

Poster presentation

Reproducibility of a high-throughput HIV-1 genotypic resistance assay over time (2001–2007)

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from Ninth International Congress on Drug Therapy in HIV Infection
Glasgow, UK. 9–13 November 2008

Published: 10 November 2008

Journal of the International AIDS Society 2008, **11**(Suppl 1):P205 doi:10.1186/1758-2652-11-S1-P205

This abstract is available from: <http://www.jiasociety.org/content/11/S1/P205>

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Background

Designed for innovative HIV-1 diagnostics and resistance analysis, a high-throughput laboratory-developed (Protease-Reverse-Transcriptase) genotyping assay was implemented at Virco (Belgium). Following regulatory guidelines, assay variability was monitored over time by means of a quarterly internal quality control (QC) panel.

Methods

The panel comprises seven well-characterized recombinant viruses that were derived from clinical isolates. Virus stocks were grown and later re-cultured as needed and aliquoted for use at each quarterly testing between Q4 in 2001 through Q1 in 2007. The nucleotide similarity among the original virus stocks and subsequent re-cultured viruses, was determined using pairwise BLAST analysis (BioEdit Sequence Alignment Editor).

Summary of results

A total of 603 and 867 pairwise BLAST comparisons were made, yielding an average within and between original and re-cultured virus stocks similarity of $99.60\% \pm 0.27$ and $99.52\% \pm 0.33$, respectively.

Conclusion

Since both BLAST values did not differ, it can be concluded that re-culturing of viruses did not lead to a significant decrease in similarity. Furthermore, despite the presence of high levels of nucleotide sequence mixtures of the original clinical isolates, the high reproducibility of

the genotyping assay (more than 99% sequence identity) was demonstrated. A combination of optimal assay design, rigorous personnel training, the testing of the internal QC panel at regular intervals and participation in a CLIA-approved 6-monthly external proficiency testing program (Accutest) ensures that quality standards are consistently met for the genotyping assay performed at Virco.