# A NOVEL HIGHLY SENSITIVE ASSAY FOR QUANTITATIVE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS-1 IN HUMAN PLASMA

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## BACKGROUND

More individuals are now living with HIV-1 infection globally with the success of antiretroviral therapy (ART). World Health Organization recommends routine viral load test as the gold standard in treatment monitoring, diagnosing, and confirming ART failure. However, the genetic heterogeneity of HIV-1 presents a significant challenge to the development of assays capable of reliably detecting and quantifying HIV-1 nucleic acid. While certain subtypes dominate in specific regions, studies show that the spread of different HIV-1 groups and subtypes is increasing globally. Although many commercial tests are currently available on the market, there is a continuous need for HIV monitoring tests with expanded variant coverage, improved workflow, reduced costs, and improved sensitivity. The NeuMoDx HIV-1 Quant Assay is highly sensitive with broad HIV-1 variant inclusivity, and is designed to be implemented on the fully automated NeuMoDx Molecular Systems. These systems provide a "sample to result" type in-vitro diagnostic workflow by incorporating efficient RNA extraction coupled with a sensitive real-time RT- PCR assay to deliver highly accurate results using all room temperature stable reagents. Performance of the NeuMoDx HIV-1 Quant Assay was characterized in plasma and is reported here.

## METHODS

Multiple primers and probes targeting two highly conserved regions in the HIV-1 genome were employed in the NeuMoDx HIV-1 Quant Assay to ensure robust detection of all groups and subtypes as well as CRF isolates. The objective of this study was to demonstrate performance of the NeuMoDx HIV-1 Quant Assay across key analytical metrics including analytical sensitivity (LoD), linearity, and HIV-1 variant coverage. Evaluation of the analytical sensitivity was performed using the 4th WHO International Standard for HIV-1, and the limits of quantitation (LLoQ/ULoQ) were determined using the TAE ≤ 1.0 criterion. The capability of the assay in detecting HIV variants, including M, O, P and N subtypes and multiple CRF and drug resistant isolates, was also evaluated with EQAPOL panels. The clinical sensitivity was demonstrated, in a pilot internal study, using residual clinical HIV-positive specimens tested with a FDA-cleared test.

## NEUMODX HIV-1 QUANT ASSAY

The NeuMoDx HIV-1 Quant Assay is designed to be a quantitative assay for the detection of HIV-1 in plasma. The assay to be implemented on NeuMoDx Molecular System incorporates automated RNA extraction coupled with real-time reverse transcription polymerase chain reaction rt-PCR to detect two targets in the HIV-1 genome. A universal exogenous sample process control, SPC2, is incorporated in each test to monitor the efficiency of RNA extraction, rt-PCR, and the presence of potential inhibitors. The entire process takes only 95 minutes with minimal hands on time.

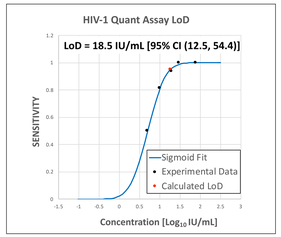
## RESULTS

The NeuMoDx HIV-1 Assay demonstrated a limit of detection (LoD) and a lower limit of quantitation (LLoQ) of 18.5 IU/mL. The NeuMoDx HIV-1 Assay showed excellent linearity across a 6-log dynamic range (R2 > 0.98) with upper limit of quantitation (ULoQ) of ~1E7 Log10 IU/mL. No cross-reactivity was observed against HIV-2 and 35 non-target pathogens tested. Equivalent performance was demonstrated across all the HIV variants, including groups K, O, P and different CRF/drug resistant isolates from the EQAPOL Genetic Diversity Panel, and Drug Resistant Panel. Finally, a preliminary method correlation study using > 70 clinical residual HIV positive plasma specimens demonstrated excellent sensitivity, 99%, and concordance between the NeuMoDx HIV-1 Quant Assay and the Roche Cobas® HIV-1 Assay with a bias <0.5 Log10 IU/mL.

## Limit of Detection and LLoQ

The Limit of Detection of the NeuMoDx HIV-1 Quant Assay was determined with pooled HIV-1 negative plasma spiked with 4th WHO International HIV-1 Standard at 6 different levels including negative samples. The limit of detection of HIV-1 was determined to be 18.5 IU/mL based on Probit style analysis.

Limit of Quantitation was defined as the concentration of target detected consistently (≥95% sensitivity) with a total analytic error, TAE (bias + 2*SD) ≤ 1. As seen from data in the Table, TAE remains < 1.0 even at 10 IU/mL (below LoD level). Since the LLoQ cannot be less than LoD, the calculated LLoQ was determined to be same as LoD and equal to 18.5 IU/mL



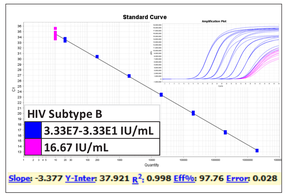

**NeuMoDx HIV-1 Assay Limit of Detection and LLoQ**

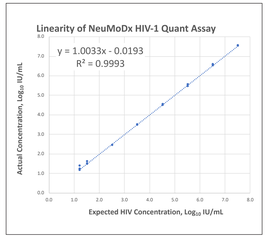
| Target Conc. (IU/mL) | Target Conc. ($Log_{10}$ IU/mL) | HIV Conc. ($Log_{10}$ IU/mL) | N | # Pos | % Pos | LoD (Probit) | SD | Abs. Bias | TAE |
|---|---|---|---|---|---|---|---|---|---|
| 78 | 1.89 | 1.99 | 16 | 16 | 100 | 18.5 IU/mL 95% CI (12.5-54.4 IU/mL) | 0.17 | 0.10 | 0.43 |
| 30 | 1.48 | 1.37 | 16 | 16 | 100 | | 0.28 | 0.11 | 0.66 |
| 20 | 1.30 | 1.29 | 16 | 15 | 94 | | 0.33 | 0.01 | 0.67 |
| 10 | 1.00 | 1.15 | 16 | 13 | 81 | | 0.26 | 0.15 | 0.67 |
| 5 | 0.70 | 1.27 | 16 | 8 | 50 | | 0.32 | 0.57 | 1.21 |
| 0 | 0 | 0.00 | 8 | 0 | 0 | | - | - | - |

## Linearity of NeuMoDx HIV-1 Quant Assay

The linearity of the NeuMoDx HIV-1 Quant Assay was determined by diluting purified HIV-1 stocks subtype B (EQAPOL) in pooled HIV-1 negative plasma to create a panel spanning 6 logs of HIV-1 concentrations ranging from 1.2 Log10 IU/mL to 7.5 Log10 IU/mL.

The NeuMoDx HIV-1 Quant Assay demonstrated excellent robust quantification linearity over a wide dynamic range (6 log units) with ULoQ determined at 7.5 Log10 IU/mL. (Fig 2, 3 and Table 2).




**NeuMoDx HIV-1 Assay Linearity in Plasma**

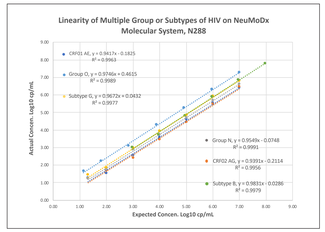
| Target Conc. ($Log_{10}$ IU/mL) | Replicate | Mean Conc. ($Log_{10}$ IU/mL) | Standard Deviation | Absolute Bias | Total Analytical Error (TAE) |
|---|---|---|---|---|---|
| 7.52 | 3 | 7.54 | 0.01 | 0.01 | 0.04 |
| 6.52 | 3 | 6.55 | 0.03 | 0.02 | 0.09 |
| 5.52 | 3 | 5.52 | 0.06 | 0.00 | 0.12 |
| 4.52 | 3 | 4.52 | 0.03 | 0.01 | 0.05 |
| 3.52 | 3 | 3.49 | 0.02 | 0.03 | 0.00 |
| 2.52 | 3 | 2.45 | 0.01 | 0.07 | 0.06 |
| 1.52 | 3 | 1.52 | 0.07 | 0.00 | 0.14 |
| 1.22 | 10 | 1.25 | 0.12 | 0.03 | 0.26 |

## CONCLUSIONS

The NeuMoDx HIV-1 Quant Assay demonstrated sensitive and accurate performance in quantitation of HIV-1 in plasma. The room temperature stable reagents coupled with the rapid turnaround times provide an extremely attractive workflow for implementation on automated system.

## HIV-1 Variant Coverage

Detection of HIV-1 variants by the NeuMoDx HIV-1 Quant Assay was evaluated with all the major HIV-1 groups and subtypes as well as multiple circulating recombinant form (CRF) variants from the External Quality Assurance Program Oversight Laboratory (EQAPOL). HIV-1 variant stocks were diluted down to the concentrations indicated in the table with negative plasma. NeuMoDx HIV-1 Quant Assay demonstrated broad variant coverage with excellent sensitivity, including type N, O, and P.




**NeuMoDx HIV-1 Quant Assay Variant Coverage (Inclusivity)**

| EQAPOL Panel # | Group/ Subtype | HIV Conc. c/mL | % POS (N=6) | EQAPOL Panel # | Group/ Subtype | HIV Conc. c/mL | % POS (N=6) |
|---|---|---|---|---|---|---|---|
| DEMA106ES002 | A1 | 20 | 100% | DEMF210CM001 | F2 | 40 | 100% |
| DEMA07UG005 | A1 | 20 | 100% | DEMG09ES002 | G | 20 | 100% |
| DEMA114PK013 | A1 | 20 | 100% | DEMG10CM008 | G | 20 | 100% |
| DEMA114PK002 | A1 | 20 | 100% | DEMG05ES001 | G | 40 | 100% |
| DEMA11KE001 | A1 | 20 | 100% | DEMG09KE001 | G | 40 | 100% |
| DEURF07UG006 | A1, D | 20 | 100% | Seracare 0800-0346 | H | 20 | 100% |
| DEMB10CN002 | B | 20 | 100% | SeraCare 0315-0062 | K | 20 | 100% |
| DEMB99JP004 | B | 20 | 100% | DE00110CN001 | CRF01_AE | 20 | 100% |
| DEMB09B0001 | B | 20 | 100% | DE00112CN011 | CRF01_AE | 20 | 100% |
| DEMB10ES003 | B | 20 | 100% | DE00208CM004 | CRF01_AE | 20 | 100% |
| DEMB08ES001 | B | 20 | 100% | DE00110CN009 | CRF01_AE | 40 | 100% |
| DEMB10TH002 | B | 20 | 100% | DE00112CN011 | CRF01_AE | 40 | 100% |
| DEMB09CN002 | B | 20 | 100% | DE00208CM004 | CRF01_AE | 40 | 100% |
| DEMB03JP004 | B | 20 | 100% | DE00109CN003 | CRF01_AE | 40 | 100% |
| DEMB10US001 | B | 20 | 100% | DE00210CM019 | CRF02_AG | 20 | 100% |
| DEMBF09ES003 | BF | 20 | 100% | DE00208CM001 | CRF02_AG | 20 | 100% |
| DEMBF09ES006 | BF | 20 | 100% | DE00212FR004 | CRF02_AG | 20 | 100% |
| DEMC07AO001 | C | 20 | 100% | DE00206AO001 | CRF02_AG | 40 | 100% |
| DEMC06ES003 | C | 20 | 100% | DE00208CM004 | CRF02_AG | 40 | 100% |
| DEMC08MW004 | C | 20 | 100% | DEURF07ES002 | A3, CRF02_AG | 40 | 100% |
| DEMC07BR003 | C | 40 | 100% | DEURF10DZ001 | CRF02_AG/ CRF06_cpx | 40 | 100% |
| DEMC09ZA009 | C | 40 | 100% | DE07010BR033 | CRF70_BF1 | 20 | 100% |
| DEMC96BW002 | C | 40 | 100% | DEURF09ES005 | URF_A1B | 20 | 100% |
| DEMC11ZM003 | C | 20 | 100% | DEURF10KE001 | URF_A1C | 20 | 100% |
| DEMD08UG001 | D | 20 | 100% | DEURF10KE003 | URF_A1C | 40 | 100% |
| DEMD07UG002 | D | 40 | 100% | DEURF09KE002 | URF_A1D | 20 | 100% |
| DEMD10CM009 | D | 40 | 100% | DEURF14PK015 | URF_A1G | 20 | 100% |
| DEMD11UG003 | D | 40 | 100% | DEN02CM062 | Group N | 20 | 100% |
| DEMF110ES001 | F1 | 20 | 100% | DEOXXDE004 | Group O | 20 | 100% |
| DEMF111BR037 | F1 | 20 | 100% | DEOXXES001 | Group O | 20 | 100% |
| DEMF110BR015 | F1 | 40 | 100% | DEOXXUS001 | Group O | 20 | 100% |
| DEURF11CM026 | F1, F2 | 40 | 100% | DEPXXFR006 | Group P | 20 | 100% |

## Analytical Specificity – Cross-Reactivity

The analytical specificity of the NeuMoDx HIV Quant Assay was evaluated with HIV negative plasma spiked with 34 non-target (or phylogenetically similar) organisms (Table 4) found in blood/plasma specimens at high concentrations as indicated in the table. No cross-reactivity was observed in any of the organisms listed in the table – demonstrated 100% analytical specificity.

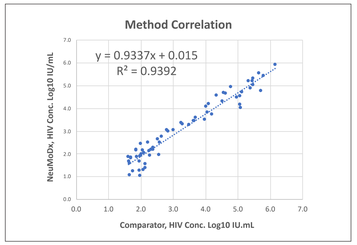
**NeuMoDx HIV-1 Quant Assay Analytical Specificity**

| Pathogen Name | Concentration | Pathogen Name | Concentration |
|---|---|---|---|
| Cytomegalovirus | 1E6 cp/mL | Hepatitis A | 1E6 TCID50/mL |
| Epstein-Barr virus | 1E6 cp/mL | Hepatitis B | 1E6 IU/mL |
| Human Herpesvirus 6B | 1E6 cp/mL | Hepatitis C | 1E6 IU/mL |
| Human Herpesvirus 8 | 1E6 cp/mL | Zika virus | 1E6 TCID50/mL |
| Rubella | 1.14E5 TCID50/mL | Yellow Fever | 1E6 TCID50/mL |
| Human adenovirus 2 | 3.58E4 TCID50/mL | Dengue 3 | 6.36E4 TCID50/mL |
| Human adenovirus 5 | 1E6 TCID50/mL | St Louis encephalitis | 1E6 cp/mL |
| Human Herpesvirus 7 | 1E6 cp/mL | Dengue 1 | 1E6 TCID50/mL |
| Dengue 4 | 1E6 TCID50/mL | Human T-lymphotropic virus 1 | 1E6 VP/mL |
| Parvo B19 | 1E6 IU/mL | Human T-lymphotropic virus 2 | 1E6 VP/mL |
| Dengue 2 | 1E6 TCID50/mL | Influenza A | 1E6 CEID50/mL |
| Human Immunodeficiency virus 2 | 1E6 cp/mL | Candida albicans | 8.1E6 CFU/mL |
| Human papillomavirus 16 | 1E6 cp/mL | Chlamydia Trachomatis | 1E6 EB/mL |
| Human papillomavirus 18 | 1E6 cp/mL | Herpes Simplex virus 1 | 1E6 cp/mL |
| Neisseria Gonorrhoeae | 8.4E6 CFU/mL | Herpes Simplex virus 2 | 1E6 cp/mL |
| Propionibacterium acnes | 6E6 CFU/mL | Staphylococcus aureus | 8.7E6 CFU/mL |
| West Nile | 1 ng/mL | Staphylococcus epidermidis | 8.7E6 CFU/mL |

## Method Correlation

The quantitative performance of the NeuMoDx HIV Test was assessed against a FDA approved comparator assay. Testing was performed internally at NeuMoDx through a single-blinded study using 77 de-identified residual clinical HIV-positive specimens obtained from reference laboratories. Specimens within the linear range common to both assays were used for the linear regression analysis as shown in Figure 4.

In addition to providing excellent sensitivity, the NeuMoDx HIV test demonstrated excellent quantitative correlation with the reference test (Table 4).




| | | Comparator | | |
|---|---|---|---|---|
| | | Pos | Neg | Total |
| NeuMoDx | Pos | 73 | 0 | 73 |
| | Neg | 1 | 3 | 4 |
| | Total | 74 | 3 | 77 |
| | Sensitivity | 99% | | |

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*The NeuMoDx HIV-1 Quant Assay is For Research Use Only. Not for use in diagnostic procedures*