WRAIR | DCB DIAGNOSTICS AND COUNTERMEASURES BRANCH

HIV DIAGNOSTICS AND REFERENCE LABORATORY SPECIMEN SUBMISSION GUIDELINES

Version: March 2024

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SUMMARY OF CHANGES

1. June 2017
   - New HDRL web page is: http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory
   - Effective 12/1/2016, Geenius HIV 1/ 2 Supplemental Assay replaced Bio-Rad Multispot HIV 1/ 2 assay in the new US Army HIV Algorithm at the HIV Diagnostics and Reference Laboratory (HDRL). Sites will not see any change in the ordering process. On the "Result Report", the Multispot HIV 1/ 2 result was replaced with the Geenius HIV 1/ 2 results.
   - Effective 6/22/2017, SOP revised and reformatted. Significant changes included in the revision are: updated mission, inclusion of order of specimen draw instruction, addition of Test Specifications, reformatting and addition of Test Request forms to the SOP, molecular testing was broken out into three Test Request Forms based upon qualitative, quantitative and send out testing procedures, and addition of HIV test algorithms to the SOP.
2. August 2017
   - Effective 8/18/2017, SOP revised. Significant change is addition of Appendix C, Packaging Instructions.
3. April 2018
   - Effective 4/29/2018, SOP revised. Addition of *Mycoplasma genitalium* test.
4. May 2018
   - Effective 5/2/2018, SOP revised. Updated the Director, US Military HIV Research Program to Robert Gramzinski, PhD.

- Clarification of specimen storage section, Section 2.8.

5. September 2018
   - Updated Assistant Laboratory Manager to CPT Jennifer Burns.
   - Addition of Appendix G, Verification Kit Instructions and Verification Kit Packing Instructions.
6. April 2019
   - Changed the name of Appendix B3 from CONUS to RV351/CONUS
   - Removed specific package inserts from the test specifications found in Appendix A.
7. August 2021
   - Updated DLDM to DCB
   - Removed MHRP logo and replaced with WRAIR DCB Logo
   - Revised format
   - Revised Points of Contact
   - Added Appendix E, "Contractor HIV Testing Algorithm"
8. February 2022
   - Revised Appendices B1 – B8 to include DoD ID *OR* FMP/SSN
   - Revised Points of Contact
9. October 2022
   - Updated section 1.2 Test menu:
     - Change test name for HIV-1 Viral Load from COBAS Ampliprep/COBAS TaqMan Assay to Hologic Panther HIV-1 Quant Dx Assay.
     - Change test name for HIV-1 RNA Qualitative Assay from COBAS Ampliprep/COBAS TaqMan to Hologic Panther HIV-1 Quant Dx Assay.
   - Appendix Updates
     - A5 Updates:
       - Update format of document for consolidation of both HIV qual/quant testing into the new singular test format (A5 +A7)
     - Retired A7-HIV-1 RNA Qualitative Analysis (APTIMA) Test Specifications
     - A6 Updates:
       - Update format of document for consolidation of both HCV qual/quant testing into the new singular test format (A6 +A8).
     - Retired A8 HCV RNA Qualitative Analysis (APTIMA) Test Specifications
     - Updated Test request forms, Appendix B4 and B5 for test names.
     - Appendix A9 HIV-1 Resistance Genotype Test specification changed to Appendix A7.
     - Appendix A10 – HIV-1 Integrase Genotype Test Specifications changes to Appendix A8.
     - Former Appendix A11 - HIV-1 Phenotype (sent out to reference laboratory) Test Specification changed to Appendix A9.
     - Former Appendix A12 - HIV-1 Trofile (sent out to reference laboratory) Test Specification changed to Appendix A10.
     - Appendix A13 HIV-1 DNA PCR, HIV-2 DNA PCR Test Specification changed to Appendix A11.
10. May 2023
    - Effective 01/23/2023, Sentosa® SQ HIV-1 Genotyping Assay replaced HIV-1 Integrase Genotype/HIV-1 Viroseq Resistance Genotype.
    - Combined Appendices A7 and A8 to Appendix A7 Sentosa® SQ HIV-1 Genotyping Assay
    - Retired Appendix A7 HIV-1 Resistance Genotype Test specification.
    - Retired Appendix A8 HIV-1 Integrase Genotype Test Specification.
    - Appendix A13 HIV-1 DNA PCR, HIV-2 DNA PCR Test Specification changed to Appendix A10.

- Replaced Appendix B4 -Molecular Viral Load (Quantitative)/Drug Resistance Test Request Form- with Drug Resistance Test Request Form to include Sentosa® SQ HIV-1 Genotyping/Integrase Assay requirement.
- Combined Appendices B4 and B5 to Appendix B3 Molecular Aptima HIV-1 and HCV Qualitative/Quantitative DX Test Request Form
- Changed Test Request Forms to fillable format.

11. March 2024
    - Updated section 1.4.5 with new Serology Technical Supervisor contact information**.**

Reference a complete list of the Summary of Changes on the HDRL website at:
http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory

HDRL Mission: The HIV Diagnostics and Reference Laboratory (HDRL), as part of the Diagnostics and Countermeasures Branch (DCB), is dedicated to defining and executing state-of-art infectious pathogen diagnostics and monitoring in support of the Department of Defense and Department of Army personnel and their beneficiaries. HDRL assures the highest quality test results within the shortest feasible turnaround time.

1 GENERAL INFORMATION

1.1 LABORATORY SERVICES

The HIV Diagnostics and Reference Laboratory (HDRL), Diagnostics and Countermeasures Branch (DCB), Center for Infectious Disease Research (CIDR), at the Walter Reed Army Institute of Research (WRAIR), is a Department of Defense – Clinical Laboratory Improvement Program (DoD-CLIP) and College of American Pathologists (CAP) accredited Reference Laboratory that offers testing to Department of Defense (DoD) Laboratories.

HDRL conducts immunological and nucleic acid testing to inform clinical patient management and in support of sponsored and non-sponsored clinical research protocols. HDRL conducts HIV screening of all U.S. Military Entrance Processing Command (USMEPCOM) applicants, confirmatory HIV testing for the Air Force and Navy, and confirmatory Hepatitis B and Hepatitis C testing for Navy Service Members per request. The Laboratory provides HIV-1 clinical monitoring (viral load) and resistance genotyping services for all DoD HIV infected service members. HDRL conducts clinical diagnostic testing in support of DoD clinical trials and provides technical oversight of retroviral diagnostics performed by the U.S. Army Force Testing Services Contract Laboratory.

NOTE: *Only the U.S. Army European Command (USAREUR) and U.S. Army Central Command (CENTCOM) are authorized to use WRAIR/HDRL to meet HIV Surveillance (Force Screen) requirements. Non-USAREUR facilities must use Service-directed test facilities (contract laboratory); special requests for assistance must be coordinated with HDRL.*

1.2 TEST MENU

Type	Tests Available	Test Specs
Serology	o HIV Combo Ag/Ab EIA	Appendix A1
	o HIV-1 Western Blot (WB) Supplemental	Appendix A2
	o Geenius HIV 1/2 Supplemental	Appendix A3
	o Hepatitis B Surface Antigen (HBsAg) Confirmatory Assay	Appendix A4
Molecular	o HIV-1 Qualitative and Quantitative NAT (Qual / Viral Load) (Hologic Panther HIV-1 Quant Dx Assay)	Appendix A5
	o HCV Qualitative and Quantitative NAT (Qual / Viral Load) (Hologic Panther HCV Quant Dx Assay)	Appendix A6
	o Sentosa® SQ HIV-1 Genotyping Assay	Appendix A7
	o HIV-1 TNA PCR, HIV-2 TNA PCR	Appendix A8

1.3 LABORATORY HOURS AND ADDRESS

1.3.1 Open Monday through Saturday from 0700 to 1830 EST for testing. Client Services available Monday through Friday from 0700 to 1600 EST at the number listed below. Closed Sundays and on Federal Holidays.

SPECIMEN SUBMISSION SHIPPING ADDRESS

HIV Diagnostics and Reference Laboratory
Diagnostics and Countermeasures Branch
Walter Reed Army Institute of Research
9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
Phone: (301) 319-3123
Fax: (301) 319-3502

1.3.2 Websites

HIV Diagnostics and Reference Laboratory (HDRL)

Reference the HDRL web page: –http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratoryfor the latest Specimen Submission Guidelines, Test Descriptions, Testing Algorithms, Test Request and Point of Contact (POC) Forms, and Accreditation Certificates (CAP and Clinical Laboratory Improvement Program [CLIP]).

1.4 POINTS OF CONTACT

1.4.1 **Director, Diagnostics and Countermeasures Branch**
Laboratory Director, HIV Diagnostics and Reference Laboratory
Sheila A. Peel, MSPH, PhD, Commercial **(301) 319-2297**

1.4.2 **Deputy Director, Diagnostics and Countermeasures Branch**
CAPT Jennifer Malia, MS, DrPH, Commercial **(301) 319-3510**

1.4.3 **Laboratory Manager, HIV Diagnostics and Reference Laboratory**
Hiwet Leghesse, BS, Commercial **(301) 319-7159**

1.4.4 **Molecular Technical Supervisor, HIV Diagnostics and Reference Laboratory**
Pierrick Nbessa, MS, Commercial **(301) 319-2030**

1.4.5 **Serology Technical Supervisor, HIV Diagnostics and Reference Laboratory**
Tung Phan, BS, Commercial **(301) 319-3173**

2 SPECIMEN COLLECTION, PROCESSING AND HANDLING

2.1 PATIENT PREPARATION

2.1.1 Verify patient identification and tests requested before specimen collection and follow local approved standard operating procedures.

2.2 ORDER OF DRAW

2.2.1 When multiple specimens are required, follow the proper order of draw (CLSI H3-A6, Section 8.10).

Order	Tube Type	Tube Stopper Color	Mix by Inverting
1	Blood Culture-SPS	Yellow	8-10 Times
2	**ACD***	Yellow	8-10 Times
3	Citrate	Light Blue	3-4 Times
4	**BD Vacutainer® SSTTM Gel Separator ***	Orange or Red/Black	5 Times
5	**Serum (Plastic Only)***	Red	5 Times
6	**Heparin**	Green	8-10 Times
7	BD Vacutainer® PST Gel Separator with Heparin	Light Green or Light Green/Black	8-10 Times
8	**EDTA* and PPT***	Lavender White	8-10 Times
9	Flouride (Glucose)	Grey	8-10 Times

** Signifies collection tubes used for tests identified in this manual*

2.3 REQUIREMENTS

NOTE: REFERENCE APPENDIX A, SPECIMEN SUBMISSION GUIDELINES FOR TEST SPECIFICATIONS AND INDIVIDUAL TEST DESCRIPTIONS, FOR SUBMISSION OF THE APPROPRIATE VOLUME AND TYPE OF SPECIMEN THAT IS REQUIRED OR TEST(S) MAY BE REJECTED.

2.3.1 WHOLE BLOOD

1. Collect whole blood according to instructions provided for the individual test referenced in Appendix A.
2. Thoroughly mix blood tubes containing additives by gently inverting the tube as noted in order of draw above, and by referencing specific test specifications.

3. Maintain the specimen at room temperature before shipping to HDRL unless instructed otherwise by Test Specification requirements.
4. **Never freeze whole blood** unless instructed to do so in accordance with a test's specimen collection and handling requirements (Appendix A). Do not place whole blood specimens in direct contact with ice/cool packs.

2.3.2 PLASMA

1. Consult the Test Specification requirements to determine the correct additive/tube to use.
2. Blood collection tubes should be inverted 8-10 times immediately after collection, followed by **centrifugation within 4 hours of draw**, ***unless otherwise specified for individual test (Refer to Assay Test Specification* Appendix A*).***
3. If the specimen is transferred to a secondary tube, indicate the specimen type, i.e., plasma, serum on the plastic screw-cap vial for transport and on the corresponding Test Request Form.

2.3.3 SERUM

1. HDRL recommends the use of serum separator collection tubes (SSTs) for most serological analyses.
2. When using a serum separator tube, invert the tube gently five (5) times. Further inversion may cause alterations in specimen integrity, ***unless otherwise specified for individual test (Refer to Assay Test Specification* Appendix A*).***
3. **DO NOT centrifuge immediately after drawing blood.** Allow the blood to clot in an upright position for at least 30 minutes, but not longer than two (2) hours, before centrifugation.
4. If specimen is transferred to a secondary tube, indicate that the specimen is serum on the plastic screw-cap vial for transport and on the corresponding Test Request Form.

2.3.4 SWAB

1. The laboratory currently conducts SARS-CoV-2 testing for research studies only; please contact the laboratory.
2. Nasopharyngeal and/or oropharyngeal swab specimens collected according to standard technique in viral transport medium (VTM). Polyester, rayon, or nylon-tipped swabs are acceptable.
3. The following types of VTMs/UTMs have been verified for use; Remel MicroTest M4, M4RT, M5 or M6 formulations; Copan Universal Transport Medium (UTM); BD Universal Viral Transport Medium on the Panther Fusion system. (This list will be updated periodically as more transport media gets validated for the specimen collection).
4. If VTM is not available, saline or PBS (phosphate buffered saline) may be used at the discretion of the Laboratory Director.
5. After collection, specimens can be stored at 2°C to 8°C for up to 96 hours.

2.3.5 CENTRIFUGATION – SERUM/PLASMA

1. Draw five (5) ml of blood for each two (2) ml of serum or plasma required in the appropriate collection tube. Ensure blood draw in appropriate order as noted in *section 2.2.1*.
2. If serum is required, allow specimen to clot for at least 30 minutes, but not longer than 2 hours, in an upright position.
3. For serum, centrifuge within two (2) hours per manufacturers guidelines, *unless otherwise specified for individual test*[*].
4. For plasma, centrifuge within four (4) hours per manufacturers guidelines, *unless otherwise specified for individual test*[*].
5. All non-gel blood collection tubes (including those that contain heparin, EDTA and non-gel serum tubes) can be centrifuged at ≤1300 RCF for 10 minutes.
6. The BD Vacutainer® SST™ and PST™ gel tubes should be spun at room temperature at a speed of 1000 to 1300 RCF for 10 minutes in a swing bucket centrifuge, or 15 minutes in a fixed-angle centrifuge.
7. Conversion of RCF to RPM is as follows:

$$\textbf{g Force (RCF)} = (\text{rpm})^2 \times 1.118 \times 10^{-5} \times r$$

$$\textbf{RPM} = \sqrt{[\text{RCF}/(r \times 1.118)]} \times 1 \times 10^5$$

2.4 LABELING OF SPECIMENS

2.4.1 Verify identification post collection and before labeling the specimens.

2.4.2 Each submitted specimen must be labeled legibly with the following information to prevent test delay:

1. Patient name
2. Unique DoD number
3. Social Security number (SSN) and family member prefix (FMP) (Required for inquiry of patient results/status in legacy LIMS) and/or DoD ID
4. Date of birth and/or barcode written exactly as it appears on the test request form
5. Date of collection/draw date
6. Specimen type (e.g., serum, plasma, whole blood, swab, etc.)

2.4.3 Failure to include required information on the specimen label may result in rejection of the specimen (section 2.6 Specimen Rejections).

2.5 SPECIMEN TEST REQUEST FORMS

2.5.1 Complete the appropriate HDRL Test Request Form (Appendix B) for each specimen:

1. Serology Clinical Test Request Form (Appendix B1)

   Suspicion of acute HIV infection or Pre-Exposure Prophylaxis Screening/acquisition of HIV Infection on PrEP: order the Acute HIV Infection / PrEP Algorithm (Appendix C) on the

Serology Clinical Test Request Form. A 4th generation HIV Ag/Ab Combo test will be performed in parallel with an HIV RNA Qualitative/Quantitative Test as an aid in diagnosis of acute or primary HIV infection.

2. HIV Verification Algorithm Test Request Form CONUS (Appendix B2)
3. Molecular Aptima HIV-1 and HCV Qualitative/Quantitative DX Test Request Form (Appendix B3)

   The Aptima HIV-1 and HCV Quant Dx assays are dual claim assays which provide a qualitative test result for detection and confirmation of presence of either HIV or HCV infection as well as a quantitative result for viral burden (viral load).
4. Molecular Drug Resistance Test Request Form (Appendix B4)
5. Vaccine Induced Sero-Reactivity Test Form (Appendix B5)

   The Vaccine Induced Sero-Reactivity (VISR) Form is for individuals in whom Sero-Reactivity is suspected due to participation in an HIV vaccine trial. A complete HIV algorithm will be performed inclusive of an HIV-1 total nucleic acid PCR (Additional testing may be performed, if required).
8. Hepatitis B Surface Antigen Test Request Form (Appendix B6)
9. HIV Verification Algorithm Test Request Form (OCONUS) (Appendix B7)
10. Panther SARS-CoV-2 currently conducted for clinical research studies only; contact the laboratory.
11. HIV-1 TNA PCR, HIV-2 TNA PCR (Appendix B8)

2.5.2 The Test Request Forms are found at HDRL website at: http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory

2.5.3 Enclose the appropriate Test Request Form(s) with patient specimen(s) in the shipment. Multiple tests may be requested, but each requested test requires a dedicated specimen.

2.5.4 Ensure that the specimens are shipped using federal, state, and international regulations as appropriate. See Appendix F, Packing Instructions, or HDRL website: http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory

2.5.5 Verbal test requests will only be accepted when an additional test is requested post submission of specimens, and only if followed by the appropriate test request form within five (5) business days.

2.5.6 Do not send e-mails containing PHI to HDRL e-mail accounts that do not end in @health.mil.

2.5.7 When sending an e-mail that is inclusive of PHI, ensure that e-mail is transmitted as an encrypted message.

2.5.8 HDRL follows test algorithms as outlined in Appendix C, Appendix D, and Appendix E. We may modify your request to run an initial screening test, then reflex, if indicated for the test you requested.

1. Appendix C: HIV Acute Infection / PrEP Algorithm
2. Appendix D: U.S. Army HIV Test Algorithm

3. Appendix E: Contract Laboratory HIV Test Algorithm

2.6 SPECIMEN REJECTIONS

2.6.1 HDRL reserves the right to reject and discard specimens that:

1. Do not meet the specimen collection and storage requirements specified on the appropriate Test Request Form(s).
2. Contain the following errors:
   - Test Request Form errors include, but are not limited to:
     - Illegible information
     - Missing draw date
     - Missing family member prefix (FMP) if applicable
     - Missing/incomplete Social Security Number (SSN) if applicable
     - Missing/incomplete DoD ID number
     - Missing/incomplete date of birth
     - Missing name of requesting physician and/or site
   - Specimen errors include, but are not limited to:
     - Provided specimen quantity not sufficient (QNS)
     - Duplicate specimen (same assay requested for same person within one 7-day period), unless authorized by the Laboratory Director
     - Illegible label
     - Incomplete information on label (at least two unique identifiers are required per regulation)
     - Leaking/Cracked specimen tube
     - Incorrect specimen type
     - Specimens collected in expired collection tubes
     - Hemolyzed, contaminated, lipemic, or coagulated specimen
     - Package not in compliance with applicable federal and state shipping standards (such shipments will be rejected)
     - Specimens not shipped at specified shipping temperature
     - Mismatch errors between Test Request Form and specimen to include, but not limited to:
       - Discrepancy between specimen label and Test Request Form
       - Incorrect type of specimen for test requested
       - Missing specimen or Test Request Form

2.6.2 The submitting laboratory will be notified of any rejected specimen either by e-mail or telephone call and will be sent a rejection report.

2.7 PACKAGING AND SHIPMENT OF SPECIMEN

2.7.1 Sites submitting specimens must comply with all applicable federal and state regulations concerning shipment for diagnostic specimens. The minimum requirements for packaging and shipping are:

1. Wrap all vials individually to avoid contact.
2. Surround individually wrapped specimens with enough absorbent material to contain spillage.
3. Package specimen in a water-tight primary and secondary container. Additional information can be found in Appendix F and on the HDRL website at: http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory.
4. Place request forms within the container but separate from the tubes.
5. Label container and ship specimens according to applicable guidelines for UN3373, Category B diagnostic specimens.
6. Mail to shipping address listed in section 1.3 Laboratory Hours and Address.
7. **Send by Fax/E-mail a FedEx/UPS tracking and/or invoice number to ensure all shipments sent to HDRL are received, IAW CAP GEN.40530 (REQUIRED OF SUBMITTING LABORATORY).**

2.7.2 If you would like your shipping boxes returned to your site, enclose a pre-paid FedEx/UPS shipping label or address slip with FedEx/UPS billing information and material to cover hazardous warning markings.

2.8 SPECIMEN STORAGE

2.8.1 To avoid hemolysis, separate serum from cells within two (2) hours of draw and separate plasma within four (4) hours of draw for EDTA or two (2) hours of draw for PPT (refer to section 2.3 Requirements).

2.8.2 Store specimen(s) according to requirements in the specimen submission guidelines (Appendix A).

2.8.3 Ship specimen(s) to HDRL in an appropriate shipping container, adhering to specimen temperature requirements listed on the Test Request Form(s) (Appendix B).

2.8.4 If shipping frozen specimens, utilize an acceptable insulated container that will allow for two (2+) lbs. of dry ice (minimum required) per day of transport.

1. **Specimen(s) must arrive frozen.**
2. HDRL also recommends the use of six **(6+) lbs. of dry ice during summer months** to ensure acceptable specimen transport requirements are maintained, as well an anticipation of shipments that may be delayed due to issues with the carrier.

2.8.5 PPT (plasma preparation) tubes can be shipped at refrigerated temperature for delivery within 24 hours of collection.

3 REPORTING OF RESULTS

3.1 HDRL will test all specimens submitted that adhere to specified Specimen Submission Guideline Test Specifications in an expeditious manner and in accordance with specified turnaround times.

3.2 HDRL generates hardcopy or electronic reports and transmits them via secured fax or encrypted e-mail to the designated Point of Contact (POC) at each submitting facility.

3.3 On a limited basis, HDRL enters results directly in CHCS for specimens received through LIO (Laboratory Interoperability).

3.4 Result reports are also sent via secure file transfer to the Service-directed test facilities for the Army and Navy and directly to USMEPCOM.

3.5 Due to the sensitive nature of our reports, only the designated POC (or alternate) can receive reports from HDRL. The facility POC is the sole manager of distribution of HDRL reports within that institution.

3.6 To designate a POC for results reporting, complete a Point of Contact Form – Results Reporting (Appendix G1).

3.7 To designate a healthcare provider POC for notification and follow-up of positive patient test results, complete a Point of Contact Form – Notification and Follow-up Form (Appendix G2). Email or Fax these forms to HDRL at (301) 319-3502.

3.7.1 New sites MUST submit these Forms before shipping specimens to HDRL for the first time.

3.7.2 Use the same forms for updates/changes to these POCs.

3.7.3 HDRL cannot/will not change or create POCs without these Forms.

4 FOLLOW-UP PROCEDURES FOR POSITIVE RESULTS

4.1 HDRL/WRAIR complies with the AR 600-110 Army HIV Surveillance Program.

4.2 An initial (first-time) HIV positive test result for an Active Duty Service member requires the submission of a second specimen for independent verification of HIV infection status. HDRL will coordinate with the site POC for shipment of a collection kit to the site as described below:

4.2.1 CONUS Active Duty Service members (Study ID RV351):

- An HIV Verification Request Kit and Form (CONUS sites only) will be shipped to your site.
- The site must complete the accompanying Test Request Form and return the kit with specimens to HDRL according to the instructions provided (Appendix H).

4.2.2 OCONUS Active Duty Service members and other Service Components:

- Complete the HIV Verification Algorithm Test Request Form found on the HDRL website (Appendix B7).
- Ship the specimen to the address provided on the Form.

4.3 If the original specimen and verification specimen are discordant, a third independent verification specimen will be required.

4.4 Per the Army Regulation, AR 600-110, each site is required to have an appointed HIV Program Manager. This individual will serve as the POC for follow-up test coordination with HDRL.

4.5 The identified Primary or Secondary POC will be notified by the HDRL or Defense Health Centers for Public Health – Aberdeen HIV Officer in accordance with HDRL notification processes.

5 SUPPORTING DOCUMENTS

Appendix A: Specimen Submission Guidelines (tests description and specification)

Appendix B: Test Request Forms

Appendix C: HIV Acute Infection Algorithm

Appendix D: U.S. Army HIV Test Algorithm

Appendix E: Contract Laboratory HIV Test Algorithm

Appendix F: Packaging and Shipment Instructions

Appendix G: Point of Contact Forms

Appendix H: Verification Kit Instructions

NOTE: All documents (including CAP and DoD-CLIP certificates and Historical Changes to HDRL testing operations) are available online at:

http://www.hivresearch.org/hiv-diagnostics-and-reference-laboratory

Appendix A: Specimen Submission Guidelines

(Tests description and specifications)

Appendix A1

HIV Combo Ag/Ab EIA Test Specification

Test Name: HIV Combo Ag/Ab EIA				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
Enzyme immunoassay: The Genetics Systems HIV Combo Ag/AB EIA is based on the principle of the sandwich technique for the qualitative detection of HIV-1 p24 Antigen and detection of HIV-1 (Group M and O) and HIV-2 antibodies in human plasma. This 4th generation HIV test has significantly reduced the serological window of detection of HIV infection. Test employed: FDA-approved, commercially available test kit: Bio-Rad GS HIV Combo Ag/Ab EIA Reference Range: Non Reactive	4 ml Serum (SST) or Plasma (PPT) or aliquoted in a screw top plastic vial Acceptable anticoagulants: EDTA, heparin, sodium citrate, CPD, CPDA-1, ACD	Shipping: Ambient (15 -30°C) Refrigerated (2-8°C) Frozen (-20°C or colder) Test must be performed within 2 days of draw if the specimen is stored and shipped at ambient temperature. **Ship frozen if sample will not be received at HDRL within 48 hours.** When shipping frozen, use 2+ lbs. dry ice per day of transport. Shipment with 6 lbs. of dry ice is recommended in case of shipment delay.	FDA approved for Screening for HIV-1 p24 Antigen and antibodies to HIV-1 (group O & M) and HIV-2.	5 business days (Clinical, Force Testing)

Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
• Tel: 301-319-3123 • Fax: 301-319-3502

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Appendix A2

HIV-1 Western Blot (WB) Supplemental Test Specification

Test Name: HIV-1 Western Blot (WB)				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
In vitro qualitative assay for detection and identification / confirmation of antibodies to HIV-1 in human serum and plasma. Test employed: FDA-approved, commercially available Western Blot Kit: Bio-Rad GS HIV-1 Western Blot. Reference Range: Negative	1 ml Serum or Plasma Acceptable anticoagulants: EDTA, heparin, sodium citrate, CPDA-1, ACD	Shipping: Ambient (15-37°C) Refrigerated (2-8°C) Frozen (-20°C) **Ship frozen if sample will not be received at HDRL within 48 hours.** Ship frozen on 2+ lbs. dry ice per day of transport. Shipping on 6 lbs. additional dry ice is recommended in case of shipment delay.	FDA approved as Supplemental Diagnostic test for confirmatory testing for HIV-1 antibodies	5 business days (Clinical, Force Testing)
Test performed on HIV EIA repeatedly reactive specimens per Army HIV Diagnostic Algorithm.				

Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
• Tel: 301-319-3123 • Fax: 301-319-3502

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Appendix A3

Geenius HIV 1/2 Supplemental Test Specification

Test Name: Geenius HIV-1/HIV-2 Supplemental Assay				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
Moderate complexity *in vitro* qualitative assay to aid in diagnosis of infection with HIV-1 and/or HIV-2 in fresh human serum and plasma. Test employed: FDA-approved, commercially available kit from Bio-Rad: Geenius HIV-1/2 Supplemental Assay. Reference Range: Negative	1 ml Serum or Plasma Acceptable anticoagulants: EDTA, sodium heparin and sodium citrate.	Shipping: Ambient (18-30°C) Refrigerated (2-8°C) Frozen (-20°C) Test must be performed within 48 hours of collection on ambient specimens and within 7 days of collection on refrigerated specimens. **If sample will not be received at HDRL for testing within 48 hours of collection, freeze serum/plasma at -20°C.** When shipping frozen, use 2+ lbs. dry ice per day of transport. Shipment with 6 lbs. of additional dry ice if shipping delays are expected.	FDA approved as a supplemental test to aid in diagnosis of infection with HIV-1 and/or HIV-2	5 business days (Clinical, Force Testing)
Test performed on HIV repeatedly reactive specimens per Army HIV Diagnostic Algorithm.				

Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
• Tel: 301-319-3123 • Fax: 301-319-3502

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Appendix A4

Hepatitis B Surface Antigen (HBsAg) Confirmatory Assay Test Specification

Test Name: Hepatitis B Surface Antigen (HBsAg) Confirmatory Assay				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
The GS HBsAg Confirmatory Assay 3.0 is a qualitative assay intended for the confirmation of HBsAg repeat reactive specimens detected by the GS HBsAg EIA 3.0 assay. Test employed: licensed commercially available: Bio-Rad GS HBsAg Confirmatory Assay 3.0 Reference Range: Negative	3 ml Serum or Plasma Acceptable anticoagulants: EDTA, heparin, sodium citrate, CPDA-1, ACD	Shipping: Ambient (15-30°C) Refrigerated (2-8°C) Frozen (-20°C) **Ship frozen if sample will not be received at HDRL within 48 hours at HDRL.** When shipping frozen, use 2+ lbs. dry ice per day of transport. Shipment on 6 lbs. additional dry ice is recommended in case of shipment delay.	GS HBsAg Confirmatory Assay 3.0 is FDA approved as a neutralization procedure for confirmatory testing of repeat reactive HBsAg EIA samples.	5 business days
Note: Test performed on HBsAg EIA repeatedly reactive specimens per CDC guidelines				

Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
• Tel: 301-319-3123 • Fax: 301-319-3502

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Appendix A5

Hologic Panther HIV-1 Quant Dx Assay Test Specifications

Test Name: HIV-1 Panther Quant Dx (Qualitative/Quantitative)				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
1. In vitro nucleic acid amplification test for qualitative detection and quantification of RNA from patients infected with HIV 1 (Group M, N and O). 2.**Intended use of the Assay:** • To aid in the diagnosis of acute and primary HIV-1 serum and plasma; specifically, qualitative detection of HIV-1 RNA in serum or plasma in individuals without antibodies to HIV-1 • As a supplemental test for confirmation of HIV-1 infection in specimens repeat reactive by U.S. Food and Drug Administration approved HIV immunoassays 3.Test employed: FDA-approved, commercially available test kit from Hologic: Panther HIV-1 Quant Dx Assay. • Analytical measurement range: 30 to 10,000,000 copies/ml **Reference Ranges** **HIV-1 Quantitative** – Not Detected Copies/mL Not Detected Log copies/mL **HIV-1 Qualitative** – Not Detected	**Quantitative Testing:** •3mLs of Plasma **Qualitative Testing:** •3 mL of Serum or Plasma **PPT Tubes:** Centrifuge immediately or within 2 hours of collection at 1100 x g for 10 minutes minimum. **EDTA Plasma:** Store blood at 25°C until centrifuged. Centrifuge at ≤1300 x g for 10 minutes within 4 hours of blood collection. **Ship frozen if transport will take longer than overnight delivery.** **Serum Tubes:** Allow blood to clot in upright position for 30-60 minutes before centrifugation. If transferring to secondary tube, indicate "serum" on plastic screw-cap tube for transport.	**EDTA / PPT or Serum:** Store serum or plasma refrigerated (2-8°C) for overnight or same day delivery. Ship frozen if transport will take longer than same day or overnight. Store plasma frozen (-70°C or colder). When shipping frozen, use 2+ lbs. dry ice per day of transport. Ship on an additional 6 lbs. dry ice in case of shipment delay.	FDA approved for patient management.	5 business days

Appendix A6

Hologic Panther HCV Quant Dx Assay Test Specifications

Test Name: HCV Panther Quant Dx (Qualitative/Quantitative)				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
1.In vitro nucleic acid amplification test for qualitative detection and quantification of RNA from patients infected with HCV (Subtypes 1-6). 2.**Intended use of the Assay:** • To aid in the diagnosis of active HCV infection in individuals with antibody evidence of HCV infection with liver disease; individuals suspected to be actively infected with HCV; and individuals with antibodies to HCV and at high risk for HCV infection. • As an aid in clinical monitoring of viral burden and effectiveness of antiretroviral therapy in plasma specimens. 3.Test employed: FDA-approved, commercially available test kit from Hologic: Panther HCV Quant Dx Assay. • Analytical measurement range: 10 to 100,000,000 International Units per mL (IU/mL) **Reference Range** **HCV Quantitative** – Not Detected Copies/mL Not Detected Log copies/mL **HCV Qualitative** – Not Detected	**Quantitative Testing:** •3mLs of Plasma **Qualitative Testing:** •3 mL of Serum or Plasma **PPT Tubes:** Centrifuge immediately or within 2 hours of collection at 1100 x g for 10 minutes minimum. **EDTA Plasma:** Store blood at 25°C until centrifuged. Centrifuge at ≤1300 x g for 10 minutes within 4 hours of blood collection. Ship frozen if transport will take longer than overnight delivery. **Serum Tubes:** Allow blood to clot in upright position for 30-60 minutes before centrifugation. If transferring to secondary tube, indicate "serum" on plastic screw-cap tube for transport.	**EDTA / PPT or Serum:** Store serum or plasma refrigerated (2-8°C) for overnight or same day delivery. **Ship frozen if transport will take longer than same day or overnight.** **Store plasma frozen (-70°C or colder).** When shipping frozen, use 2+ lbs. dry ice per day of transport. Ship with an additional 6 lbs. dry ice in case of shipment delay.	FDA approved for patient management.	5 business days

Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910
• Tel: 301-319-3123 • Fax: 301-319-3502

Version: May 2023

Appendix A7

Sentosa® SQ HIV-1 Genotyping Assay *Test Specification*

Clinical Significance	Specimen Requirements	Transport/Storage Temperature	Test Approved For	Turn Around Time
Sentosa® SQ HIV-1 Genotyping is a Next Generation Sequencing (NGS) Assay based on *in vitro* diagnostic (IVD) test intended for use in detecting HIV-1 genomic mutation in protease, reverse transcriptase and integrase regions of the pol gene as an aid in monitoring and treating HIV-1 infection. Sentosa® SQ HIV-1 Genotyping Assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease progression and as an assay to monitor or assess viral response to antiretroviral treatment. The test is available for all patients who are: (1) initiating drug therapy; (2) not responding to antiretroviral drug therapy (low viral RNA level at 1,000 to 3,000 copies/ml); or (3) failing their antiretroviral regimen. Sentosa® SQ HIV-1 Genotyping is an FDA-approved test that uses commercially available test kit from VELA Sentosa SQ HIV-1 Genotyping Assay.	Three (3) vials of EDTA plasma (1 ml per tube). Centrifuge at room temperature at 1900 x g for 10 minutes at 2 to 8°C following the steps below: EDTA: Invert 8-10 times. Store the tubes upright at room temperature, spin tubes within 2 hours of collection. Centrifuge at 1900 x g for 10 minutes at 2 to 8°C to remove plasma. Using a pipette, immediately transfer the resulting supernatant (Plasma) to clean polypropylene tubes while ensuring 2-8°C when handling the samples. **Please note:** Patients presently on antiretroviral drug therapy should still be on their drug regimen when specimen is collected.	EDTA Plasma: Store refrigerated (2-8°C) for overnight or same day delivery. If transport longer than overnight or same day, aliquot plasma, freeze at -70°C, then ship frozen. Store plasma frozen (-70°C or colder). Use 2 lbs. dry ice per day of transport. Shipment with an additional 6 lbs. of dry ice is recommended in case of shipment delay.	The Sentosa® SQ HIV-1 Genotyping Assay is FDA-approved for therapeutic (HIV-1 resistance genotype) monitoring of HIV-infected individuals.	10 business days after receipt at HDRL.

Please note:

1. Viral load MUST BE ≥ 1000 copies/ml and result must have been obtained within the past 30 days.
2. When requesting Sentosa® SQ HIV-1 Genotyping Assay, the requesting laboratory must provide most recent Viral Load result on the request form at time of submission.
3. If the patient has not had a Viral Load determination within the past 30 days, request a HIV-1 Viral Load along with the HIV-1 Genotype.
4. Any specimen without a Viral Load reported (or a Viral Load requested) on the request form will need resolution and may affect Turn Around Time.
5. Duplicate specimens will be discarded.
6. Treatment Decision should be made in consideration of all relevant clinical and laboratory findings and the prescribing information of the drug in question.

Appendix A8

HIV-1 TNA PCR, HIV-2 TNA PCR Test Specification

Test Name: HIV-1 TNA PCR / HIV-2 TNA PCR				
Clinical Significance	**Specimen Requirements**	**Transport/Storage Temperature**	**Test Approved For**	**Turn Around Time (After Receipt at HDRL)**
HIV-1 TNA PCR: Qualitative HIV-1 TNA test for detection of HIV-1 infection in infants up to 18 months of age, and detection of HIV-1 Total Nucleic Acid. HIV-2 TNA PCR: Qualitative TNA test to distinguish between HIV-1 and HIV-2 infection.	3 ml (0.5 ml minimum) whole blood in EDTA (lavender top) tubes Store at room temperature for overnight or same day delivery.	**Ship ambient within 24 hours of collection.**	Not FDA approved Intended for use in screening of infants 18 months of age or less, born to HIV-1 infected mothers; and/ or difficult to resolve infection status cases.	10 business days

Please note:

1. Maternal antibodies may persist for the first 18 months of life, confounding diagnosis in the infant; however, maternal antibodies do not interfere with nucleic acid testing.
2. This test may be used to detect HIV-1 in patients with acute infection prior to seroconversion (antibody formation), as well as in patients with agammaglobulinemia.
3. It is recommended that positive results be confirmed on two separate blood samples with one or a combination of virus-specific tests. Repeatedly reactive HIV-1 EIA results with a supplemental confirmatory test are required to confirm the diagnosis HIV-1 infection.
4. HIV-2 TNA PCR is a laboratory developed test designed and validated by HIV Diagnostics and Reference Laboratory in September 2019.
5. The HIV-2 TNA assay is real-time qualitative RT-PCR assay for detection of highly conserved regions within the Long Term Terminal Repeat (LTR) of the virus; it provides a highly sensitive assay for all major HIV-2 Groups (A, B).

Appendix B: Test Request Forms

Appendix B1

Serology Clinical Test Request Form

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **HIV Algorithm** ☐ **Acute / PrEP HIV Algorithm**	☐ 4 ml serum (SST Tubes) or ☐ 4 ml plasma (PPT EDTA preferred, (Na Citrate, CPDA, ACD-1 plasma is acceptable.)	**SST Tubes** – Invert 5X and allow to clot for 30 min (no more than 2 hrs.) post-collection. Centrifuge 10 minutes at 1000-1300 RCF in a swing bucket centrifuge. **NOTE:** Tubes MUST be allowed to clot for 30 minutes. **PPT tubes** – Invert 8-10X. Spin tubes within 2 hrs. of collection. Centrifuge in swing-out rotor centrifuge at 1100 RCF for a minimum of 10 min. Freeze plasma aliquot at -20°C.	☐ **Ambient 15-30°C** – SST tube must be received at HDRL within 48 hours of collection. ☐ **Refrigerated 2-8°C** – SST tube must be immediately stored and shipped in cold box with ice packs and received at HDRL within three (3) days of collection. ☐ **Frozen -20°C** – Ship frozen **aliquoted** sample with dry ice if sample will not be received at HDRL within three (3) days of collection.

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

PROCESSING LABORATORY (For HDRL use only)

BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR SER Version: June 2023

Appendix B2 – RV351/CONUS

HIV Verification Algorithm Test Request Form

TESTS	SPECIMEN REQUIREMENT	DRAW TUBE
☐ **HIV Algorithm**	4 ml plasma (Cold Pack)	PPT

SHIP FROZEN PLASMA IF SAMPLE WILL NOT BE RECEIVED AT HDRL WITHIN 72 HOURS.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient Stamp must include: Full Name*, FMP*/SSN* or DoDID*, DOB*	**POC*** _____ **Physician Name*** _____ **Clinic / Center*** _____ **Center Address*** _____ _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____
Specimen Draw Date / Time*: _____ Ship Date: _____ Sample Storage (circle): **Frozen / Refrig / Ambient** Sample Shipping (circle): **Dry Ice / Cold Pack**	

***Required**

PROCESSING LABORATORY (For internal use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED/INITIALS

TUBES DRAWN	SPECIMEN REQUIREMENT	INSTRUCTIONS
☐ **4 PPT s**	Aliquot PPL into NINE (9) cryovials - 2, 1.0 mL each - 2, 0.5 mL each - 1, 2mL - 2, 1.20 mL each - 1, 2 mL - 2, remaining volume	Freeze at -80°C or lower Forward one vial of plasma at 1 ml to HDRL for confirmatory
☐ **2 CPTs**	Collect Cell Pellets and store into SEVEN (7) cryovials - 2, 5 million cells each - 5, 1 million cells each	Freeze at -80°C or lower

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR VA Version: June 2023

Appendix B3

Molecular Aptima HIV-1 and HCV Qualitative/Quantitative Dx Test Request Form

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **Qual / Quant HIV-1** (Viral Load) ☐ **Qual / Quant HCV** (Viral Load)	☐ 3 ml plasma (PPT or EDTA)	**PPT tubes** – Invert 8-10X. Spin tubes within 2 hrs of collection. Centrifuge ≤1300 RCF for 10 minutes. Freeze plasma aliquot at less than -20°C. **EDTA tubes** – Store blood at 25°C until centrifuged. Centrifuge ≤1300 RCF for 10 minutes within 4 hours of blood collection. Freeze plasma aliquot at less than -20°C.	☐ **Frozen -20°C** – Ship frozen **aliquoted** specimen with dry ice if specimen will be received at HDRL after 24 hours of collection. **RECOMMENDED**: Ship on 6 lbs. dry ice in case of shipment delay.

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

PROCESSING LABORATORY (For HDRL use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Appendix B4

Drug Resistance Test Request Form

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **Sentosa® SQ HIV-1 Genotyping/Integrase Assay**	☐ 3 ml plasma (EDTA only) Viral load **MUST BE ≥ 1000** Copies/ml and result must have been obtained within the past 30 days.	**EDTA:** Invert 8-10 times. Store the tubes upright at room temperature, spin tubes within 2 hours of collection. Centrifuge at 1900 RCF for 10 minutes at 2 to 8°C to remove plasma. Freeze plasma aliquot at -20°C. Viral Load_____ Date Performed_____	☐ **Refrigerated 2-8°C** - Must be stored at 2-8°C post-centrifugation, shipped in cold box with ice packs and received at HDRL within 24 hours of collection. ☐ **Frozen** - Ship frozen **aliquoted** sample with dry ice if sample will be received at HDRL after 24 hours of collection. **RECOMMENDED:** Ship on 6 lbs. dry ice in case of shipment delay.

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name**_____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name_____ Alternate POC Phone _____

PROCESSING LAB (For HDRL use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Appendix B5

Vaccine Induced Sero-Reactivity Test Request Form

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **VISR Algorithm**	☐ 3 ml serum (SST Tubes) or ☐ 3 ml plasma (EDTA, PPT preferred) or ☐ 15 ml whole blood (3 x 5ml EDTA tubes)	**SST Tubes** – Invert 5X and allow to clot for 30 min (no more than 2 hrs) post-collection. Centrifuge 10 minutes at 1000-1300 RCF in a swing bucket centrifuge. **NOTE:** Tubes MUST be allowed to clot for 30 minutes. **PPT tubes** – Invert 8-10X. Spin tubes within 2 hrs of collection. Centrifuge in swing-out rotor centrifuge at 1100 RCF for a minimum of 10 min. Freeze plasma aliquot at -20°C. **EDTA tubes** – Store at room temperature for overnight or same day delivery; must be received within three (3) days of collection.	☐ **Ambient 15-30°C** – SST tube and EDTA tubes must be shipped ambient within 24 hours of collection and received within three (3) days of collection. ☐ **Refrigerated 2-8°C** – SST tube must be immediately stored and shipped in cold box with ice packs and received at HDRL within two (2) days of collection. ☐ **Frozen -20°C** – Ship frozen **aliquoted** specimen with dry ice if specimen will be received at HDRL after 24 hours of collection. **RECOMMENDED**: Ship on 6 lbs. dry ice in case of shipment delay.

Vaccine Construct if known: _____

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

PROCESSING LABORATORY (For HDRL use only)

BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR MSO Version: May 2023

Appendix B6

Hepatitis B Surface Antigen Test Request Form

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **HBsAg Confirmatory**	☐ 3 ml serum (SST Tubes) or ☐ 3 ml plasma (EDTA, Na Heparin, Na Citrate, CPDA and ACD-1 plasma is acceptable.)	**SST Tubes** – Invert 5X and allow to clot for 30 min (no more than 2 hrs) post-collection. Centrifuge 10 minutes at 1000-1300 RCF in a swing bucket centrifuge. **NOTE:** Tubes MUST be allowed to clot for 30 minutes. **PPT Tubes** – Invert 8-10X. Stable at room temperature up to 6 hrs. Centrifuge in swing-out rotor centrifuge at 1100 RCF for a minimum of 10 min. Freeze plasma aliquot at -20°C.	☐ **Ambient 15-30°C** – SST tube must be received at HDRL within 7 days of collection. ☐ **Refrigerated 2-8°C** – SST tube must be shipped in cold box with ice packs and received at HDRL within 7 days of collection. ☐ **Frozen -20°C** – Ship frozen **aliquoted** plasma with dry ice if specimen will be received at HDRL after 7 days of collection.

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

PROCESSING LABORATORY (For HDRL use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR HBsAg Version: June 2023

Appendix B7

HIV Verification Algorithm Test Request Form (OCONUS)

Test Requested	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **HIV Algorithm**	☐ 4 ml serum (SST Tubes) or ☐ 4 ml plasma (PPT preferred. EDTA, Na Heparin, Na Citrate, CPDA and ACD-1 plasma is acceptable.)	**SST Tubes** – Invert 5X and allow to clot for 30 min (no more than 2 hrs.) post-collection. Centrifuge 10 minutes at 1000-1300 RCF in a swing bucket centrifuge. **NOTE:** Tubes MUST be allowed to clot for 30 minutes. **PPT tubes** – Invert 8-10X. Spin tubes within 2 hrs. of collection. Centrifuge in swing-out rotor centrifuge at 1100 RCF for a minimum of 10 min. Freeze plasma aliquot at -20°C.	☐ **Frozen -20°C** – Ship frozen **aliquoted** specimen with dry ice if specimen will be received at HDRL after 7 days of collection. **RECOMMENDED**: Ship on 6 lbs. dry ice in case of shipment delay.

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

PROCESSING LAB (For HDRL use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR HIVVER Version June 2023

Appendix B8

HIV-1 TNA PCR, HIV-2 TNA PCR Test Request Form

Test Requested (Check one)	Specimen Requirement	Draw Tube	Shipping Conditions (Check one)
☐ **HIV-1 TNA PCR**	☐ 18 ml whole blood (EDTA only)	**EDTA:** Store at room temperature for overnight or same day delivery.	☐ **Ambient** - Ship ambient within 24 hours of collection.
☐ **HIV-2 TNA PCR**	☐ 18 ml whole blood (EDTA only)		

Please fill the request form completely to ensure timely specimen processing.

PATIENT IDENTIFICATION	CONTACT INFORMATION
Patient identifiers MUST INCLUDE: **Full Name** _____ **DoDID** _____ OR **FMP/SSN** _____ **DOB** _____ **Specimen Draw Date / Time:** _____ Ship Date: _____	**POC** _____ **Physician Name** _____ **Clinic / Center** _____ **Center Address** _____ _____ _____ _____ Telephone Number _____ Fax Number _____ (Commercial # only; please include area/country code) Alternate POC Name _____ Alternate POC Phone _____

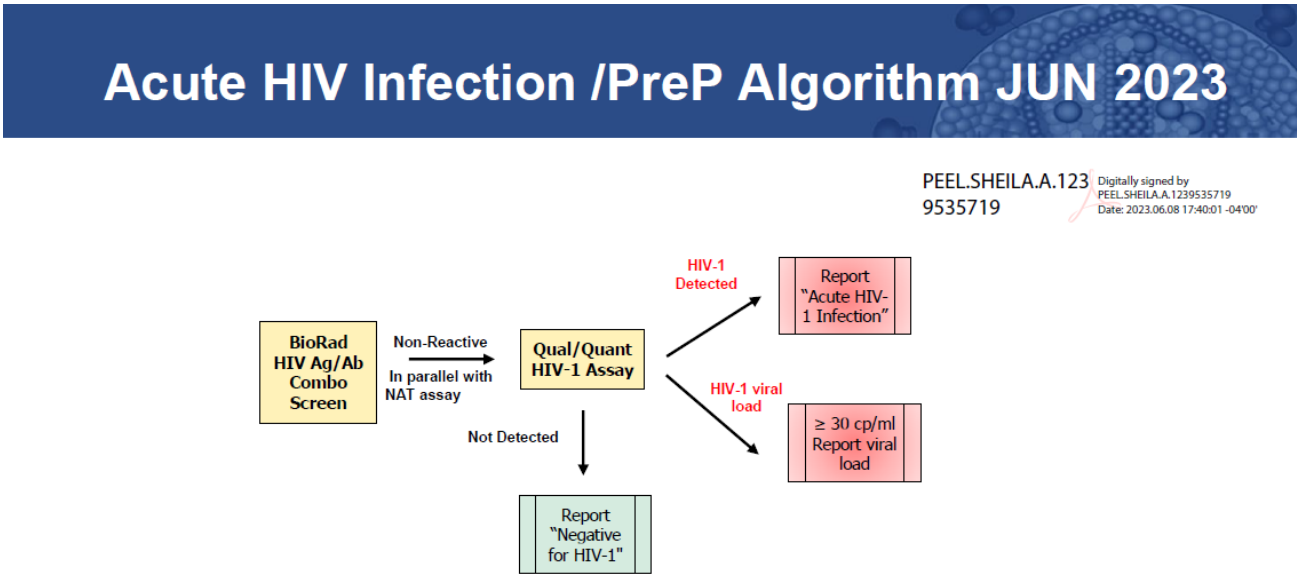
PROCESSING LABORATORY (For HDRL use only)		
BARCODE	DATE RECEIVED	QUANTITY & TYPE RECEIVED / INITIALS

Fax/Email a FedEx tracking and/or invoice number to ensure all shipments sent to the HIV Diagnostics and Reference Laboratory are received, IAW CAP GEN.40530
Shipping Address: 9100 Brookville Road, BLDG 508, Silver Spring, MD 20910

Form # TR MSO Version July 2023

Appendix C: HIV Acute Infection Algorithm

Appendix C



Algorithm available upon request; suspect ARS or increased risk/exposure event

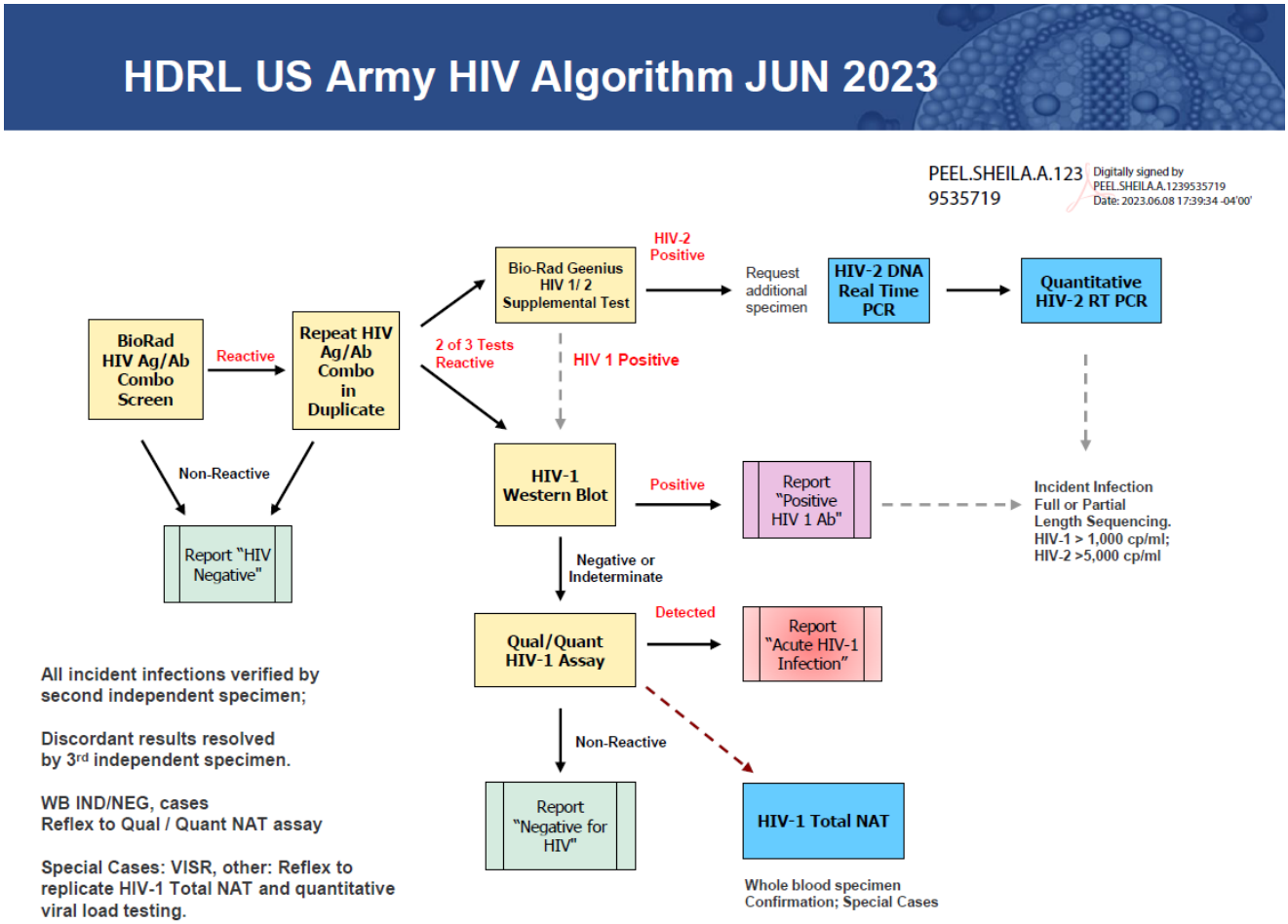
All incident infections verified by second independent specimen;

All AHI specimens are run in parallel by 4th generation EIA and dual intended use assay for qualitative detection of HIV-1 and quantitative report of viral load for specimens with ≥ 30 cp/ml

Provider contacted to submit test request

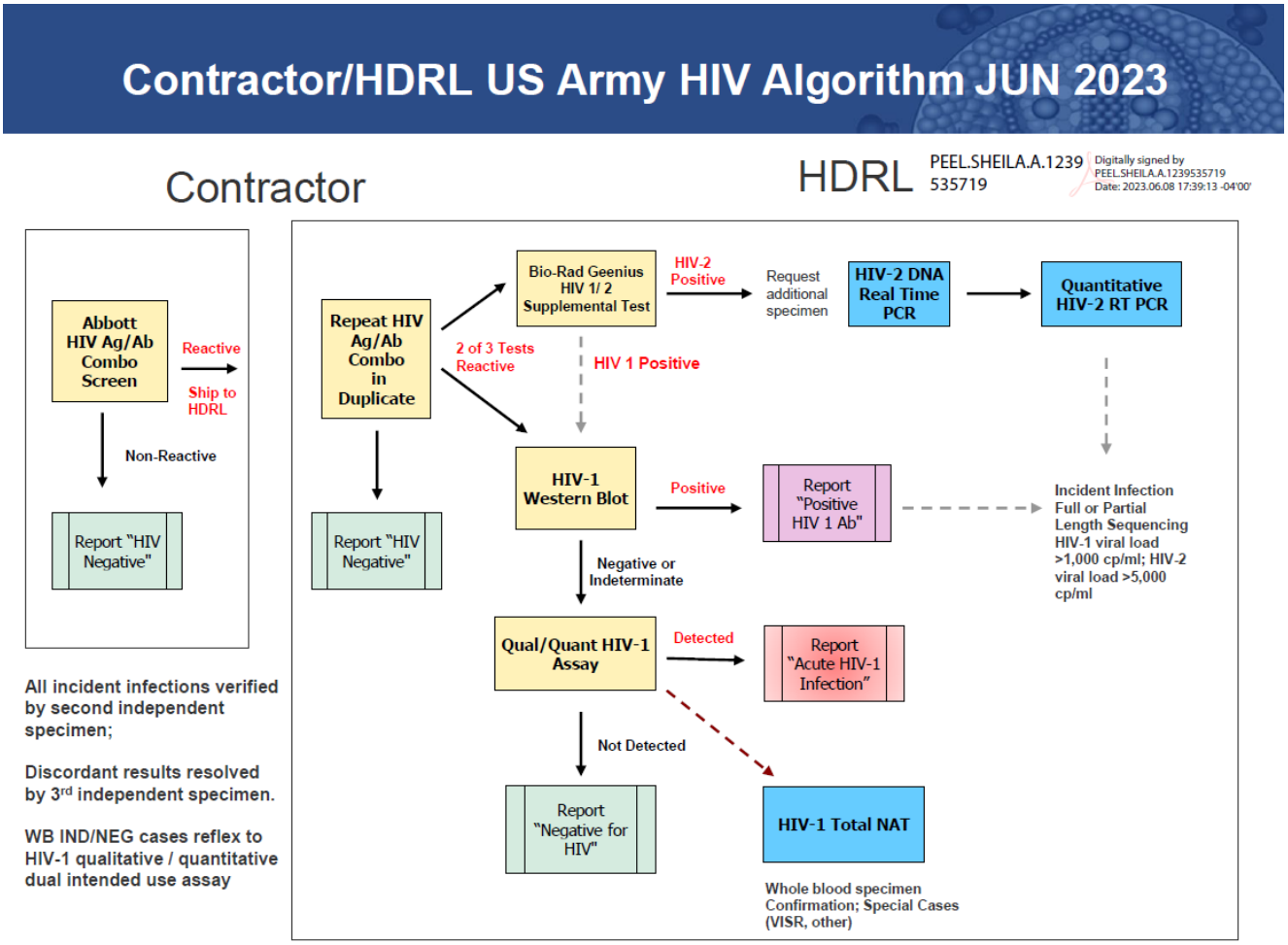
Appendix D: U.S. Army HIV Test Algorithm

Appendix D


HDRL US Army HIV Algorithm JUN 2023
PEEL.SHEILA.A.1239535719
Digitally signed by PEEL.SHEILA.A.1239535719
Date: 2023.06.08 17:39:34 -04'00'
BioRad HIV Ag/Ab Combo Screen
Reactive
Repeat HIV Ag/Ab Combo in Duplicate
Non-Reactive
Report "HIV Negative"
2 of 3 Tests Reactive
Bio-Rad Geenius HIV 1/ 2 Supplemental Test
HIV-2 Positive
Request additional specimen
HIV-2 DNA Real Time PCR
Quantitative HIV-2 RT PCR
HIV 1 Positive
HIV-1 Western Blot
Positive
Report "Positive HIV 1 Ab"
Incident Infection Full or Partial Length Sequencing. HIV-1 > 1,000 cp/ml; HIV-2 >5,000 cp/ml
Negative or Indeterminate
Qual/Quant HIV-1 Assay
Detected
Report "Acute HIV-1 Infection"
Non-Reactive
Report "Negative for HIV"
HIV-1 Total NAT
Whole blood specimen Confirmation; Special Cases
All incident infections verified by second independent specimen;
Discordant results resolved by 3rd independent specimen.
WB IND/NEG, cases Reflex to Qual / Quant NAT assay
Special Cases: VISR, other: Reflex to replicate HIV-1 Total NAT and quantitative viral load testing.

Appendix E: Contract Laboratory HIV Test Algorithm

Appendix E


Contractor/HDRL US Army HIV Algorithm JUN 2023
Contractor
HDRL
PEEL.SHEILA.A.1239535719
Digitally signed by PEEL.SHEILA.A.1239535719
Date: 2023.06.08 17:39:13 -04'00'
Abbott HIV Ag/Ab Combo Screen
Reactive
Ship to HDRL
Non-Reactive
Report "HIV Negative"
All incident infections verified by second independent specimen;
Discordant results resolved by 3rd independent specimen.
WB IND/NEG cases reflex to HIV-1 qualitative / quantitative dual intended use assay
Repeat HIV Ag/Ab Combo in Duplicate
Report "HIV Negative"
2 of 3 Tests Reactive
Bio-Rad Geenius HIV 1/ 2 Supplemental Test
HIV-2 Positive
Request additional specimen
HIV-2 DNA Real Time PCR
Quantitative HIV-2 RT PCR
HIV 1 Positive
HIV-1 Western Blot
Positive
Report "Positive HIV 1 Ab"
Incident Infection Full or Partial Length Sequencing HIV-1 viral load >1,000 cp/ml; HIV-2 viral load >5,000 cp/ml
Negative or Indeterminate
Qual/Quant HIV-1 Assay
Detected
Report "Acute HIV-1 Infection"
Not Detected
Report "Negative for HIV"
HIV-1 Total NAT
Whole blood specimen Confirmation; Special Cases (VISR, other)

Appendix F: Packaging and Shipment Instructions

Appendix F

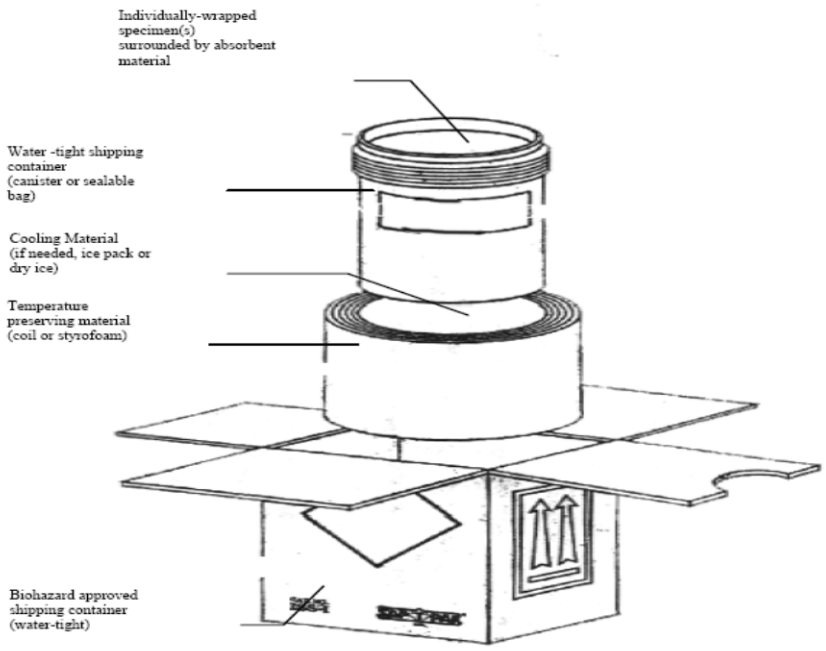
PACKAGING AND SHIPMENT OF SPECIMEN

DO NOT SHIP SPECIMENS FOR ARRIVAL ON WEEKENDS OR FEDERAL HOLIDAYS.

Sites submitting specimens must comply with all applicable Federal and State regulations concerning shipment for UN3373 Biological Substances, Category B. Minimum requirements for packaging and shipping follow:

A. Package sample in a watertight primary container (i.e. Sample tube) and secondary container.
B. Surround individually wrapped specimens with enough absorbent material to absorb entire content of tube if spillage occurs during transport.
C. Wrap all vials individually to avoid contact with other tubes.
D. Place each tube in a biohazard bag to contain spillage and avoid contaminating other tubes being shipped in the secondary container.
E. Place request forms within the container in a waterproof sleeve separate from the tubes.
F. Label container and ship specimens according to applicable guidelines (ie 49CFR and IATA regulations, UN3373 Biological Substances, Category B) for shipment of diagnostic specimens.
G. Ship to: **9100 Brookville Road, BLDG 508, Silver Spring, MD 20910**
H. **FAX/EMAIL/CALL TO NOTIFY THE LAB OF THE COMMERICAL COURIER TRACKING AND/OR INVOICE NUMBER TO ENSURE ALL SHIPMENTS ARE RECEIVED.**

Note: If you would like your shipment boxes returned to your site, please enclose a prefilled FedEx or address slip with FedEx billing information and material to cover hazardous warnings.



DELETION OR SUBSTITUTION OF ANY REQUIRED COMPONENTS MAY RENDER PACKAGES ILLEGAL FOR SHIPPING INFECTIOUS SUBSTANCES

Version: May 2023

Shipping Address: 9100 Brookville Road* BLDG. 508 * Silver Spring * MD 20910
Tel: 301-319-3123* Fax: 301-319-3502

Appendix G: Point of Contact Forms

Appendix G1

POINT OF CONTACT FORM – RESULT REPORTING

To maintain the integrity of our database and services, the HIV Diagnostics and Reference Laboratory (HDRL) requests new or updated information from your site. Please review and enter any corrections.

This information pertains to the delivery of **(please check ALL applicable reports):**

- ☐ Serology (HIV EIA, Geenius HIV1/2, Western Blot)
- ☐ Qual / Quant HIV-1 RNA
- ☐ Qual / Quant HCV RNA
- ☐ HIV Viral Load
- ☐ HCV Viral Load
- ☐ Sentosa® SQ Sentosa® SQHIV-1 Genotyping
- ☐ Other _____

All Fields are required for Primary POC. Please provide information for Secondary POC if applicable.

Primary POC: _____	Secondary POC: _____
Phone Number: _____ (Commercial Only)	Phone Number: _____ (Commercial Only)
Fax Number: _____	Fax Number: _____
Is this fax secure (in a private office)? ☐ Yes ☐ No	Is this fax secure (in a private office)? ☐ Yes ☐ No
Email Address: _____	Email Address: _____

Mailing Address: _____
(Including organization name) _____
_____

Send Results By:
☐ Fax ☐ Fed Ex ☐ Both
☐ Secure File Transfer

Reason for POC change (if applicable): _____
(e.g., new account, POC moved, different testing, yearly update)

_____ _____
Primary POC authorizing signature **OIC or Dept. Manager authorizing signature**

Signatures of the primary POC and Officer in Charge or Department Manager are required to update this information.

Due to the sensitive nature of HDRL's reports, only the designated POC (or alternate) can receive reports from HDRL. The facility POC is responsible for posting results and distributing any other mailings HDRL provides them.

HDRL may contact POCs if questions arise during the receipt and/or processing of specimens.

Fax completed forms to 301-319-3502. For questions, call 301-319-3123.

Form # POC RR Version June 2023

Appendix G2

POINT OF CONTACT FORM – NOTIFICATION AND FOLLOW-UP

In order to ensure proper follow-up on reported HIV positive test results, we need information from your site. The Primary POC should be the Provider responsible for discussing the result with the patient. This is to ensure compliance with CAP regulations for report of HIV test results.

Primary POC: _____	**Secondary POC:** _____
Phone Number: _____	**Phone Number:** _____
(Commercial Only)	(Commercial Only)
Fax Number: _____	**Fax Number:** _____
Is this fax secure (in a private office)?	Is this fax secure (in a private office)?
Yes No	Yes No
Email Address: _____	**Email Address:** _____
Mailing Address: _____	**Mailing Address:** _____
_____	_____
_____	_____

Printed name and position title: _____ (Printed name) _____ (Title)

Signature authorizing designation of POCs: _____ (Signature) _____ (Date)

HDRL requires at least two (2) POCs, full addresses, and telephone and fax numbers.

Due to the sensitive nature of HDRL's reports, the POCs will be the only persons HDRL will consult for follow-up information. HDRL may also contact the POCs if questions arise concerning specimens.

Result reports will continue to go through the current mechanisms, but NLT 10 working days post reporting a positive result, HDRL will conduct a follow-up with the primary POC to ensure notification.

Please fax this information to (301) 319-3502. Additionally, keep this form on file and update HDRL with any changes regarding the POCs, addresses, or phone numbers.

For questions, please contact the HDRL Associate Laboratory Director @ 301-319-9938

Form# POC NF Version: June 2023

Appendix H: Verification Kit Instructions

Appendix H1

Verification Specimen Kit Instructions

You are receiving this package because a patient at your site had a first-time positive test result for HIV. This shipment contains everything you need to draw the blood for an HIV verification test and to ship to the Specimen Processing Laboratory/BioRepository (SPL/BioR) within the Diagnostics and Countermeasures Branch (DCB).

PLEASE FOLLOW ALL INSTRUCTIONS CAREFULLY

Blood should ONLY be drawn MONDAY THROUGH THURSDAY.
DO NOT schedule any draws or send kits out Friday, Saturday, or Sunday.

Use kit BEFORE (mm/dd/yyyy)_____DO NOT use expired tubes.
Samples should be processed and shipped on the same day of the blood draw.

1. **This Kit Includes:**
   a. Two (2) CPT Tubes (blue and black tops)
   b. Four (4) PPT Tubes (white top) for a minimum of 4 mL of plasma
   c. Three (3) Cold Packs
   d. One (1) absorbent sheet STP-711
   e. One (1) kPa Bag
   f. One (1) foam block
   g. One (1) UN3373 Biological Substance label
   h. Return FedEx air bill
   i. Test Request Form
   j. Packing Instructions
   k. Example of a completed Test Request Form
2. **Procedure:**
   a. Label tubes with Patient NAME, SSN, DOB. Un-labeled or mislabeled tubes won't be processed.
   b. Complete Test Request Form, ALL fields need to be completed.
   c. Draw tubes to maximum fill point. Invert 8-10 times to mix.
   d. Tube Processing:

	Centrifugation	Shipment
CPT	Spin tubes within 2 hours of collection. Centrifuge in swing-out rotor centrifuge at 1500-1800 RCF for a minimum of 20 min.	Tubes must be immediately shipped in cold box with ice packs and received at the SPL/BioR within 24 hours. **PLEASE EMAIL FEDEX TRACKING NUMBER TO splab@hivresearch.org and Anais Valencia-Ruiz (Laboratory Director) avalencia-ruiz@hivresearch.org**
PPT	Spin tubes within 4 hours of collection. Centrifuge in swing-out rotor centrifuge at 1100 RCF for a minimum of 10 min.	

   e. Prep original shipment box by following the included Packing Instructions.
   f. Fill in the fields of the Packing Instructions form.
   g. Affix the UN3373 Biological Substance Label to the outside of the box.
   h. Affix the supplied Return FedEx airbill to the box, and schedule a pick up with FedEx.

For questions about the blood draw, or shipment information, please contact **Anais Valencia-Ruiz (Laboratory Director) avalencia-ruiz@hivresearch.org (301) 251 - 3032.**

Form # KIT INS Version August 2021

Appendix H2

VERIFICATION KIT
PACKING INSTRUCTIONS

IMPORTANT

- **Upon receipt** place Cold Packs in refrigerator (4°C)
- Once kit is packed up, call FedEx to schedule pick up.

Pack Date:_____

Time Samples Added and Box Sealed:

_____AM/PM

Initials of Packer: _____

1. Place One (1) Chilled Cold Pack in bottom of Styrofoam lined kit.

2. Place One (1) Chilled Cold pack against two opposite side walls of the Styrofoam lined kit.

3. Place grey foam containing specimen Tubes into kPa Bag with absorbent sheets and seal bag. (Remove as much air as possible).

4. Place Sealed bag with tubes (with tubes up) between the 4 frozen side bricks on top of the bottom brick.

5. Replace lid. Ensure the filled in Test Request Form and Packing Instructions are placed on top of the lid. Seal box for shipping.

Form # PK INS

Version August 2021