Unique Testing Experience for Acute HIV Infection:
The Dallas County NAAT Program

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A pooling method (Figure 2) was developed and validated at DCHHS to help reduce the cost of the assay. If the pooled sera test result is NAAT nonreactive, then all specimens included in the specific pool are considered to be nonreactive for 23% Limit of Detection for individual specimen: 30 HIV copies per 1 ml gp41 74% 10% If HIV-1 RNA is detected in the pooled sera, that specific pool is considered NAAT reactive. Once a reactive pool is detected, the pool is 72 100% An indeterminate on the Western Blot is a result containing one of the following:

- Minor Bands (ex. band p18)
- Major Bands (ex. band gp160)

Following the DCHHS testing algorithm (Figure 1), a Western Blot is performed for confirmation on repeatedly reactive EIA specimens. If the Western Blot result is nonreactive or indeterminate then a HIV-1 RNA test is performed.

The data shows a higher probability for a HIV-1 RNA (NAAT) result to be reactive following repeat testing, the specimen is reported as reactive for HIV-1 RNA.

The number of HIV-1 RNA (NAAT) reactive specimens found demonstrates the spread of HIV-1 will remain an ongoing fight for public health departments. The early detection can prevent a possible second generation of HIV infections. The early detection can prevent a possible second generation of HIV infections. The early detection can prevent a possible second generation of HIV infections.
Abstract

Routine antibody assays for HIV-1 (Enzyme Immunoassays) are usually nonreactive during the first four to five weeks after infection. Dallas County Health and Human Services Laboratory (DCHHS) integrated HIV-1 RNA Qualitative Assay (Aptima Gen-Probe Inc.), an HIV-1 Nucleic Acid Amplification Test (NAAT), in July 2009 making possible detection of HIV-1 within two weeks of infection. Dallas County Laboratory defines acute HIV-1 infection (AHI) as an antibody negative and RNA positive specimen. This testing methodology is critical in HIV prevention as individuals are the most infectious during the acute stage.

A total of 118 HIV-1 RNA reactive specimens were detected out of 113,843 specimens analyzed. In 2009, 18,957 specimens were tested, with 12 HIV-1 RNA reactive specimens detected. In 2010, 36,760 specimens were tested, with 44 HIV-1 RNA reactive specimens detected. In 2011, 58,126 specimens were tested, with 62 HIV-1 RNA reactive specimens detected. Additionally, 22% of the HIV-1 RNA reactive specimens were found to have a dual diagnosis of syphilis, and 12% were found to have a chlamydia and/or gonorrhea infection.

The antibody assay (EIA) did not detect any antibodies for HIV on 52% (61/118) of the HIV-1 RNA reactive specimens. The remaining 49% (57/118) HIV-1 RNA reactive specimens were reactive on the antibody assay with a nonreactive or indeterminate on the Western Blot.

Improving the detection of an AHI is crucial for HIV prevention, because without the advancement in technology 118 patients could have received a negative or indeterminate test result prior to the HIV-1 RNA qualitative assay. We feel the increase in detection of an AHI warrants implementing the new technology for HIV-1 detection.
Methods:
Dallas County Health and Human Services Laboratory (DCHHS) performs HIV testing for local clinics and additional submitters across the state of Texas.
- Detection of antibodies is performed by Bio-Rad’s Human Immunodeficiency Virus Type 1/2 (recombinant and Synthetic Peptide) GS HIV-1/HIV-2 plus O EIA and Clearview HIV 1/2 STAT Pak Rapid Kits. Confirmation of a reactive is performed using the Bio-Rad Human Immunodeficiency Virus type 1 GS HIV-1 Western Blot (WB).
- Following the DCHHS Algorithm (See Figure 1, pg 5), specimens that are EIA and/or Rapid nonreactive, repeatedly reactive EIA with nonreactive or indeterminate Western Blot and outside submitters (ex. San Antonio Metropolitan Health District) have additional testing performed by Gen Probe’s Aptima HIV-1 RNA Qualitative Assay, an HIV-1 Nucleic Acid Amplification Test (NAAT).

Pooling Method:
- A pooling method (See Figure 2, pg 6) was developed and validated at DCHHS to help reduce the cost of the assay.
- The specimens are categorized as either High Risk (10 specimens per pool) or Low Risk (20 specimens per pool) based on the submitters.
  - High Risk category is primarily specimens from the STD clinics, jails, and clinics providing testing to patients with high risk behaviors and/or demographics.
  - Low Risk category is primarily specimens from the Family Planning Clinics (Parkland Hospital).
- Pool of 10: 100µl from each of the 10 specimens are manually pipetted into a labeled pool tube (total volume: 1ml). (See Figure 2, pg 6)
  - Limit of Detection for a pool of 10: 300 HIV copies per 1 ml
- Pool of 20: 50µl from each of the 20 specimens are manually pipetted into a labeled pool tube (total volume: 1ml).
  - Limit of Detection for a pool of 20: 600 HIV copies per 1 ml

Nonreactive Pool:
- If the pooled sera test result is NAAT nonreactive, then all specimens included in the specific pool are considered to be nonreactive for HIV-1 RNA.

Reactive Pool:
- If HIV-1 RNA is detected in the pooled sera, that specific pool is considered NAAT reactive. Once a reactive pool is detected, the pool is deconstructed into the individual specimens that comprise the reactive pool and are tested to identify the reactive specimen(s). Once the reactive specimen(s) is identified, a second NAAT test is required before any reactive result is reported. If the suspected specimen(s) test result is reactive following repeat testing, the specimen is reported as reactive for HIV-1 RNA.
**Individual Specimen(s)**

- Following the DCHHS testing algorithm (See Figure 1, pg 5), a Western Blot is performed for confirmation on repeatedly reactive EIA specimens. If the Western Blot result is nonreactive or indeterminate then a HIV-1 RNA test is performed.

- An indeterminate on the Western Blot is a result containing one of the following:
  - 1 major band (ex. gp160, gp120, p24, p41)
  - Minor bands with no major bands
  - 1 major band with additional minor bands

- Specimen(s) requesting additional analysis for a HIV-1 RNA are tested individually and are not subject to the pooling methods. (Ex. Specimen with a nonreactive and/or indeterminate Western Blot result, and any specimen received from the outside submitters)

- Specimens contained in a reactive pool are tested individually.
  - Limit of Detection for individual specimen: 30 HIV copies per 1 ml

The antibody EIA and RNA testing are performed concurrently offering the unique ability of a reactive result to be reported within 72 hours from the time of collection (TOC) and nonreactive result within 24 hours of the TOC.
Figure 1: Dallas County HIV Algorithm

Blood sample received for HIV test
ELISA or EIA is performed

EIA + (Test repeated X2)
EIA Nonreactive

Western Blot
Western Blot Reactive
HIV Reactive
Western Blot Indeterminate
NAAT Testing
NAAT Reactive
AHI Protocol
NAAT Nonreactive
HIV – (Retest based on risk factors)

Pooled NAAT Testing
NAAT Reactive
AHI Protocol
NAAT Nonreactive
Repeat HIV testing in 1 month

Western Blot Nonreactive
NAAT Testing
NAAT Reactive
AHI Protocol
NAAT Nonreactive
Repeat HIV testing in 1 month

Bio-Rad Multispot HIV-1/HIV-2 Rapid Test
10 individual specimens are assigned to a specific pool number.

100 μl of serum is pipetted into labeled pool tube.

Figure 2: Pooling Methods
Results:
The Dallas County Health and Human Services Laboratory defines an Acute HIV infection (AHI) as a specimen with no antibodies present with only HIV-1 RNA detected (NAAT reactive).

Dallas County Health and Human Services HIV-1 RNA Program Summary:

<table>
<thead>
<tr>
<th>Year</th>
<th>Specimens Tested with the HIV-1 RNA:</th>
<th>Specimens HIV-1 RNA Reactive:</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>18,957</td>
<td>12</td>
</tr>
<tr>
<td>2010</td>
<td>36,760</td>
<td>44</td>
</tr>
<tr>
<td>2011</td>
<td>58,126</td>
<td>62</td>
</tr>
<tr>
<td>Total number of Specimens:</td>
<td>113,843</td>
<td>118</td>
</tr>
</tbody>
</table>

Number of AHI detected (Per DCHHS definition): 61 out of 113,843

  - 8 HIV-1 RNA reactive specimens detected in 7,279 specimens analyzed

The antibody assay (EIA) did not detect any antibodies for HIV on 52% (61/118) of the HIV-1 RNA reactive specimens. The remaining 48% (57/118) HIV-1 RNA reactive specimens had antibodies detected with the EIA and either a nonreactive or indeterminate result on the confirmation with the Western Blot.

Additionally, 22% of the HIV-1 RNA reactive specimens were found to have a dual diagnosis of syphilis, and 12% were found to have a chlamydia and/or gonorrhea infection.

Of interesting note, the WB gp160 band was more likely to be present for HIV-1 RNA reactive specimens than the WB p24 band (Table 1). It was also observed that when HIV-1 RNA specimens were nonreactive, the WB p24 band was more likely to be present than the WB gp160 band (Table 2).

The DCHHS laboratory observed 26 HIV-1 RNA (NAAT) reactive specimens that had repeatedly reactive EIA and Indeterminate Western Blot results. In addition, there were 231 specimens that were HIV-1 RNA (NAAT) nonreactive, EIA repeatedly reactive and Western Blot nonreactive.
Graph 1

Graph 1: Western Blot Bands Present on Indeterminate Specimens

- **p24**: 6 NAAT Reactive, 72 NAAT Nonreactive
- **gp160**: 17 NAAT Reactive, 11 NAAT Nonreactive
- **gp41**: 1 NAAT Reactive, 5 NAAT Nonreactive
- **Minor Bands**: 2 NAAT Reactive, 10 NAAT Nonreactive
Table 1: HIV-1 RNA detected:

<table>
<thead>
<tr>
<th>Western Blot Bands Present</th>
<th>Number of specimens</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band p24</td>
<td>6</td>
<td>23%</td>
</tr>
<tr>
<td>Band gp160</td>
<td>17</td>
<td>65%</td>
</tr>
<tr>
<td>Band gp41</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Minor Bands (ex. band p18)</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>Total Number of Specimens</td>
<td>26</td>
<td>100%</td>
</tr>
</tbody>
</table>

The observed percentage of a HIV-1 RNA (NAAT) reactive specimen with the gp160 band present is 65% compared to 23% of the HIV-1 RNA (NAAT) reactive specimen with the p24 band. The data shows a higher probability toward a HIV-1 RNA (NAAT) reactive specimen when the gp160 band is present on an indeterminate Western Blot confirmation.

Table 2: No HIV-1 RNA detected:

<table>
<thead>
<tr>
<th>Western Blot Bands Present</th>
<th>Number of specimens</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band p24</td>
<td>72</td>
<td>74%</td>
</tr>
<tr>
<td>Band gp160</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>Band gp41</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>Minor Bands (ex. Band p18)</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>Total Number of Specimens</td>
<td>98</td>
<td>100%</td>
</tr>
</tbody>
</table>

The observed percentage of an HIV-1 RNA (NAAT) nonreactive when the indeterminate Western Blot result contains the p24 band is 74% comparing to 11% when the indeterminate Western Blot result contains the gp160 band. The difference suggests when the p24 band is present there is a higher probability for a nonreactive HIV-1 RNA (NAAT) result.
Picture 1: HIV-1 RNA (NAAT) Laboratory Set-up

Work Flow is Right to Left

Results ← ← ← ← Begin Assay ← Pooling
Public Health Follow-up for 2010-2011

DCHHS Disease Intervention Specialist performed the public health follow-up (PHFU) for 69 of the 106 HIV-1 RNA reactive cases (65%). Other jurisdictions in Texas conducted PHFU for 34 cases (32%). Ninety-eight (98) cases were located and interviewed (92%), which resulted in the initiation of 205 partners and 103 high risk clusters. A cluster is defined as a non-infected sex partner, suspect, or associate related to the original patient or sex partners to the original patient, or suspected STD patients who have signs/symptoms of a disease and are engaged in high risk behaviors.

Results of PHFU:
- 72 partners and 11 clusters were previous positives
- 6 partners and 1 cluster were previous negative, new positives
- 3 partners were not previously tested, new positives
- 63 partners and 71 clusters were not previously tested, new negatives
- 25 partners and 7 clusters were unable to be located

Twelve (12) new HIV infections were identified from PHFU.
- Ten (10) new HIV cases were identified from interviewing the AHI positives, termed “first generation” cases.
- In addition, 2 new HIV infections were identified by interviewing the first generation HIV cases and are termed “second generation” cases. The second generation cases did not yield any new HIV positives.

Disclaimer: 2010-2011 Public Health Follow-up data is preliminary.
HIV-1 RNA Reactive Demographics, 2010 - 2011

**Age Group**

- 15-24: 40%
- 25-34: 34%
- 35-44: 14%
- 45+: 12%

**Race**

- White: 54%
- Black: 43%
- Other: 3%

**Risk Behavior**

- Male-to-Male Sexual Contact: 74%
- High Risk Heterosexual: 19%
- Injection Drug Use: 1%
- Unknown: 6%
AHI Protocol

- Laboratory Director or Assistant is notified immediately

- Assistant STD/HIV Program Manager is notified immediately

- Surveillance Administrative Support Staff enters case immediately

- Program Manager notified if Assistant Manager is not available

- Surveillance Administrative Support Staff enters case immediately

- Field Record is created immediately

- Field Operations Manager receives copy of record

- DIS Unsuccessful in interviewing case within 72 hours

- Field Operations Manager notifies Assistant Program Manager

- Field Operations Manager attempts to interview case

- Assistant Program Manager documents unsuccessful interview

- Enhanced Partner Notification

- Field Operations Manager notifies Assistant Program Manager

- Field Operations Manager attempts to interview case

- Assistant Program Manager documents unsuccessful interview

- DIS Successfully Interviews Case within 72 hours of test result

- Case is assigned to a DIS immediately

- DIS Successfully Interviews Case within 72 hours of test result

- Immediate Enrollment into Early Intervention Clinic (within 24 hours)

- Medical clinic appointment made for case to outside provider

- AHI patient is seen by area medical provider within 21 days of testing date

- Review of cases where AHI patient is not seen by medical provider within 21 days of test date by Medical Directors
**Enhanced Partner Notification**

**DIS elicits names of high risk contacts of AHI cases**

**High risk contacts are interviewed within 72 hours**

- High risk contact has had unprotected sex and/or shared needles with AHI case within the last 72 hours
  - Perform Rapid HIV test either in the field or in the STD Clinic
    - Rapid HIV test +
      - Refer to Early Intervention as a presumptive positive
    - Rapid HIV test -
      - Notify Medical Director for potential referral for possible PEP
  - High risk contact has had unprotected sex and/or shared needles with AHI case within 72 hours – 4 weeks
    - Perform Rapid HIV test either in the field or in the STD Clinic
      - Rapid test +
        - Refer to Early Intervention as a presumptive positive
      - Rapid test -
        - Recommend retesting in 1 month if negative
  - High risk contact has had unprotected sex and/or shared needles with AHI case > 4 weeks
    - Perform Rapid HIV test either in the field or in the STD Clinic
      - Rapid test +
        - Refer to Early Intervention as a presumptive positive
      - Rapid test -
        - Perform HIV testing
Conclusion:
The number of HIV-1 RNA (NAAT) reactive specimens demonstrates the spread of HIV-1 will remain an ongoing fight for public health departments. The HIV-1 RNA assay is a valuable tool in the prevention of HIV due to its ability to find Acute HIV infections. The early detection can provide an opportunity to counsel the patient on prevention when viral load is the highest and the virus is most infectious.

The implementation of the HIV-1 RNA program was crucial since the current antibody assay (EIA) did not detect any antibodies for HIV on the majority of the HIV-1 RNA (NAAT) reactive specimens. Additionally, the data from table 1 and 2 demonstrate an interesting subject of discussion in reference to the new generation of antigen/antibody assays approved by the FDA.

The increase in detection of an AHI warrants implementing the new technology for HIV-1 detection.

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