Multiplex Assay for Concurrent Diagnoses and Detection of HIV-1, HIV-2, and Recent HIV-1 Infection in a Single Test

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ABSTRACT

Reliable assays that can accurately distinguish recent (occurring within the past year) from long-standing (>1 year) HIV infections are crucial for providing up-to-date information on HIV transmission dynamics in a population. However, often these efforts are confounded by inaccurate HIV-1 diagnosis and the presence of HIV-2 in the population being surveyed. Our study describes the detection of recent HIV-1 infection in a single assay.

HIV-1 and HIV-2 serotyping were accomplished by coupling beads with an HIV-1 p24-gp41 fusion protein and HIV-2 peptide from gp120 immunodominant region, respectively. HIV-1 recent infection detection was accomplished by coupling beads with limiting amounts of mouse-rat anti-HIV-1 immunodominant proteins, reconstituted immunodominant region, group M-395-655. Also, Multispot (for HIV-2) and LAg-Avidity EIA (for recency classification) were further performed to classify specimens as recent or long-term using the LAg-Avidity EIA.

The specimens had a diagnostic ability to diagnose HIV infection, perform serotyping and separate recent from long HIV infections, all in a single assay. It is the first test to have the potential to significantly reduce variance by reducing the multiplex steps that are otherwise required.

MATERIALS AND METHODS

Specimens:
- Included stored plasma samples from our specimen bank collected over a period of 10 years from various sources including the blood bank and commercial sources.
- The specimens were characterized by 3rd generation Gen-Probe EIA + Western blot detection. Multispot HIV-1 for serology, with additional characterization of multiple HIV-1 positive specimens as recent or long term using the LAg-Avidity EIA.

Sample preparation:
- All specimens were equilibrated at room temperature (-16°C, except the EIA).
- Bead coupling was performed as recommended by the bead manufacturer with slight modifications.

Antigen:
- After coupling, the supernatant was removed and the mixed beads washed twice with PBS (pH 7.2, 150 mM NaCl).
- Twenty microliters of serum or buffer containing R1/2 were added onto 50,000 beads for 20 minutes at room temperature.

Plasma samples were diluted 1:50 in assay buffer and added onto the plate wells containing the beads, then incubated and washed prior to staining for 1 hr at room temperature.
- All specimens were analyzed on a plate reader. Data were acquired using the Luminex MagPix instrument according to the manufacturer’s instructions with the background of each bead region determined from wells with no sample added.

RESULTS

Table 1. Parameter panel used for the development, optimization and evaluation of the Multiplex Assay: 1-Specificity of the Multiplex Assay.

Table 2. Diagnostic performance of the diagnostic and serotyping components of the multiplex assay with results from the standard algorithms (X-DA, X-Late and X-Recent) and the performance of the multiplex assay at a cut-off of 1000 MFI compared with the LAg-Avidity EIA at a cut-off of 1.5 ODn for positive (X-7OS).

Figure 1. Schematic representation of the Multiplex Assay. The scheme shows the serological parameters and logical approach that was used for diagnostics, serotyping and recent infection determination and the antigen used for each parameter.

Figure 2. A scatter plot showing multiple results for panel 4 (HIV-1 Recent) demonstrating effective separation of specimens and distribution of NFT using beads coated with p24-gp41 (left), HIV-2 IDR (middle) and rIDR-M (right). The results show the tentative cutoffs that separate the two groups within each parameter and the middle horizontal line within each group indicates the mean value for the group. NFI, net fluorescence intensity.

Figure 3. A scatter plot showing the distribution and antibody reactivity levels of p24-gp41 (left), HIV-2 IDR (middle) and rIDR-M (right) with Panel 4 (X-7OS). The horizontal line (black) shows the tentative cutoffs that separate the two groups within each parameter and the middle horizontal line within each group indicates the mean value for the group.

Figure 4. A) Comparing the performance of the diagnostic and serotyping components of the multiplex assay with results from the standard algorithms (X-DA, X-Late and X-Recent) and the performance of the multiplex assay at a cut-off of 1000 MFI compared with the LAg-Avidity EIA at a cut-off of 1.5 ODn for positive (X-7OS). These show in red represent cutoff results.

Figure 5. A scatter plot showing the distribution and antibody reactivity levels of p24-gp41 (left), HIV-2 IDR (middle) and rIDR-M (right) with Panel 4 (X-7OS). The horizontal line (black) shows the tentative cutoffs that separate the two groups within each parameter and the middle horizontal line within each group indicates the mean value for the group.

Figure 6. Three-dimensional representation of the multiplex data for panel 4 (X-7OS) showing the performance of the different analytes using cut-offs of 2500 (HIV-1 recent infection positive, negative). 2000 MFI for HIV-2 positive and negative specimens; and 1250 MFI U for recent and long-term infection detection. Three-dimensional plot with shadow on the wall (red arrow above) for proper visualization of the separations between the different parameters. The z-axis MFI U is represented as 1, 10, 100, 1000, 10,000, 100,000.

CONCLUSIONS

- The study shows that HIV diagnosis, serotyping and separation of recent from long-term infections can be simultaneously performed in a single assay in a single well.
- The assay is robust and precise with low inter- and intra-assay variability.
- Simultaneous classification of individuals as positive or negative, with HIV-1 on HIV-2, HIV and HIV-1, recent or long term would have a major implication in HIV surveillance.
- This methodology is suitable for adding new biomarkers; therefore it presents a great opportunity to reposition/multiply multi-disease detection.

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