**Abstract**

Patients who have recently been infected with HIV contribute disproportionately to the spread of the disease. Viral loads are high in the first few weeks after infection, and newly infected patients are unlikely to be aware that they are infected and can spread the disease to others before they are diagnosed. Therefore, early detection of acute HIV infection is of great importance for public health. PCR methods are the gold standard with respect to sensitivity, they can detect as few as 60 HIV RNA copies per mL of serum or plasma (30 virus particles per mL). However, PCR technology is complex and expensive, and therefore not suitable for all settings. Immunoassays are simpler and cheaper, but the detection limit of current, 4th generation p24 immunoassays is only about 10 pg/mL or approximately 250 million capsid proteins per mL. On a per virus basis, these immunoassays are several thousand times less sensitive than PCR testing, despite the fact that there are about 2,000 p24 capsid proteins per virus.

A next-generation electrochemiluminescence assay format based on MSD’s MULTIARRAY™ technology was developed and its performance characterized. The detection limit for this novel p24 immunoassay was approximately 1 fg/mL – 10,000 fold more sensitive than current p24 immunoassays. A sensitivity of 1 fg/mL corresponds to less than 1 virus particle in our sample volume of 25 µL. The lower and upper limits of quantitation were 3 fg/mL and 38,000 fg/mL, respectively. Within-plate CV was 7%, and total CV 13%. Spike recovery and dilution linearity were between 89% and 120%. p24 was undetectable in the serum of plasma of 32 apparently healthy donors. A Seracare p24 “Mixed Titer Panel” (12 samples) showed good correlation between our p24 assays and commercial p24 immunoassays. Two seroconversion panels were tested: Seracare PRB948 (days 0 and 18, PCR negative; days 22 and 23, PCR positive) and PRB982 (days 0 and 18, PCR negative; days 7, 9, 14, and 17, PCR positive). In both cases, the MSD® p24 assay result was negative for all PCR-negative samples and positive for all PCR-positive samples, and infection was detected well before conventional p24 immunoassays.

In conclusion, we developed a next-generation p24 immunoassay that is 10,000 times more sensitive than the current limits of p24 ELISAs and comparable in sensitivity to PCR assays. The assay does not require specialized equipment and can be run on the MesoQuickPlex SQ 120, and all Meso SECTOR Imagers.

**Methods**

Meso Scale Diagnostics (MSD)’s electrochemiluminescence detection technology uses SULFO-Tag™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTIARRAY and MULTI-SPOT microplates. We developed the S-PLEX assay platform, a next-generation MULTIARRAY technology with significantly higher sensitivity. S-PLEX assays do not require specialized equipment and can be run on the MesoQuickPlex SQ 120, and all Meso SECTOR Imagers.

**Electrochemiluminescence Technology**

- Minimal non-specific background and strong responses to analyze yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

The performance of the HIV p24 assay was characterized. Essentially all experiments had the following plate layout:

- Point-symmetrical plate layout; calibrators, QC samples and unknowns measured in duplicates.
- 7 calibrator levels + zero calibrator, 7x serial dilutions.
- 3 QC samples spanning the assay range and a plasma pool control (QC-4).

Performance characterization included determination of limit of detection, upper and lower limit of quantitation; within plate and total reproducibility, spike recovery and dilution linearity. Serum and plasma samples from apparently healthy donors and from well-characterized HIV patients were tested.

**Assay Range**

![HIV p24 Calibration Curve](image)

**Spike Recovery, Dilution Linearity**

![Spike Recovery Chart](image)

**Seroconversion**

Three serum samples, EDTA plasma samples, and heparin plasma samples from apparently healthy donors were spiked with calibrator at three concentrations. The assay does not require specialized equipment and can be run on the MESO QuickPlex SQ 120, and all Meso SECTOR Imagers.

**Reproducibility**

Six plates were run over a period of 10 days. Each plate included an 8-point calibration curve (duplicates) and two replicates of each of four QC samples. The plate layout was point-symmetrical with calibrators in columns 1 and 12, and QC samples in columns 2 and 11. Total CV ranged from 5% to 17%.

To assess within-plate reproducibility, one 96-well plate was run at a single mid-range calibrator concentration. Within-plate CV was 7%.

**Serum/Plasma from Cases and Controls**

Serum and plasma samples from 32 apparently healthy donors were tested. All measured HIV p24 concentrations were below the detection limit (4 fg/mL in this experiment). The table above shows the ECL range. The table on the right shows data obtained with a SeraCare p24 “Mixed Titer Panel” (12 samples). Columns 2, 3, and 4 show results reported by Seracare for three commercial assays (Numbers marked in red are positive). Columns 5 and 6 show results obtained with the S-PLEX assay. S-PLEX results correlate well with results obtained using commercially available methods.

The two tables below show results for two Seroconversion panels obtained from Seracare. Each panel contains a series of plasma samples from a single donor before and after HIV seroconversion, and results from five or six commercial HIV assays (four p24 immunoassays and a Roche PCR assay). Numbers marked in red indicate a positive result. The last two columns show p24 concentrations and ECL counts for the S-PLEX assay. For both seroconversion panels, the S-PLEX assay is as sensitive as PCR: turning positive between day 18 and day 20 for panel 1, and between day 2 and day 7 for panel 2.

**Conclusion**

We developed a next-generation p24 immunoassay that is 10,000 times more sensitive than the current limits of p24 ELISAs and comparable in sensitivity to PCR assays. The assay does not require specialized equipment and can be run on the MesoQuickPlex SQ 120, and all Meso SECTOR Imagers.