Evaluation of an EIA-based Testing Algorithm in Antenatal Survey Using Dried Blood Spot Specimens from South Sudan

Hakim I[1], Patel HK[2], Duong YT[2], Katoro J[3], Gregory W[1], Farouk I[1], Hakim A[2], and Parekh B[2]

[1]National Public Health Laboratory, Ministry of Health, Juba, South Sudan; [2]Centers for Disease Control and Prevention, Atlanta, GA; [3]Centers for Disease Control and Prevention, Juba, South Sudan

ABSTRACT

BACKGROUND: Due to high sensitivity of enzyme immunoassays (EIA) some levels of false positivity is expected when used alone or in EIA-based testing algorithm for HIV diagnostic testing. This is problematic for ANC surveys where data are used for surveillance purposes as it can lead to inflated prevalence estimates. DBS are preferred specimen for HIV surveillance studies as they are simple, inexpensive, and can be prepared in non-laboratory settings. However, use of DBS specimens require modification and optimization of EIA procedure. Here, we present results from an EIA testing algorithm compared to the US Centers for Disease Control (CDC) external quality assurance (EQA) testing algorithm applied to specimens from the South Sudan 2012ANC surveillance survey. **METHODS:** DBS specimens were collected for South Sudan 2012 HIV Antenatal Clinic Survey and tested at the Juba Teaching Hospital Laboratory. A total of 1059 HIV-negative and 285 HIV-positive specimens were sent to CDC Atlanta for EQA testing. As part of the South Sudan testing algorithm, the specimens were screened on Vironostika Uni-form II plus EIA (Biomerieux, France) and reactives were confirmed by Murex HIV-1.2.O EIA (Abbott, Germany). The retesting at CDC was performed using an optimized DBS elution protocol on Genetic Systems HIV-1/2 plus O (GS 1-2-O, Bio-Rad, California) followed by confirmation of positives on Cambridge Biotech HIV-1 Western Blot (WB) (Maxim Biomedical, Maryland). Specimens with discrepant final classification between the two algorithms were further confirmed by WB. **RESULTS**: Of the 1344 of specimens tested, the overall agreement of the South Sudan and CDC algorithms was 97.5%; however, 97.2% and 89.1% agreements were observed amongst the negatives and positives, respectively. A total of 33 specimens (2.5%) were discrepant between the algorithms and of those, 29 (88%) specimens were negative and 4 (12%) specimens were indeterminate according to WB results. The optical density values of most false positive (FP) specimens were low on EIAs indicating inherent false positivity of EIAs that is magnified when DBS specimens are used. **CONCLUSION**: Our result suggests a high FP rate with EIA-testing algorithm alone. These results highlight the need to incorporate more specific assay such as WB or similar test in the testing algorithm to confirm the HIV status and ensure the accuracy of HIV prevalence data. Use of DBS specimens requires additional optimization steps to ensure accurate results.

INTRODUCTION

Use of highly sensitive 3rd and 4th generation EIA is common in surveys and DBS are preferred specimen for HIV surveillance as they are easy to prepare in the field and transport to central laboratory. However, both use of EIAs and DBS specimens pose some challenges in terms of accuracy of survey results. We present results from an EIA-based testing algorithm compared to the US Centers for Disease Control (CDC) external quality assurance (EQA) testing algorithm, that incorporates confirmatory testing, applied to specimens from the South Sudan 2012ANC survey.

METHODS

DBS specimens collected for the South Sudan 2012 HIV ANC Survey were tested at the Juba Teaching Hospital Laboratory. Of the 11,155 specimens collected in the ANC survey, 1059 HIV-negative and 285 HIV-positive specimens were sent to CDC Atlanta for EQA testing. As part of the South Sudan testing algorithm, the specimens were screened on Vironostika Uni-form II plus EIA (Biomerieux, France) and reactives were confirmed by Murex HIV-1.2.O EIA (Abbott, Germany). Retesting at CDC was performed using an optimized DBS elution protocol on Genetic Systems HIV-1/2 plus O (GS 1-2-O, Bio-Rad, California) followed by confirmation of positives on Cambridge Biotech HIV-1 Western Blot (WB) (Maxim Biomedical, Maryland). Specimens with discrepant final classification between the two algorithms were further confirmed by WB.

RESULTS

Figure 1. A flow chart describing comparison of EIA-based testing algorithm (left) and EIA/WB testing algorithm (right). Of the 1344 of specimens sent to CDC for EQA, 285 were reactive by EIA-based testing algorithm. CDC testing algorithm (EIA/WB) showed 269 EIA-reactive specimens of which 254 were confirmed as HIV-positives. A total of 33 specimens were discrepant between the algorithms and of those, 29 were negative and 4 specimens were indeterminate according to WB results.

Table 1. All EIA's had sensitivity of 100%, however, the specificity of GS 12O was 98.7% and 97.2% both for Vironostika and Murex EIAs (Table 1) for the samples tested, with PPV ranging from 95% to 91%.

			Predictive Values (%)	
Test Names	Sensitivity	Specificity	Positive	Negative
1. Gentic System HIV- 1 2 O	100	98.7%	95.1%	100
2. Vironostika Uni-form II Plus O	100	97.2%	90.8%	100
3. Murex HIV 1.2.O	100	97.2%	90.8%	NA

Table 1. Performance of various EIA tests on DBS specimens, true positive or true negative, as defined by Western blot

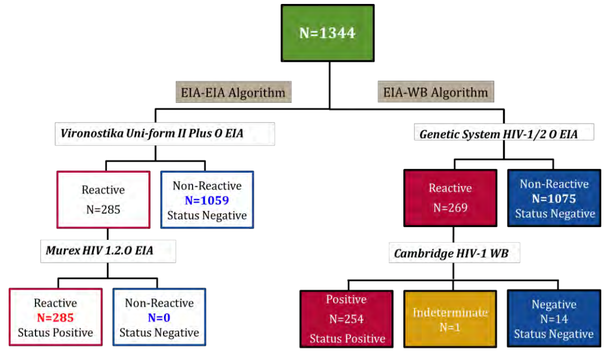
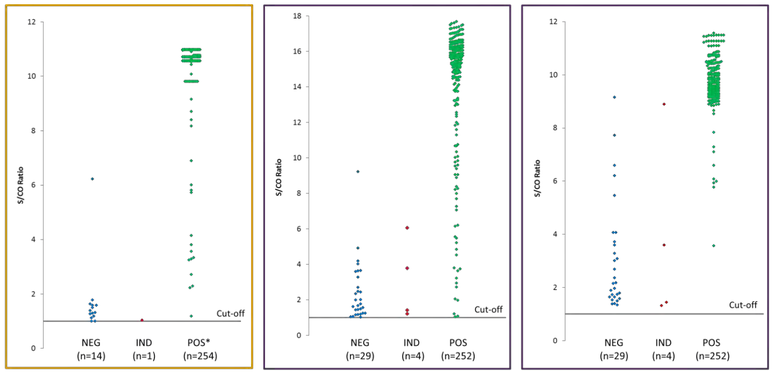


Figure 1. Results of HIV testing performed using EIA-based algorithm and EIA/WB algorithm on DBS specimens.

Figure 2. Distribution of EIA-reactive specimens based on their signal to cut-off ratio (S/CO) on each EIA plotted according to their final HIV status as negative, indeterminate and positive. HIV-positive specimens usually clustered with high S/CO ratio on all EIAs while HIV-negative or indeterminate specimens generally exhibited low S/CO ratios on EIAs. There were 14 false-positive (FP) specimens on GS 12O EIA that had low OD values, ranging from 0.10 – 0.58 with a mean of 0.165 and median of 0.138. The Vironostika EIA optical densities (OD) of the 29 FP specimens ranged from 0.17-1.74 with a mean and median of 0.445 and 0.317, respectively, while the Murex EIA had an OD range of 0.48–3.25 with a mean and median of 1.108 and 0.775, respectively. High OD values for some of the FP on Murex indicates complexity and lack of specificity of some EIAs.

Figure 2. Distribution of EIA positive samples according to Western Blot results for DBS specimens



CONCLUSIONS AND IMPLICATIONS

1. Our result suggests a high false positive rate when using only EIA-based testing algorithm in surveys.
2. These results highlight the need to incorporate a more specific assay such as WB or similar test (e.g. Geenius or Multispot) in the testing algorithm to confirm the HIV status and ensure the accuracy of HIV prevalence data.
3. Additionally, if the DBS specimens are used in surveys, additional optimization steps should be conducted with each EIA to ensure accurate results.
4. UNAIDS modeling data to estimate HIV burden, which rely on ANC or DHS surveys where EIA-based algorithm is routinely used, may be impacted by falsely elevated HIV prevalence amounting to 10%-20% of the positives.
5. This may have further implications on the first 90 as we strive to achieve UNAIDS 90-90-90 goals.

CONTACT INFORMATION

Hetal Patel, MSc. hpatel@cdc.gov
Bharat Parekh, Ph.D. bparekh@cdc.gov

Centers for Disease Control & Prevention
1600 Clifton Road, NE.
Atlanta GA 30329
404-639-4156

www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info
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