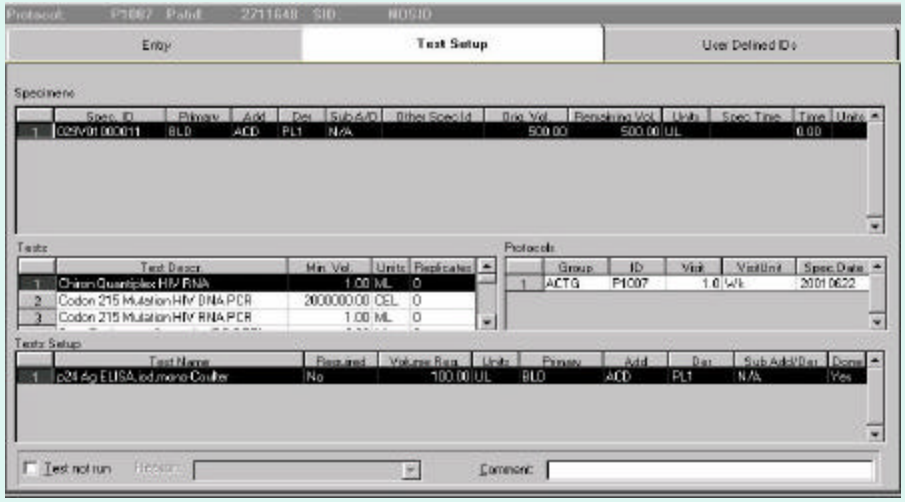
- Vaginal fluid slides prepared for Gram stain evaluation at the HPTN Central Lab will be entered into LDMS but will **not** be labeled with LDMS-generated labels
- VAG/NON/SWB

Specimen Management Module

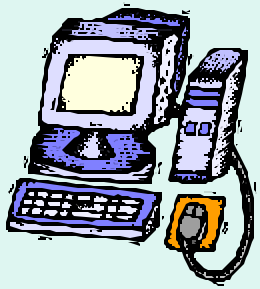




- **Enter primary specimen information**
- **Enter aliquot processing tube information**
- **Search the database for specimen records**
- **Order tests on aliquot tubes**



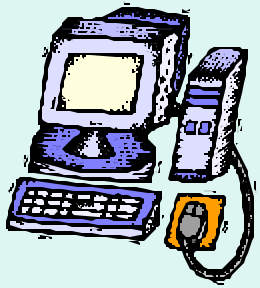
Computer/LDMS System



- **Storage of specimens must be entered into the LDMS specimen storage module, so that their location is accessible.**

- **All LDMS corrections must be made according to the LDMS corrections standard operating procedure found at:**
- **http://www.fstrf.org/ldms/hptn/hptn1.html**
- **Use your HPTN lab ID number to log in**

Shipping Module



- View shipping history
- Search for specimens to ship
- Create shipping manifest and box map
- Import specimens from another LDMS

Batch# 20 Status: Pending Address: Yes

View Shipment | Setup Shipment | Shipment Destination | Import

	Batch #	Type	Shipped	Setup Date	Ship Date	Ship Rec	Address	Sending Lab
4	5	Sent	Yes	02/22/2000	03/03/2000		Yes	
5	6	Sent	Yes	03/03/2000	03/03/2000		Yes	
6	7	Sent	Yes	08/23/2000	08/23/2000		Yes	
7	9	Sent	Yes	08/28/2000	08/28/2000		Yes	
8	10	Sent	Yes	08/28/2000	08/28/2000		Yes	
9	11	Sent	Yes	10/19/2000	02/01/2001		Yes	
10	12	Batched	No	01/03/2001			Yes	
11	14	Sent	Yes	02/07/2001	02/07/2001		Yes	
12	15	Received				07/19/2002		15 Univ. of Washington (15)
13	16	Received				07/19/2002		29 Rush Presb./St. Lukes (2
14	17	Sent	Yes	08/02/2002	08/02/2002		Yes	
15	18	Sent	Yes	08/02/2002	08/02/2002		Yes	
16	19	Sent	Yes	08/02/2002	08/02/2002		Yes	
17	20	Batched	No	09/30/2002			Yes	
18								

Sort By: Protocol

Manifest Report | Storage Report | Shipping Box Report

Shipping Box Layout

Batch Number 20

9 X 9 Box(es)

Box *001 (* indicates shipping box)

500\V02000042 ACTG 356 09/25/2002 NOSID BLD EDT PL2 1.00 ML 1 333333I 1,1	500\V02000042 ACTG 356 09/25/2002 NOSID BLD EDT PL2 1.00 ML 2 333333I 1,2	500\V02000042 ACTG 356 09/25/2002 NOSID BLD EDT PL2 1.00 ML 3 333333I 1,3	500\V02000043 ACTG 356 09/25/2002 NOSID BLD HEP PL1 1.50 ML 4 333333I 1,4	500\V02000043 ACTG 356 09/25/2002 NOSID BLD HEP PL1 1.50 ML 5 333333I 1,5	500\V02000044 ACTG 356 09/25/2002 NOSID CSF NON FLD 4.50 ML 6 333333I 1,6	500\V02000037 ACTG 356 09/06/2002 3660098J BLD EDT PL2 1.00 ML 7 333333I 1,7	500\V 8
500\V02000046 ACTG 356 07/01/2002 NOSID BLD EDT PL1 1.00 ML 10 333333I 2,1	500\V02000048 ACTG 356 07/01/2002 NOSID BLD ACD PL1 1.00 ML 11 333333I 2,2	500\V02000048 ACTG 356 07/01/2002 NOSID BLD ACD PL1 1.00 ML 12 333333I 2,3	500\V02000051 ACTG 356 07/15/2002 NOSID BLD ACD PL1 1.00 ML 13 333333I 2,4	500\V02000051 ACTG 356 07/15/2002 NOSID BLD ACD PL1 1.00 ML 14 333333I 2,5	500\V02000062 ACTG 356 07/15/2002 NOSID BLD EDT PL1 1.00 ML 15 333333I 2,6	500\V02000052 ACTG 356 07/15/2002 NOSID BLD EDT PL1 1.00 ML 16 333333I 2,7	

Shipping Manifest **Batch # 20**

Setup Date: 09 / 30 / 2002 **Shipped:** NO **Ship Date:**

From

Lab ID: 113
Lab Name: Johns Hopkins (113) Ad./Ped. Vir. ATL/Contract
Pathology Building
600 N. Wolfe Street
Baltimore MD 21287-6417
Contact: ESTELLE PIWOWAR-MANNING
Phone #: (410) 502-5296
Fax #: (410) 614-0430
E-mail: ACTGVJHOP2@tfstf.org

Ship To

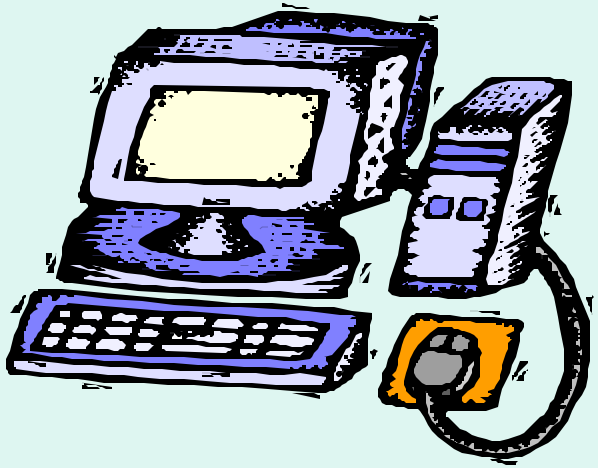
Lab ID: 999
Lab Name: BIOMEDICAL RESEARCH INSTITUTE (BRI)
12264 WILKINS AVENUE, BAY F
ROCKVILLE MD 20852
Contact: Chris Kennell
Phone #: (301) 881-7636
Fax #: (301) 770-9811
E-mail:

# of Specimens: 16
Comment:

Spec ID	Group/Prot	PID/ID 1	SID/ID 3	VID	Site	Spec Date or Harvest Date	Spec Time	Prim Der	Add	Sub A/D	Current Volume
500\V02000042	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	08:00	BLD PL2	EDT	N/A	1.00 ML
500\V02000042	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	08:00	BLD PL2	EDT	N/A	1.00 ML
500\V02000042	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	08:00	BLD PL2	EDT	N/A	1.00 ML
500\V02000043	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	08:00	BLD PL1	HEP	N/A	1.50 ML
500\V02000043	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	08:00	BLD PL1	HEP	N/A	1.50 ML
500\V02000044	ACTG 356	333333I	NOSID	0.00 Wk	301	09/25/2002	07:30	CSF FLD	NON	N/A	4.50 ML

- Specimens used for testing (ie HSV) need to be appropriately removed from the LDMS.

LDMS Laboratory Specimen Processing



- The following table should be used as a guide when logging in 035 specimens. Please use the LDMS codes listed below when logging in specimens for each test listed.

LDMS Laboratory Specimen Processing

Test	Primary	Additive	Derivative	Sub Add/Derv	Primary Volume	Aliquot Volume
Plasma archive	BLD	EDT	PLA	N/A	10ml 5ml	1.0ml 1.0 ml
Gram Stain Slide	VAG	NON	SWB	N/A	100ul	100ul
Swab for Multiplex PCR	GLU	NON	SWB	N/A	100ul	100ul

LDMS Quality Control/Monitoring

- **At various time points throughout the study, the local LDMS laboratory data manager will be requested to send LDMS data to SCHARP and/or FSTRF. A comparison between the data entered into LDMS by the site with data entered on DataFax forms will be done.**

- In addition, prior to PPD site monitoring, SCHARP will provide PPD with a list of randomly selected specimens stored within the LDMS system. PPD will then verify that these specimens are stored at the site as indicated by the information in LDMS

- **REVIEW THE MOP for details**!

Quality Control And Quality Assurance Procedures

- The HPTN CL will retest all HIV antibody-positive seroconversion specimens. The HPTN CL will test an equal number of HIV negative samples for HIV antibody. SCHARP will inform site staff of the samples selected for quality assurance testing, and site staff will ship the selected specimens to the CL. All specimens will be shipped in accordance with the HPTN Manual of Laboratory Operations and IATA specimen shipping regulations. All shipments will be documented using the HPTN LDMS.

- The CL will also retest a random 10% of entry samples