# High-Sensitivity Immunoassays for IVD Applications: Evaluation of the Analytical Performance Characteristics of the Simoa HD-1 Automated Platform

*V. Benoit, B. Seignères, M. Lesénéchal, N. Cayet, C. Tourneur, M. Richard, M.C. Bastide, C. Pretis, C. Pease, B. Beyaert, J.M. Dugua, M. Hausmann*

*bioMérieux S.A., Marcy l'Etoile, France*

## Principle of Simoa Technology

In a Simoa assay (Figure 1), analyte protein molecules are captured onto antibody-coated paramagnetic beads, the captured proteins are labeled with an enzyme label, and individual beads are isolated and sealed in arrays of femtoliter wells in the presence of a fluorogenic enzyme substrate.

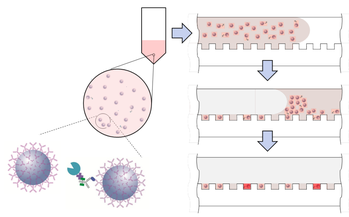

*Figure 1. Basic principle of Simoa digital immunoassay technology.*

Confining individual labeled immunocomplexes and substrate in fL-sized wells allows to overcome the sensitivity limitations stemming from diffusion and dilution in conventional (analog) immunoassays, and can provide a sensitivity enhancement of up to 1000-fold over traditional ELISA.

High-resolution fluorescence imaging of Simoa beaded arrays is used to determine both the fraction of beads associated with at least one enzyme and the fluorescence intensity from each well. The Simoa unit of measurement is the average number of enzymes per bead (AEB), calculated using Poisson's distribution law at ultra-low analyte concentrations (digital readout mode) or the average fluorescence intensity at higher concentrations (analog readout mode).

## The Simoa HD-1 System

The Simoa HD-1 floor-standing analyzer (Figure 2) is a fully-automated instrument (samples in, results out) that integrates microbead-based ELISA robotics with a Simoa imaging module. The system consists of five main functional areas: (1) input bays for addition of disposables (tips, assay cuvettes, array discs), reagents, and samples; (2) a system bay for onboard storage of liquid resources and waste handling; (3) the user control interface (computer and touch-screen monitor); (4) a module for performing immunological reactions (incubation and wash steps); and (5) the Simoa load-seal-image (LSI) readout module for the processing of Simoa arrays. Pipetting operations are provided by two x-y-z pipettors: a disposable-tip pipettor for sample handling and transfer of bead-substrate solution to the array disc, and a fixed-tip pipettor for reagent pipetting functions.


*Figure 2. The Simoa HD-1 immunoanalyzer (Research Use Only version).*

The instrument is designed to minimize operator intervention. Results are automatically calculated and can be displayed in real time upon completion of each sample's assay. The touch-screen provides the user with access to all instrument functionalities, including run setup, system status overview and result reports. The system accommodates 5 mL, 7 mL, and 10 mL primary tubes as well as 1 mL pediatric sample cups.

## References

Clinical Laboratory Standards Institute, Document EP6-A, **2003**, 23 (16).
Clinical Laboratory Standards Institute, Document EP5-A2, **2004**, 24 (25).
Rissin, D. *et al,* Nature Biotech. **2010**, 28, 595-599.
Rissin, D. *et al,* Anal. Chem. **2011**, 83, 2279-85.
Kan, C. *et al,* Lab Chip **2012**, 12 (5), 977-985.
Clinical Laboratory Standards Institute, Document EP17-A2, **2012**, 32 (8).
Wilson, D. *et al,* J. Lab. Aut. **2015**, 1-15.

## Acknowledgements

The authors acknowledge the contributions from Imen Canova (Biostatistics), Thierry Prentout (System Development) and their teams at bioMérieux S.A., Marcy l'Etoile, France.

## Background & Objectives

While immunoassays remain the mainstay for the automated, routine diagnostics testing of patient samples, early clinical diagnosis often hinges on the ability to reliably measure medically relevant biomarkers at very low-concentrations, beyond the reach of current conventional immunoanalyzer capability.

Simoa™ (Single Molecule Array) technology provides the ability to measure protein analytes with unprecedented sensitivity, down to the femtomolar range. Quanterix's HD-1 system offers full automation of Simoa digital immunoassay technology for life science research applications. This RUO (Research Use Only) system is available with a menu of assays (currently about 30) showing up to 1000x higher sensitivity compared to current best-in-class automated analog immunoassays.

In the prospect of developing an IVD-compliant (regulatory approved) version of the Simoa system, key analytical performance characteristics were evaluated, based on guidelines from the Clinical and Laboratory Standards Institute™ (CLSI), using two prototype assays for the detection of PSA (prostate-specific antigen) and HIV p24 (human immunodeficiency virus p24 antigen) in human serum or plasma. In the context of these studies, the two prototype assays served as tools for system performance evaluation.

## Methods

Evaluation of the analytical performance of the system consisted in a set of studies, based on CLSI EP5-A2, EP17-A2 and EP6-A guidelines, designed to determine precision, sensitivity and linearity. Three instruments were used for the evaluation.

Precision determination was based on the PSA prototype assay. 10 replicate runs (of 96 measurements each) were performed on each instrument over a period of 5 days. Each run included a full calibration curve (comprised of 8 calibrator levels, measured in triplicate). Calibrators were prepared from WHO standard in a diluted serum matrix. Concentration values for specimens were calculated using the calibration curve from the run. Additionally, concentration values for runs 2-9 were re-calculated using the calibration curve from run 1, in order to evaluate the consistency of the calibration curve over time (over multiple runs). Seven specimens (human serum samples), ranging from approximately 1 pg/mL to 80 pg/mL, were assayed in triplicate as part of each run. A nested ANOVA (Analysis of Variance) was performed, on concentration values, to determine within-run and between-run imprecision.

Sensitivity evaluation was conducted using both the PSA and p24 prototype assays. For PSA sensitivity evaluation, 10 samples (prepared from WHO standard) and 6 human serum specimens, covering a range of approximately 0.03 pg/mL to 0.3 pg/mL, were assayed in 6 separate runs on each of the 3 instruments. Limits of blank (LoB), limits of detection (LoD) and limits of quantitation (LoQ) were determined as per CLSI EP17-A2. LoQ was derived from the precision profile, after nonlinear regression fitting of the data, as the analyte concentration corresponding to a CV (coefficient of variation) of 20%.

HIV p24 seroconversion sensitivity was determined for an associated specificity level of 95% (i.e., > 95% observed specificity on repeatedly reactive samples). The sensitivity/specificity cut-off was determined from a set of samples at 30, 40, 50, 100, 150 and 300 copies/mL (viral load obtained from the Abbott ART method) prepared by dilution in negative serum of HIV-1 seroconversion panels. In addition, p24 LoB, LoD and LoQ were determined using the prescribed approach for quantitative assays. A low-range precision profile was determined, on each instrument, from 6 viral lysate samples and 2 diluted seroconversion panel samples covering the range 0-50 fg/mL (as determined from the bioMérieux Vidas p24 II kit).

Linearity was evaluated on the p24 prototype assay. Linear measuring range was determined as per CLSI EP6-A, using 3 series of 10 samples prepared by dilution of viral lysates in a negative native sample.

All datasets were inspected for the presence of outlier results. In the precision and sensitivity studies, the Tukey test was applied to AEB values (i.e., prior to concentration determination) in order to identify statistical outliers, defined as datapoints below (resp. above) the lower (resp. higher) quartile minus (resp. plus) 3 times the interquartile range. In the linearity study, outlier detection was performed as described in CLSI EP6-A.

## Results

Between-run imprecision levels (CV) of 2.6%-9.4% are obtained for human serum specimens over the range 1-80 pg/mL (Figure 3). Importantly, precision is maintained when a calibration curve from a different run was used for sample concentration determination (Figure 4).

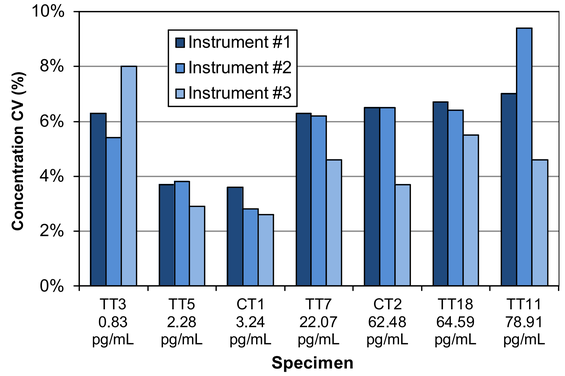


*Figure 3. Between-run imprecision for 7 human serum specimens measured on 3 instruments (10 runs on each instrument), showing single-digit CV values for all instruments over the PSA concentration range 1-80 pg/mL. Assay calibration was performed as part of every run. (Concentration values indicated on the x-axis represent the mean value over the 3 instruments).*

| Specimen | Per-run calibration | | Stored calibration curve | |
|---|---|---|---|---|
| | PSA concentration (pg/mL) | CV (%) | PSA concentration (pg/mL) | CV (%) |
| **TT3** | 0.830 | **8.0%** | 0.829 | **5.8%** |
| **TT5** | 2.284 | **2.9%** | 2.285 | **4.1%** |
| **CT1** | 3.269 | **2.6%** | 3.271 | **3.1%** |
| **TT7** | 22.78 | **4.6%** | 22.76 | **4.5%** |
| **CT2** | 62.44 | **3.7%** | 61.74 | **6.0%** |
| **TT18** | 66.12 | **5.5%** | 65.07 | **3.8%** |
| **TT11** | 81.55 | **4.6%** | 79.91 | **4.3%** |

*Figure 4. Illustration of the precision capability of the Simoa HD-1 system: reported PSA concentrations and between-run imprecision (CV) determined for 7 human serum specimens covering the concentration range 1-80 pg/mL, on Instrument #3, showing comparable concentration values and CV levels between (a) assay calibration performed with every run and (b) assay calibration using the calibration curve from the first run.*

Precision performance at ultra-low concentrations (in the fg/mL range) is illustrated by the PSA assay precision profiles calculated on the 3 individual instruments and across all 3 instruments (Figure 5).

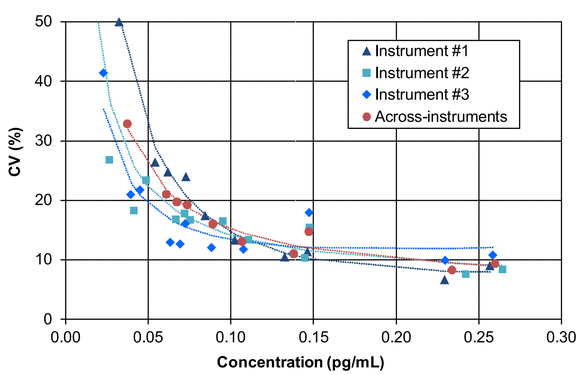


*Figure 5. PSA assay precision profiles for 3 instruments (6 runs on each instrument), determined from 10 samples and 6 female human serum specimens.*

PSA assay sensitivity performance is characterized by LoQ values of 0.048-0.076 pg/mL (Figure 6). Sensitivity performance of the p24 assay, considered here as a quantitative assay for the purpose of sensitivity evaluation, is characterized by LoQ values of 0.0060-0.0109 IU/mL (Figure 7).

| | Instrument #1 | Instrument #2 | Instrument #3 | Across instruments |
|---|---|---|---|---|
| **PSA LoB (pg/mL)** | 0.015 | 0.019 | 0.010 | **0.019** |
| **PSA LoD (pg/mL)** | 0.042 | 0.037 | 0.024 | **0.046** |
| **PSA LoQ (pg/mL)** | 0.076 | 0.059 | 0.048 | **0.076** |

*Figure 6. Illustration of the detection capability of the Simoa HD-1 system: LoB, LoD and LoQ for the prototype PSA assay, determined as per CLSI EP17-A2.*

This suggests that the Simoa HD-1 system is capable of 100-1000x higher sensitivity compared to conventional (non-digital) immunoanalyzers based on analog fluorescence, chemiluminescence or electrochemiluminescence detection.

| | Instrument #1 | Instrument #2 | Instrument #3 | Across instruments |
|---|---|---|---|---|
| **p24 LoB (IU/mL)** | 0.0011 | 0.0012 | 0.0008 | 0.0012 |
| **p24 LoD (IU/mL)** | 0.0027 | 0.0027 | 0.0026 | 0.0031 |
| **p24 LoQ (IU/mL)** | 0.0060 | 0.0061 | 0.0109 | 0.0109 |

*Figure 7. Illustration of the detection capability of the Simoa HD-1 system: LoB, LoD and LoQ values for the prototype p24 assay, determined as per CLSI EP17-A2.*

HIV p24 seroconversion sensitivity is illustrated in Figure 8. 100 copies/mL sensitivity is achieved, based on the cut-off determined across the 3 instruments. When setting the specificity level (lower limit of the 95% confidence interval) at 94.82%, 50 copies/mL sensitivity is reached (data not shown). Sensitivity levels obtained for the p24 prototype assay suggest that the Simoa HD-1 system has the potential to provide NAT-level sensitivity for HIV screening with a low-cost digital immunoassay.

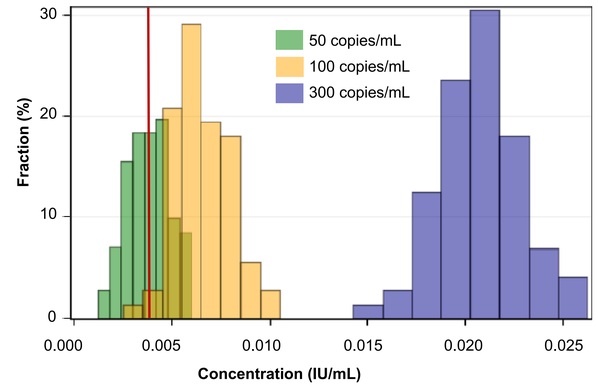


*Figure 8. Sample distribution histogram illustrating HIV p24 seroconversion sensitivity (at 95% specificity) for the prototype p24 assay. Red vertical bar: cut-off determined across the 3 instruments.*

Results from the p24 assay linearity study are shown in Figure 9. A linear measuring range in excess of 4 logs is demonstrated on the 3 instruments.

| | Instrument #1 | Instrument #2 | Instrument #3 | Across instruments |
|---|---|---|---|---|
| **Linear range (IU/mL)** | 0.0003 - 45.802 | 0.0010 - 44.232 | 0.0007 - 50.035 | 0.0007 - 46.689 |
| **Number of logs** | 5.2 | 4.6 | 4.9 | 4.8 |

*Figure 9. Linear measuring range for the Simoa p24 prototype assay, determined as per CLSI EP6-A (<12% deviation from linearity).*

## Conclusions & Outlook

Superior analytical performance of the automated Simoa HD-1 system has been confirmed, with ultra-high sensitivity capability and IVD-compliant precision levels demonstrated on 3 instruments, using prototype PSA and p24 assays as model assays for system performance evaluation.

The Simoa HD-1 system provides a unique platform for the translation of ultrasensitive immunoassays from the research laboratory to the IVD setting and to clinical practice. This capability is expected to greatly contribute to the improvement of standard of care in various clinical areas, including inflammatory disease, oncology, neurology, cardiovascular disease and infectious diseases.