8th Conference on Retroviruses and Opportunistic Infections February 4-8, 2001

O18e

NucliSens EasyQ® HIV-1
A Novel Viral Load Assay Based on Real Time NASBA

Bert Top[1], Mike Cronin[2] and Paul van de Wiel[1],

[1]Organon Teknika, Department of Nucleic Acid Diagnostics, Boxtel, The Netherlands. [2]Organon Teknika Corporation, Durham (NC)

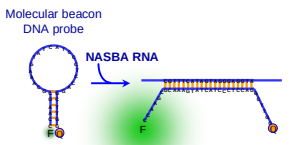
For more information please mail to b.top@organonteknika.nl

Visit us at www.nuclisens.com

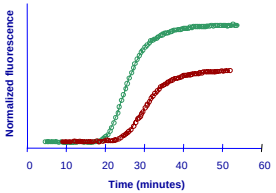
Technology Description

We have developed a novel assay for the quantitation of HIV-1 viral load in clinical specimens. This assay combines the NASBA amplification - with homogeneous Molecular Beacon based detection. The combination of these technologies has resulted in a real-time amplification assay for HIV-1 viral load monitoring that meets the increasing demands for diagnostic quality, throughput, flexibility and user convenience.

Schematics



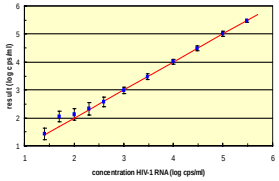
Kinetic curve example



Two differently labeled molecular beacon probes are added into a standard NASBA reaction. One probe is directed against the HIV-1 viral sequence whereas the other probe is directed against the (in vitro) calibrator RNA. The probes will hybridize to the anti-sense RNA transcripts generated by the NASBA process. Beacon probe hybridization causes the separation of fluorophore and quencher molecules, resulting in the production of fluorescence at two different wavelengths.

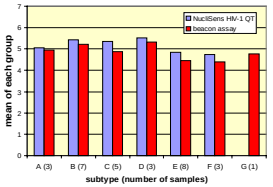
Quantitation of the assay is based on the fact that the relative amounts of HIV-1 RNA and the calibrator RNA present at the start of the NASBA reaction results in two fluorescent signal curves. The characteristics of these curves are then used in an algorithm to calculate the HIV-1 copy number originally present in the clinical sample.

HIV-1 Assay Characteristics



Dynamic Range

The analytical performance was tested on the VQC panel (CLB, The Netherlands). The HIV-1 concentration in these panel samples ranges from 25 - 300.000 copies/ml.



HIV-1 subtype quantitation

Comparison between NucliSens HIV-1 QT and the new NucliSens EasyQ HIV-1assay.

Quantitation of both assays is comparable for clades A-F. But even the HIV-1 subtype G sample was now easily detected and quantitated with EasyQ HIV-1.

Quantitation of the first WHO HIV-1 RNA International Standard

Concentration (log IU / ml)	Avg. result	SD
3.00	3.06	0.18
4.00	4.02	0.07

This standard has been prepared by the NIBSC and is available in lyophilized format at a concentration of 100.000 IU / vial. The standard contains HIV-1 subtype B.

Summary

Real Time NucliSens EasyQ HIV-1 features

* A combination of state of the art technologies
* > 4 log dynamic range (on the panel tested)
* No cross reactivity with HTLV-1 and HTLV-2
* Quantitation of HIV-1 M-Group subtype A-H
* Accurate quantitation of WHO standard (subtype B)
* Internally controlled test procedure
* No post amplification steps eliminates contamination risks allowing same lab testing
* High throughput with minimal hands on time

NucliSens EasyQ System Approach for Isothermal Real Time Quantitation

Clinical sample ▶ **Lysed Sample** ▶ **Nucleic Acid Extraction** ▶ **Purified RNA** ▶ **Set-up reaction** ▶ **Real time amplification and detection. Automated data reduction and reporting**

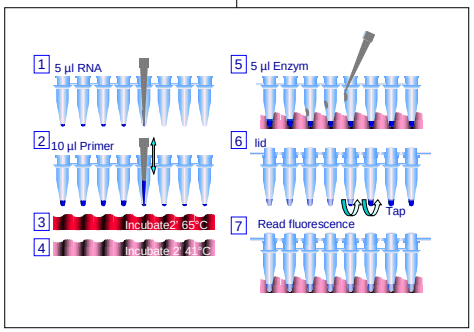
Add to lysis buffer — Add calibrator (internal control)

The new test procedure combines three state of the art technologies: (1) Silica based Nucleic Acid Extraction (Boom procedure), (2) NASBA Amplification and (3) Molecular Beacon based detection.

The addition of in -vitro generated calibrator RNA to the sample prior to extraction ensures complete monitoring of all steps of the procedure.

The amplification/detection reaction set-up consists of three, easy to perform pipetting steps for the subsequent addition of (1) RNA, (2) reagents and (3) enzymes.

With this procedure the total assay time for amplification and detection will be less than 2 hours for 48 samples with only 45 minutes of hands on time.



NucliSens EasyQ Analyzer