Shipping and Submission Instructions

Thank you for participating as a submitting site for APHL/CDC’s Demonstration Project for HIV Nucleic Acid Testing (NAT) Referral. The objective of the demonstration project is to provide PHLs using the HIV Diagnostic Testing Algorithm with access to HIV NAT testing in a shared service model. Your public health laboratory should have received a unique submitting site identification number in the email associated with this document. Please include this identification number on your requisition form and specimens submitted for NAT referral.

Specimens that are repeatedly reactive by a screening immunoassay for HIV-1/2 (antibody (3rd Gen) or antigen/antibody combo (4th Gen)) and non-reactive, negative or indeterminate using a supplemental antibody assay such as an HIV-1/HIV-2 differentiation assay (e.g. Multispot or Geenius), Western Blot, or Immunofluorescent Assay (IFA)) should be submitted to the designated referral laboratory for testing with the APTIMA HIV-1 RNA Qualitative Assay. NY State Department of Health Wadsworth is now also offering qualitative HIV-2 NAT for all enrolled submitting sites for specimens that meet those requirements. For detailed information about HIV-2 NAT see Appendix A.

In order to collect additional data for potential updates to the HIV diagnostic testing algorithm, both referral laboratories will also accept specimens that were Reactive for HIV Ag-Ab with Reactive for HIV-1 Ag using the BioPlex 2200 HIV Ag-Ab Assay without additional supplemental antibody testing and will perform the Hologic APTIMA HIV-1 RNA Qualitative assay. To clarify further, this only applies for submitting laboratories that are performing the BioPlex assay and have a specimen that only has HIV-1 antigen reactivity with no HIV-1 or HIV-2 antibody reactivity.

Upon receipt, the referral laboratories will examine specimens for quality and the test requisition form for completeness. The referral laboratories will perform the APTIMA HIV-1 RNA Qualitative Assay and automatically reflex to HIV-2 NAT as necessary (See Section I for more information). Referral laboratories will report results to the submitting laboratories with patient identifiers.

Following reporting of the results, all patient identifiers will be disassociated from the specimen. If there is sufficient specimen volume remaining, the samples may be used to evaluate new HIV diagnostic tests to generate data that could be used to inform updates to the HIV Diagnostic Testing Algorithm.

Your public health laboratory will be submitting specimens requiring HIV-1 NAT to:
Florida Department of Public Health, Bureau of Laboratories
Sally Fordan, MT (ASCP)
Retrovirology Supervisor
FL Bureau of Public Health Laboratories
1217 Pearl St.
Jacksonville, FL 32202

SECTION ONE: REQUIRED SPECIMENS AND LABELING SPECIMENS

Required Specimens
Specimens that are repeatedly reactive by a HIV-1/HIV-2 Screening Immunoassay (antibody (3rd Gen) or antigen/antibody combo (4th Gen)) but non-reactive, negative or indeterminate by a supplemental antibody assay should be submitted to the designated referral laboratory for testing with the Hologic APTIMA HIV-1 RNA Qualitative Assay. BioPlex 2200 HIV-1 antigen only reactive specimens (Result: Reactive for HIV Ag-Ab: Reactive for HIV-1 Ag) may also be sent without performing the supplemental antibody assay. If specimen requires HIV-2 NAT (see Table 1) Florida will submit specimens with sufficient quantity (at least 150 uL) directly to NYSDOH-Wadsworth on behalf of the submitting laboratory. The submitting laboratory will not need to do anything else. If quantity is not sufficient to perform HIV-2 NAT, the submitting laboratory will be notified and a new specimen and test request form should be submitted directly to NYSDOH-Wadsworth following the instructions in Appendix A.

Table 1: Specimens requiring HIV-1 NAT

<table>
<thead>
<tr>
<th>Screening Assay Result</th>
<th>Geenius Result</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Repeatedly Reactive</td>
<td>Nonreactive, Indeterminate: HIV-1 indeterminate</td>
<td>Request HIV-1 NAT</td>
</tr>
<tr>
<td>2 Repeatedly Reactive</td>
<td>Indeterminate: HIV Indeterminate</td>
<td>Request HIV-1 NAT, automatically reflexed to HIV-2 NAT if HIV-1 NAT negative</td>
</tr>
<tr>
<td>3 Repeatedly Reactive</td>
<td>Indeterminate: HIV-2 Indeterminate</td>
<td>Request HIV-1 NAT, automatically reflexed to HIV-2 NAT if HIV-1 NAT negative</td>
</tr>
<tr>
<td>4 Reactive for HIV Ag-Ab, Reactive for HIV-1 Ag (BioPlex)</td>
<td>Not Required</td>
<td>Request HIV-1 NAT</td>
</tr>
</tbody>
</table>

In order to comply with package insert requirements, the following specimen criteria are mandatory:
- Plasma (serum is acceptable but not preferred)
Specimens must meet one the following storage requirements:
- Whole blood, plasma or serum stored for less than 72 hours from time of draw at 2°C to 25°C
- Plasma or Serum removed and stored at 2°C to 8°C for up to 5 additional days (up to 8 days total)
- Plasma or Serum removed and stored at <-20°C

**Specimen Volume**

<table>
<thead>
<tr>
<th>Specimen Volume Submitted</th>
<th>Assays Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>550 uL Serum or Plasma</td>
<td>Hologic APTIMA HIV-1 Qualitative Assay</td>
</tr>
</tbody>
</table>

**Labeling Specimens**

- Label specimens with labels generated by your facility and follow your facility’s procedures for proper specimen labeling
- In addition to unique patient identifiers, please include the unique submitting site identification number assigned to you for your project on the tube.

**SECTION TWO: PACKAGING SPECIMENS**

- Preferred: Specimens should be frozen and shipped overnight on dry ice and packaged according the relevant packaging requirements.
- Alternative: If you plan on sending specimens on cold packs, freeze the specimen (serum or plasma) first, then transfer to cold packs at the time of shipping. Regardless of the method, specimen still meets the minimum storage requirements listed above.
- The specimen should be clearly labeled with the unique patient identifier(s), the laboratory name, and the unique submitting site identification number associated with this project.
- The completed requisition form should be included in the shipment.

**SECTION THREE: REQUISITION FORMS**

Include the APHL HIV-1 NAT Demonstration Project Test Requisition form with the shipment. See Appendix B for instructions on utilizing the new test requisition form. The following information MUST be recorded on the requisition form and is consistent with data routinely collected by referral laboratories:

- Submitting Laboratory ID
• Unique patient identifier(s);
• Date of collection;
• Date and time of receipt in the laboratory;
• Conditions and specimen type received;
• Specimen storage conditions from point of specimen receipt;
• Name of IA and supplemental test performed;
• Results of IA (if BioPlex) and supplemental antibody assays;
• Date of shipment

Additional information on specimen collection and storage should be recorded if available. The completed requisition form should be included in the shipment.

SECTION FOUR: SHIPPING SPECIMENS

Prior to shipment the submitting laboratory should notify the point of contact at the testing laboratory that they will be a submitting a specimen. Specimens should be sent overnight and include a copy of the test requisition form.

Point of contact: Sally H. Fordan
Email: sally.fordan@flhealth.gov
Phone: (904) 791-1531

The submitting site is to ensure that all Federal regulations for shipping infectious substances under Division 6.2 are met.

Specimen shipments are to be scheduled and made through your FedEx Account

2. Once logged in, select the “Ship” tab and a drop down menu will appear. Then click on “Prepare Online Shipment”.

   **NOTE:** If FedEx does not make daily pick-ups at your laboratory, then you will need to click the “Schedule a Pickup” option, pick the “Express Service” option, and be sure to change the pick-up address to your laboratory.

3. You will be directed to a screen that allows you to enter recipient information, billing details, package and shipment details, and e-mail notification information.
4. Fill in recipient information for the laboratory conducting the supplemental testing:

Florida Department of Public Health, Bureau of Laboratories
Sally Fordan, MT (ASCP)
Retrovirology Supervisor
FL Bureau of Public Health Laboratories
1217 Pearl St.
Jacksonville, FL 32202

5. Under the Billing details section, open the drop down menu under “Bill transportation to” and select “Third Party”

6. Once “Third Party” is selected, in the “Account Number” field enter APHL’s account number: 131240619

7. In the Package and shipment details section, please select “Standard Overnight” in the “Service type” box. Then select “Your Packaging” in the “Package type” box. You will then need to provide the dimensions and weight of your package in the appropriate boxes. (See above)

8. Under the E-mail Notifications section, please enter anne.gaynor@aphl.org in the “Other 1” box. Then click the “Ship” and “Delivery” checkboxes so that APHL will receive a notice of shipment pick-up and delivery.

9. Click and verify that all of the information is correct in the pop-up box. If a pop-up box does not appear, please make sure that your pop-up blocker is disabled.

10. Click and your electronic waybill will be generated on the next screen.

SECTION FIVE: RESULTS REPORTING

Referral laboratories will report results from the APTIMA HIV-1 RNA Qualitative Assay to the submitting laboratories with patient identifiers. Positive results will be reported via a fax within 24 hours of test completion along with an email alert to the submitting laboratory and followed with results followed by a mailed paper report. Submitting laboratories can expect to receive a mailed paper report containing results from the APTIMA HIV-1 RNA Qualitative Assay for all specimens submitted.

<table>
<thead>
<tr>
<th>HIV IA</th>
<th>Hologic APTIMA HIV-1 Qualitative Assay</th>
<th>Result Reported (according to Hologic package insert)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Repeatedly Reactive by Screening HIV IA</td>
<td>Reactive</td>
<td>HIV-1 RNA Detected</td>
</tr>
<tr>
<td>Specimen Repeatedly Reactive by Screening HIV IA</td>
<td>Nonreactive</td>
<td>HIV-1 RNA Not Detected</td>
</tr>
<tr>
<td>Specimen Repeatedly Reactive by Screening HIV IA</td>
<td>Invalid</td>
<td>Invalid</td>
</tr>
</tbody>
</table>
SECTION SIX: ADDITIONAL DATA REPORTING

During the project period, submitting laboratories will be asked to record data on the total number of specimens tested by a screening immunoassay for HIV-1/2 (antibody (3rd Gen) or antigen/antibody combo (4th Gen)), the number that were singly and repeatedly reactive, and among those that were repeatedly reactive, report the number that had each of the potential supplemental antibody assay result used by the submitting laboratory. APHL will provide a template for reporting results.

Data should be submitted to APHL (anne.gaynor@aphl.org) by the submitting laboratory within 30 days of the announcement of the end of the project period.

QUESTIONS

If you have any questions or problems with specimen packaging or shipment, please send an email to or call one of the following contacts:

Primary APHL Contact
Anne Gaynor
Anne.gaynor@aphl.org
240-485-2739

Secondary APHL Contact
Kelly Wroblewski
kelly.wroblewski@aphl.org
240-485-2728
APPENDIX A
SPECIAL TESTING CIRCUMSTANCES – HIV-2 NAT Testing

New York State Department of Health, Wadsworth Center is now offering qualitative HIV-2 NAT testing for specimens meeting specific criteria submitted to the HIV NAT Program (Florida Department of Public Health, Bureau of Public Health Laboratories will forward specimens meeting criteria to NYSDOH, Wadsworth). Due to these changes we have compiled additional information for when and how to request HIV-2 NAT.

There are two circumstances where HIV-2 NAT can be requested directly from NYDOH-Wadsworth (Table 2). The first circumstance is for specimens listed in Table 1 in the main section that require an automatic reflex to HIV-2 NAT but there is insufficient quantity. The submitting laboratory would then follow the instructions in this appendix to submit the new specimen for HIV-2 NAT testing. The second circumstance will only impact those laboratories that are performing the BioPlex HIV Ag-Ab Assay and the screening result is: Reactive for HIV Ag-Ab, Reactive for HIV-2 Ab which is not confirmed by the supplemental HIV-1/HIV-2 antibody differentiation test meaning that the screening result indicates that HIV-2 is present in the specimen but the supplemental test is discordant. If this is the case the submitting laboratory would then follow the instructions in this appendix to submit the specimen for HIV-2 NAT testing.

Table 2: Specimen Criteria for HIV-2 NAT Testing

<table>
<thead>
<tr>
<th>Screening Assay Result</th>
<th>Geenius Result</th>
<th>HIV-1 NAT Result</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Repeatedly Reactive</td>
<td>Indeterminate: HIV Indeterminate or Indeterminate: HIV-2 Indeterminate</td>
<td>Negative, QNS for HIV-2 NAT</td>
<td>Request HIV-2 NAT</td>
</tr>
<tr>
<td>2</td>
<td>HIV-2 not confirmed (Indeterminate: HIV, HIV-1, or HIV-2 indeterminate; Nonreactive)</td>
<td>N/A</td>
<td>Request HIV-2 NAT, automatically reflexed to HIV-1 NAT if quantity sufficient</td>
</tr>
</tbody>
</table>
SECTION 1: REQUIRED SPECIMENS AND LABELING SPECIMENS

Required Specimens

Only specimens meeting Criteria 1 and 2 in Table 2 will be accepted for HIV-2 NAT testing at NYSDOH, Wadsworth. Prior to shipping HIV-2 NAT specimens, the submitting laboratory should notify the point of contact and alternate point of contact at the referral laboratory (NYSDOH-Wadsworth) and at APHL to confirm that the sample requires HIV-2 NAT only.

The HIV-2 NAT that will be performed is a laboratory-developed, qualitative RNA assay. The assay has been validated by the NYSDOH Wadsworth Center Bloodborne Viruses Laboratory and approved for clinical use by the NYSDOH Clinical Laboratory Evaluation Program (CLEP). In order to comply with standard operating procedure (SOP) requirements of the assay, the following specimen criteria are mandatory:

- Plasma (serum is acceptable but not preferred)
- Specimens must meet one the following storage requirements:
  - Whole blood, plasma or serum stored for less than 72 hours from time of draw at 2°C to 25°C
  - Plasma or Serum removed and stored at 2°C to 8°C for up to 5 additional days (up to 8 days total)
  - Plasma or Serum removed and stored at <-20°C

Specimen Volume Requirements for HIV-2 NAT

<table>
<thead>
<tr>
<th>Specimen Volume Submitted</th>
<th>Assays Completed</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL Serum or Plasma preferred. Minimum of 150 uL is acceptable.</td>
<td>Qualitative HIV-2 RNA real time PCR assay</td>
<td>In addition to volume needed for HIV-1 NAT</td>
</tr>
<tr>
<td>550 uL Serum or Plasma</td>
<td>Hologic APTIMA HIV-1 Qualitative Assay</td>
<td></td>
</tr>
</tbody>
</table>

SECTION TWO/THREE: FOR PACKAGING AND REQUISITION FORMS REFER TO INSTRUCTIONS IN MAIN DOCUMENT

SECTION FOUR: SHIPPING SPECIMENS DIRECTLY TO NYSDOH FOR HIV-2 NAT (FOR SUBMITTING HIV-1 NAT REFER TO MAIN DOCUMENT)
Prior to shipment of HIV-2 NAT specimens, the submitting laboratory should notify the point of contact and alternate point of contact at the referral laboratory (NYSDOH-Wadsworth) and at APHL to confirm that the sample requires HIV-2 NAT only. Specimens should be sent overnight and include a copy of the test requisition form.

APHL Point of Contact: Anne Gaynor
Email: anne.gaynor@aphl.org
Phone: (240) 485-2739

NYSDOH-Wadsworth Point of contact: Linda Styer
Email: linda.styer@health.ny.gov
Phone: (518) 473-6007 or (518) 474-2163

NYSDOH-Wadsworth Alternate Point of contact: Monica Parker
Email: monica.parker@health.ny.gov
Phone: (518) 474-2444 or (518) 474-2163

The submitting site is to ensure that all Federal regulations for shipping infectious substances under Division 6.2 are met.

Specimen shipments are to be scheduled and made through your FedEx Account

2. Once logged in, select the “Ship” tab and a drop down menu will appear. Then click on “Prepare Online Shipment”.

   NOTE: If FedEx does not make daily pick-ups at your laboratory, then you will need to click the “Schedule a Pickup” option, pick the “Express Service” option, and be sure to change the pick-up address to your laboratory.

3. You will be directed to a screen that allows you to enter recipient information, billing details, package and shipment details, and e-mail notification information.
4. Fill in recipient information for the laboratory conducting the supplemental testing:

   Wadsworth Center—New York State Department of Health
   Bloodborne Viruses Laboratory, Attn: APHL NAT Project
   Wadsworth Center-NYSDOH David Axelrod Institute
   120 New Scotland Avenue
   Albany, NY 12208

5. Under the Billing details section, open the drop down menu under “Bill transportation to” and select “Third Party”
6. Once “Third Party” is selected, in the “Account Number” field enter APHL’s account number: 131240619

7. In the Package and shipment details section, please select “Standard Overnight” in the “Service type” box. Then select “Your Packaging” in the “Package type” box. You will then need to provide the dimensions and weight of your package in the appropriate boxes. (See above)

8. Under the E-mail Notifications section, please enter anne.gaynor@aphl.org in the “Other 1” box. Then click the “Ship” and “Delivery” checkboxes so that APHL will receive a notice of shipment pick-up and delivery.

9. Click and verify that all of the information is correct in the pop-up box. If a pop-up box does not appear, please make sure that your pop-up blocker is disabled.

10. Click and your electronic waybill will be generated on the next screen.

RESULTS REPORTING

Referral laboratories will report results from the qualitative HIV-2 NAT testing to the submitting laboratories with patient identifiers. If HIV-1 NAT testing is performed and HIV-2 NAT is reflexed, it will be noted on the initial result that HIV-2 NAT is pending. Positive results will be reported to the submitting laboratory via a telephone call within 24 hours of test completion and/or secure fax or secure file transfer program (sFTP) and followed with a mailed paper report. Results that are not positive, may also be reported by telephone, secure fax and/or secure FTP. A paper report will be returned by mail for all results. Submitting laboratories can expect to receive a mailed paper report containing results from the HIV-2 NAT Assay for all specimens submitted.

Specimen Results Reported Based on Tests Performed for HIV-2 NAT
(For Hologic APTIMA HIV-1 Qualitative Assay see main document)

<table>
<thead>
<tr>
<th>HIV-2 RNA Qualitative Assay</th>
<th>Result Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (P)</td>
<td>HIV-2 RNA Detected</td>
</tr>
<tr>
<td>Negative (N)</td>
<td>HIV-2 RNA Not Detected</td>
</tr>
<tr>
<td>Inhibited (INH)</td>
<td>Indeterminate due to PCR inhibition</td>
</tr>
<tr>
<td>Inconclusive (INC)</td>
<td>Indeterminate, low positive with insufficient sample for repeat testing unconfirmed low positive result</td>
</tr>
<tr>
<td>Unconfirmed (UNC)</td>
<td>Indeterminate, low positive on initial run that did not confirm on repeat</td>
</tr>
</tbody>
</table>
APPENDIX B
TEST REQUEST FORM INSTRUCTIONS

The APHL HIV-1 NAT Demonstration Project Test Requisition form has been updated to improve data collection. The form is now a fillable form in Microsoft Excel with drop-down menus for many fields to eliminate unnecessary typing.

Instructions:
1. Please only modify the fields are marked with gray shading and those that are further highlighted with a red box. Cells with a red box are required for submission.

2. Each submitting laboratory may enter the information that will stay the same and save that as their own template to further save time on submitting the form.

3. The following fields contain drop-down menus. Please select the appropriate response from the drop down list.
   - Test Request
   - Conditions on Receipt
   - Specimen Type Received
   - Screening Immunoassay Manufacturer
   - Supplemental Antibody Test
   - Multispot Interpretation
   - Geenius Interpretation
   - Western/Blot IFA Interpretation
   - Specimen Shipping Conditions

4. All other fields you may enter free text. When the form is printed it will have the project name on the top and a space at the bottom to re-enter your submitting laboratory ID.