Extending HIV Avidity Recency Detection by Addition of p24 Ag Detection

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INTRODUCTION

Currently available assays for detecting recent HIV infection generally utilize characteristics of the humoral antibody response.

This means that specimens have to be HIV antibody positive before they can be subjected to a recency assay despite HIV specific IgG antibody being one of the last markers of HIV infection to appear.

The ability to determine the presence of low avidity HIV antibody, which is a marker of recent HIV infection, within an assay which can also identify HIV p24 Ag, allows both the early antigen signal and the low avidity antibody to be used to determine the period in which recent infection can be identified.

Using an FDA licensed assay we demonstrated that alongside the excellent diagnostic performance of the assay, the assay could be modified to identify recent HIV infection.

Uniquely, the ability of the assay to distinguish antibodies from HIV-2 from HIV-1 meant that we could confirm that the specimens tested were anti-HIV-1 positive (currently there is no incidence assay validated for HIV-2) and identify antibody negative but p24 Ag positive individuals who are truly recently infected but would normally be discarded by current HIV incidence assays due to the lack of antibody.

METHODS

Specimens were tested using the BioPlex® 2200 HIV Ag-Ab assay.

This '5th generation' assay allows separate detection and identification of anti-HIV 1, anti-HIV-2 and HIV p24 Ag unlike 4th generation assays which give a single result.

An antibody avidity modification using 1M guanidine allowed determination of an avidity index.

Various aspects of assay performance was investigated including:

1: Its diagnostic performance on specimens from an HIV reference laboratory.
2: The effect of chaotrope on the p24 Ag component of the assay.
3: Development of an avidity index in seroconversion panels and use of p24 Ag to lengthen recency period.
4: Determination of an avidity index in specimens of known periods of infection and comparison to a market leading incidence assay in clinical specimens (HIV Limiting Antigen - LAg).

Diagnostic Performance

Sensitivity

HIV-1: 100% Sensitivity
95% CI: 98.7 – 100%
284 reactive / 284 tested

HIV-2: 100% Sensitivity
95% CI: 84.6 – 100%
22 reactive / 22 tested

Specificity

Initial Reactive Rate 0.5%
95% CI: 0 – 2.9%
1 reactive / 191 tested

Final Reactive Rate 0%
95% CI: 0 – 1.9%
0 / 191

P24 Ag detection – compared to standalone p24 Ag assay

Category	Total tested	Number positive by BioPlex
P24 only	15	14

Figure 1. Diagnostic performance of BioPlex® 2200 HIV Ag-Ab assay

RESULTS

Figure 1 summarizes the diagnostic performance of the BioPlex® 2200 HIV Ag-Ab assay.

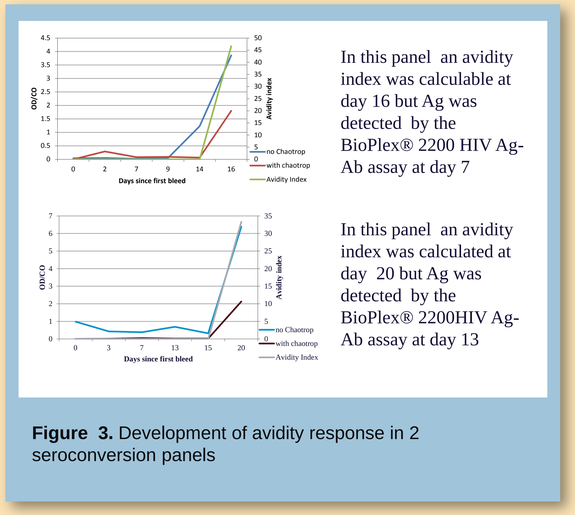
In a clinical environment the assay showed excellent performance with 100% sensitivity for HIV-1 and HIV-2 and a final specificity of 100%

When compared to a standalone p24 Ag assay the BioPlex® 2200 HIV Ag-Ab detected 14 of 15 antigen only positive specimens

Antigen signal w/o chaotropic Agent	Antigen signal with chaotropic Agent
27.53	20.48
34.27	21.86
184.13	99.83
2.32	2.17

Figure 2. Reduction in signal following addition of chaotropic agent

- Avidity modifications of assays often involve a pre-dilution of the specimen (usually1:10) in PBS for the control well and a chaotropic agent for the test well
- Dilution of specimens for avidity modifications reduces but does not eliminate the p24 Ag signal. For those using 4th generation assays where the antigen signal is not differentiated from the antibody signal, users should be aware that an antigen signal may be contributing to their avidity index.
- Addition of a chaotropic agent (in this case 1M guanidine) leads to an average reduction in signal compared to a PBS diluent of approximately 28% (Figure 2)
- Figure 3 examines the development of an avidity index in two seroconversion panels.
- In the first panel detectable antibody signal was not detected until day 14 and an avidity index could not be calculated until day 16. However p24 Ag was detected in this specimen panel on day 7 showing that the use of the BioPlex® 2200 HIV Ag-Ab assay added 9 days to the duration in which recent infection could be determined
- In the 2nd panel confirmed detectable antibody was present in some assays at day 13 but a reliable avidity index could not be confirmed until day 20. A p24 Ag signal was detected at day 13 which meant that using p24 Ag lengthened the duration that recent infection could be identified by 7 days
- Figure 4 shows the comparative performance of the BioPlex® 2200 HIV Ag-Ab modified avidity assay against the market leading HIV incidence assay
- When compared with specimens with known longstanding infection and treatment-naive the assay correctly identified them as longstanding.
- The BioPlex® 2200 HIV Ag-Ab modified assay correctly identified 14 of 15 specimens as recently infected. One specimen could not be classified as its reactivity fell below the limit of detection of the assay suggesting that in assays where an additional dilution is made specimens with very low antibody titers may not be detected.



In this panel an avidity index was calculable at day 16 but Ag was detected by the BioPlex® 2200 HIV Ag-Ab assay at day 7

In this panel an avidity index was calculated at day 20 but Ag was detected by the BioPlex® 2200HIV Ag-Ab assay at day 13

Figure 3. Development of avidity response in 2 seroconversion panels

When evaluated using specimens received in a national reference laboratory the BioPlex® 2200 HIV Ag-Ab assay had similar performance to that of the market leading HIV incidence assay. The specimens evaluated have no background information attached to them so it is not possible to determine whether the LAg or BioPlex® 2200 HIV Ag-Ab result is 'correct'.

Using an avidity index cut-off of 80% the assay had a >95% concordance with the LAg avidity assay.

Of the 4 specimens that were mismatched from the clinical cohort two were very close to the cut-off suggesting that refinements to the assay cut-off may improve concordance with the LAg assay.

Currently we have no data that examines whether there is a HIV subtype effect on the performance of the modified avidity assay.

Similarly we do not have any data on whether antiretroviral treatment has a negative impact on the performance of the avidity assay

Category	LAg Avidity	BioPlex® 2200HIV Ag-Ab
Known longstanding infections	35	35
Known recent infections	15	14*
Clinical specimens identified as recent by assay	20	24
Clinical specimens identified as longstanding by assay	47	43

* One Positive specimen when diluted for avidity testing fell below assay limit of detection

Figure 4. Comparison of LAg avidity and BioPlex® 2200 avidity results

DISCUSSION

A major drawback of routine HIV incidence testing is the need to confirm serology on specimens that have low reactivity due to low antibody levels in your incidence assay.

Furthermore, failure to add a specimen will lead to a false result and may not be readily detected in some assays without further screening.

In addition specimens from those infected with HIV-2 need to be removed from your evaluation and those with no antibody signal but potentially recently infected and p24 Ag positive are routinely removed from evaluations.

The assay described here while developed as a diagnostic screening test it is able to identify specimens for recency testing. Moreover, it is able to identify those specimens that should be excluded from incidence testing, such as those with HIV-2 (this can be performed as part of the normal use of the platform and obviates the need for a further discriminatory assay). Sample addition verification confirms the presence of specimen during testing and the identification of p24 Ag-only positive samples adds to the pool of true recents detected. This assay has the potential to expand the use of Incidence testing into routine laboratory use.

CONCLUSIONS

- BioPlex® 2200 HIV Ag-Ab assay has a sensitive and specific HIV diagnostic capability and potential to allow HIV recency discrimination on the same platform.
- Initial results show performance comparable to the market leading incidence assay in those anti-HIV positive specimens.
- However, the ability to identify Ab negative/Ag positive specimens increases the duration in which 'true' recent infection can be determined. This increases duration of detectable recent infection by up to two weeks but importantly identifies 'true' recent infections that may otherwise be discarded.
- Our data demonstrate that assays containing an antigen detection component are compatible with use in HIV incidence methodologies but that users should be aware of the impact of a signal from that antigen component may have on their assay.
- The ability of this assay to be able to provide specific diagnostic differentiation of HIV-1 from HIV-2 and p24 Ag, and, with modification, to identify recent HIV infection may change HIV diagnostic algorithms to allow the incorporation of recency testing into routine practice.
- Further work needs to be performed to determine the avidity-modified assay false recent ratio and the duration in which it accurately categorises recent infection. In addition, evaluation of known confounding factors of HIV incidence assays must be evaluated for this assay.

ACKNOWLEDGEMENTS

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