Evaluating HIV Ag-Ab Combination Assays for Agreement and Specificity

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Abstract

Background

We compared three HIV Ag-Ab combination assays that provide a single result without differentiating Ag from Ab reactivity and two assays that provide separate antigen-and antibody results for agreement and clinical specificity in a large community hospital setting.

Methods

Following IRB approval, we deidentified 618 consecutive patient specimens which were submitted for HIV testing. We also included 139 previously tested HIV negative specimens from patients with a variety of conditions, including Hepatitis B and C, CMV, EBV, and pregnant women to assess clinical specificity. We included 32 Zeptrometrix specimens. All specimens were tested with the Abbott ARCHITECT HIV Ag/Ab (4th gen) assay, the Bio-Rad GS HIV Combo Ag/Ab EIA (4th gen), , the Siemens ADVIA Centaur HIV Ag/Ab Combo (CHIV) (4th gen) Assay, the Abbott Alere Determine HIV Ag/Ab combo assay and the BioPlex 2200 HIV Ag-Ab (5th gen) assay. Repeatedly reactive specimens on any of the assays were tested on the Bio-Rad Geenius antibody differentiation assay.

Results

763/789 (96.7%) specimens agreed on all assays
48/789 (6.1%) had at least 1 reactive result.
26/48 (54.2%) reactive specimens agreed on all methods
136/139 (97.8%) specimens from the cross-reactivity sample were negative on all methods.
22/23 reactive patient specimens were reactive for HIV-1 antibodies with the Geenius™ differentiation assay.
17/22 (77%) reactive specimens that disagreed were low positives on the quantitative method(s) that were reactive
6 reactive specimens that were Geenius negative were BioPlex antigen positive. 4/6 were Alere antigen positive.
3 specimens that were HIV Ag repeatedly reactive on the BioPlex assay were negative on all other assays

Conclusions

Fourth and Fifth generation assays perform similarly on strongly positive specimens. The BioPlex and Alere assays identify antigen reactivity specifically which must be taken into account in developing a new algorithm. Antigen sensitivity appears greater on the BioPlex than the Alere. Weakly reactive specimens on one assay may be negative on other methods. Our data support using more than one antigen/antibody testing method on low reactive specimens while suggesting that strongly reactive specimens may not benefit from additional testing. PCR testing would be useful for antigen reactive only specimens.

Introduction

- A weakness of diagnostic HIV antibody assays has always been the "antibody negative window", i.e. the time between viral exposure and a positive test. The early HIV tests had a window period approaching 8-12 weeks, while the third generation HIV antibody tests decreased the test negative window to approximately three weeks post infection. The p24 antigen assay could detect infection approximately 2 weeks post exposure, however, the test would become negative following antibody production. The fourth generation HIV test combines HIV antibody and antigen detection into a single result and a specific algorithm for confirming reactive results has been developed. Summa Health has been performing the 4th generation HIV assay using the Abbott Architect method since December of 2010. When the fifth generation procedure which also detects both HIV antibody and antigen but provides separate results for each component, became available, we decided to compare that procedure to the available 4th generation assays to determine which procedure would be most approbation for our laboratory. At present, no specific algorithm is available for the fifth generation procedure.
- We compared the available fourth and fifth generation HIV assays on a cohort of clinical specimens and specimens with known reactivity toward HIV and other diseases. The project received expedited review approval from the Summa Health IRB.

HIV Combo Method	Seroconversion Panels Viral load of 1st reactive specimen	Specificity (n=139)
Abbott Architect	101,800 copies/ml	97.8%
Alere Card Test	101,800 copies/ml	97.8%
BioPlex 2200	15,280 copies/ml	97.8%
Bio-Rad GS Combo EIA	101,800 copies/ml	95.0%
Siemens Centaur	101,800 copies/ml	97.8%

Materials and Methods

Specimens

618 Consecutive Summa patient specimens were deidentified prior to being run in this study.
139 HIV negative specimens were provided by Bio-Rad to assess specificity. 89 of these specimens had potential interfering substances or antibodies. The specific characterizations are below

Normal Blood Donors	50
EBV +	10
CMV +	10
HCV +	10
HBV sAb+	10
HAV +	10
HTLV +	8
hCG >100	10
HSV 1 +	10
RF +	11

We also tested 32 Zeptometrix specimens from 3 separate HIV seroconversion panels, HIV 9012 (8 specimens), HIV 9018 (11 specimens) and HIV 12008 (13 specimens)

Assays

Abbott (Architect) HIV Ag/Ab Combo, predicate method at Summa (4th Gen)
Bio-Rad GS HIV Combo Ag/Ab EIA (4th Gen)
Abbott Alere HIV Ag/Ab Combo Card test (4.5 gen)
Siemens Centaur HIV Ag/Ab Combo (4th Gen)
BioPlex 2200 HIV Ag/Ab Assay (5th gen)
Bio-Rad Geenius HIV 1/2 Supplemental Assay

The Siemens Centaur assay was performed at the Louis Stokes VA Medical Center in Cleveland, OH. All other assays were performed in the Dept. of Pathology and Laboratory medicine at Summa health Akron City Hospital in Akron, OH.
Bio-Rad Assays , the BioPlex 2200 and the Geenius instrument were provided by Bio-Rad. The remaining assays were purchased from the manufacturers.
All assays were performed according to manufacturers instructions. Reactive specimens were repeated in duplicate and, if repeatedly reactive, assayed on the Bio-Rad Geenius HIV 1/2 Supplemental assay, as recommended in the CDC's HIV 4th generation algorithm

Results

Overall reactivity- 48/789 (6.1%) had at least 1 reactive result with one or more of the testing methods evaluated, 26 (54.2%) of these were reactive on all specimens. 12/15 (80%) of reactive specimens that disagreed were low positives on the quantitative method(s) that were reactive.;
Clinical Specimens- 22/618 (3.6%) clinical specimens were repeatedly reactive on all testing methods and were HIV-1 reactive on the Geenius. 1 additional clinical specimen was ELISA repeatedly reactive only and was negative on the Geenius. 13 specimens were initially reactive on the ELISA but negative upon repeat duplicate testing. Those were negative on all other assays. 2 Specimens were low reactive on the Centaur and negative on all of the other assays.
Specificity using specimens with potential Interfering substance- 3 specimens were reactive on all assays, including the Geenius; 2 of these were from documented Hepatitis C antibody positive patients and 1 from a documented Hepatitis A positive specimens. The possibility that these specimens represent true positive should be considered. An additional 4 specimens were reactive only on the ELISA test. 2 of those were from normal blood bank donors, one was positive for HSV-1 IgG antibody and one was rheumatoid factor positive.
Zeptometrix seroconversion specimens- the 3 Zeptometrix seroconversion panels had 8-13 specimens in each panel. Viral load values for specimens ranged from undetectable to >750,000 copies/ml. The BioPlex 2200 assay became positive 2-3 days prior to the other assays (see the table to the left).
P24 specific antigen reactivity- 6 Specimens that were reactive on all of the tests except the Geenius showed antigen reactivity on the BioPlex. 4 of those specimens showed antigen reactivity on the Alere

Method Strengths and Weaknesses

The fourth generation HIV assays were developed to reduce the test negative window following infection. A weakness of the fourth generation's assays is the inability to differentiate antigen from antibody reactivity. This "combination" result led the CDC to develop a 3 step algorithm to be used on 4th gen reactive specimens that are antibody follow-up test negative. The 4th gen automated methods performed similarly in our test, with low positives being found on a small percentage of each assay. The Alere card test does differentiate antigen from antibody results, although its antigen sensitivity was below the BioPlex's level. While the card assay is useful for rapid exposure or resource limited testing, it is not a practical procedure for routine testing in high volume laboratories. The 5th generation assay is an automated procedure that does differentiate Ag from Ab reactivity and HIV-1 Ab from HIV-2 Ab, requiring a new algorithm. We see no need to perform an antibody differentiation assay on a specimen that is only antigen positive, for example. Our assay did not address whether or not molecular testing may be helpful on specimens that are 5th gen antibody positive yet antibody supplemental testing negative. In addition to assay performance, however, the choice of an assay (or assays) to be used in any specific laboratory may be dictated by cost, available personnel, turnaround time concerns and available equipment.

Conclusions

- Fourth and Fifth generation HIV assays perform similarly on strongly positive specimens.
- The ELISA assay had the lowest specificity in our sample and the highest number of low level, non-repeatedly reactive results.
- Specific Antigen sensitivity was greater on the BioPlex than the Alere test .In the seroconversion panels, the BioPlex method became positive 2-3 days earlier than the other methods.
- Weakly reactive specimens on one assay may be negative on other HIV tests, suggesting the possibility of false positive results.
- Our data support using more than one antigen/antibody testing method on low reactive specimens while suggesting that strongly reactive specimens may not benefit from being assayed by additional antibody or antigen testing methods. Patients with strongly reactive results on one of the quantitative methods may benefit by being triaged immediately to an appropriate clinic or physician for CD4 and HIV viral load testing.

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