Evaluating HIV Ag-Ab Combination Assays for Agreement and Specificity
Thomas S. Alexander, Robert Pantaleon-Vasquez, Linda Define, Gary Dixon, Jackie Beach and M. Qasim Ansari. Dept of Pathology and Laboratory Medicine, Summa Health, Akron, OH and the Louis Stokes VA Medical Center, Cleveland, OH

Materials and Methods

Specimens
618 Consecutive Summit patient specimens were deidentified prior to being run in this study. 139 specimens were included with the Abbott Architect (4th Gen) assay, the Bio-Rad Abbott G6 Ag/Ab Combo assay, the Siemens Centaur G6 Ag/Ab Combo assay and the BioFlex 2200 HIV Ag/Ab Assay. All assays were performed in the Dept of Pathology and Laboratory Medicine at Summit Health Akron City Hospital in Akron, OH. All assays were performed in the CDC 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 (56.7%) of these reactives were on all Summit specimens. 12/13 (92%) of reactive specimens that were discordant (reactive in 1 test and non-reactive in another) in the SIEMENS Centaur G6 Ag/Ab Combo assay had 8-13 specimens in each panel. Viral load specimens for values ranged from undetectable to >750,000 copies/ml. The BioFlex 2200 assay became positive 2-3 days prior to the other assays (see the table to the left). 324 specific antigen reactivity. 6 Specimens that were reactive on all of the tests except the G6 assay showed antigen reactivity on the BioFlex. 4 of these specimens showed antigen reactivity on the Alera.

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 its inability to differentiate antigen from antibody reactivity. This "combination" result led to the development of 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 study, with low positives being found on a small percentage of each assay. The Alera card test does differentiate antigens from antibody reactivity, although its antigen sensitivity was below the BioFlex 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 4th 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 a antibody supplemental testing negative. In addition to our assays, we only 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-reactively reactive results.
• Specific Antigen sensitivity was greater on the BioPlex than the Alera 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 benefit from being assayed by additional antigen or antibody 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.

Acknowledgement

We thank Bio-Rad, Inc for providing the BioFlex 2200 instrument, Bio-Rad assay kits and financial support for the study.

Abstract

We compared four HIV Ag-Ab combination assays that provide a single result without differentiating Ab and Ag to determine antigen and antibody results for agreement and disagreement in a large community hospital setting.

Introduction

A variety of diagnostic HIV antibody assays have always been the "antibody negative window," i.e. the time between viral exposure and a positive test. The early HIV tests had a window of undetectability approaching 8-12 weeks, while the third generation HIV antibody tests decreased the test negative window to approximately three weeks post infection. The 2nd generation assay could detect infection approximately 2-3 weeks post exposure, however, the test would become negative following viral latency and antibody resolution. The fourth generation HIV test combines HIV antibody and antigen detection into a single result and a specific algorithm for confirming reactivity has been developed. Summit Health has been performing the 4th generation HIV assay using the Abbott Architect method since December of 2010. When the 4th generation algorithm was released in 2010, the 2nd generation assay was no longer considered to be adequate for this laboratory. At present, no specific algorithm is available for the fifth generation assay.

We compared the available fourth and fifth generation HIV assays on a cohort of clinical specimens and specimens from previous laboratory research on viral load and other diseases. The project received expedited review approval from the Summa Health IRB.

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 its inability to differentiate antigen from antibody reactivity. This "combination" result led to the development of 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 study, with low positives being found on a small percentage of each assay. The Alera card test does differentiate antigens from antibody reactivity, although its antigen sensitivity was below the BioFlex 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 a antibody supplemental testing negative. In addition to our assays, we only 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.

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