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

Brian Emerson; Shayma Haq; Jennifer McCarver; Barry Mullins; Daniel Serinaldi; Edward Bannister PhD

Dallas County Health and Human Services, Dallas, Texas

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 syphillis, 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 48% (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


HIV - 1 RNA Laboratory Set-up

Dallas County Health and Human Services (DCHHS) Laboratory 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 (Figure 1), specimens that are EIA and/or Rapid nonreactive, repeatedly reactive EIA with nonreactive or indeterminate Western Blot 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 (Figure 2) 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).
- 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 (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.
- An indeterminate on the Western Blot is a result containing one 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)
- 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

HIV-1 RNA Program Summary

YEAR	Specimens tested with the HIV-1 RNA (NAAT)	Specimens HIV-1 (NAAT) reactive
2009	18,957	12
2010	36,760	44
2011	58,126	62

Graph 1: Western Blot Bands Present on Indeterminate Specimens

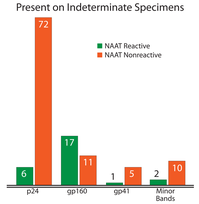


Figure 2

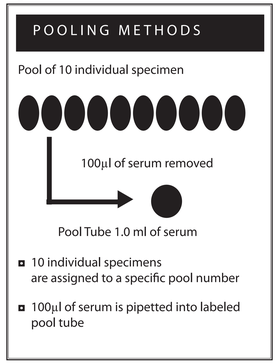


Table 1: HIV-1 RNA detected

Table 1: NAAT Reactive Specimens

Western Blot Bands Present	Number of specimens	Percentage
Band p24	6	23%
Band gp160	17	65%
Band gp41	1	4%
Minor Bands (ex. band p18)	2	8%
total	26	100%

The observed percentage of a HIV-1 RNA (NAAT) reactive specimen with the gp160 band present is 65% compared to 23% of the reactive specimens 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 2: NAAT Nonreactive Specimens

Western Blot Bands Present	Number of specimens	Percentage
Band p24	72	74%
Band gp160	11	11%
Band gp41	5	5%
Minor Bands (ex. band p18)	10	10%
total	98	100%

The observed percentage of a HIV-1 RNA (NAAT) nonreactive specimen with the p24 band present is 74% compared to 11% of the nonreactive specimens with the gp160 band. The difference suggests when the p24 band is present on an indeterminate Western Blot confirmation there is a higher probability for a HIV-1 RNA (NAAT) result to be nonreactive.

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). Number of AHI detected (Per DCHHS definition): 61

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 Western Blot result on the confirmation.

Additionally, 22% of the HIV-1 RNA reactive specimens were found to have a dual diagnosis of syphillis, 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.

PUBLIC HEALTH FOLLOW-UP

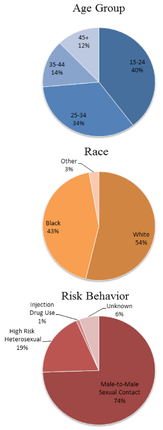
DCHHS Disease Intervention Specialists performed the public health follow-up (PHFU) for 69 of the 106 HIV-1 RNA reactive cases (65%). Other jurisdictions in Texas conducted PHFU for 34 cases (32%). Ninety-eight (98) cases were located and interviewed (92%), which resulted in the initiation of 205 partners 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 sexual 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



CONCLUSION

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 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 provides an opportunity to counsel the patient on prevention when the 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 Tables 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.

ACKNOWLEDGEMENTS: We would like to thank the Texas Department of State Health Services, (Dr. Nick Curry, Jim Lee, and Deborah Carr), Gen-Probe Inc. for the opportunity to attend the conference and endless of hours of technical support, Marisa Gonzales, and the DCHHS personnel for all their help.