Performance of the Maxim Limiting Antigen Avidity EIA for use with Dried Blood Spot Specimens

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BACKGROUND

- Limiting Antigen (LAg) Avidity EIA is a single-well, avidity-based HIV-1 incidence assay that is commercially available from two manufacturers, Sedia Biosciences and Maxim Biomedical.
- The assay was validated using plasma/serum and was shown to accurately estimate HIV-1 incidence from cross-section specimens.1

OBJECTIVES

- To assess the performance of the Maxim LAg Avidity EIA DBS Kit which has DBS controls and calibrator specimens
- To determine the correlation of matched DBS and plasma/serum specimens on the Maxim LAg Avidity EIA and compare the LAg Avidity EIA classification of DBS and plasma/serum specimens
- To determine the inter-operator variability and reproducibility of the Maxim LAg Avidity EIA

METHODS

A total of 447 matched plasma and DBS specimens were used for this evaluation. Of these, 133 plasma units were purchased from Boca Biosciences (Concord, CA) and 314 from SeraCare Life Sciences (Westport, MA). Matched DBS were prepared by spotting 100 μl of mock blood (plasma mixed 1.1 with packed red blood cells) onto Whatman 903 Filter paper (Whatman plc, United Kingdom). The remaining 225 specimens were collected as unidentifiable random whole blood specimens from HIV-positive patients of the Atlanta-based AIDS drug and matched DBS and plasma were prepared by spotting whole blood onto Whatman 903 Filter paper followed by plasma separation. For DBS specimens, 505 μl of specimen-dissuent was used to elute antibodies overnight from a single 6 mm punch and 100 μl was added to the assay plate. Matched plasma/serum and DBS specimens were assayed on the same plate along with the corresponding DBS and plasma controls. All specimens were tested independently by two operators using the Maxim HIV-1 LAg Avidity EIA (Rodsville, MA) according to the manufacturer’s instructions. Raw OD values were inputted into the LAg Avidity EIA data management tool where validity of plasma/serum controls (negative control [NC], calibrator [CAL] low positive control [LPC], and high positive control [HPC]), normalized OD values (ODn), and mean/long-term classification for samples tested were determined and reported. ODn values were calculated using the following formula: raw specimen OD/Median CAL OD. The cut off for determining recent/long-term classification was 1.5 ODn.

RESULTS

A total of 447 matched DBS and plasma/serum specimens were assayed on the Maxim LAg Avidity EIA. A 6mm punch from DBS specimens was eluted in 500 μl of sample diluent overnight at 4°C and 100 μl of DBS or plasma/serum dilution and corresponding controls were added to the assay plate the following day. The LAg Avidity EIA was performed according to the manufacturer’s protocol. Data represent the ODn of two independent runs performed by two operators. The blue lines represent the assay cut-off (1.5 ODn) defining recent and long-term HIV-1 infection.

CONCLUSIONS

- Correlation between matched DBS and serum/plasma specimens was excellent (R² = 0.94).
- The respective serum/plasma and DBS Maxim LAg Avidity EIA kits each classified matched specimens with high concordance (88.7%) with both kits identifying 85 recent and 362 long-term infections.
- Reproducibility of HIV positive DBS controls and inter-operator variability both had CV<10% for ODn values
- This evaluation demonstrates the Maxim LAg Avidity DBS kit performs similarly to the Maxim LAg Avidity serum/plasma kit, allowing DBS specimens to be used in surveillance activities for the estimation of HIV incidence.