Validation of “DeepChek SingleRound® RT-PCR and Sequencing PR/RT Assay v.2” kit as HIV-1 Drug Resistance Test for routine laboratory

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Background

Genotypic resistance test (GRT) is a key diagnostic tool for a proper therapeutic approach aimed at prolonged maintenance of virological suppression in HIV infection. GRT is also crucial in patients receiving HAART with low-level viremia (LLV). In fact, many studies have shown that LLV can be predictive of progressive viral rebound thus increasing subsequent virological failure.
Giving that, the clinical management of such patients certainly deserves novel resistance testing solutions which must be very sensitive, reliable and easy to be performed in routine laboratory.
With this regard we evaluated and validated the “DeepChek® SingleRound RT-PCR and Sequencing PR/RT Assay v2” and “DeepChek® Nested-PCR and Sequencing PR/RT Assay v2”, a new resistance testing solutions to amplifying and sequencing PR and RT of HIV-1.

Materials and Methods

A total of 45 plasma samples from HIV-1 infected patients on cART, collected at the Laboratory of Microbiology and Virology of the San Raffaele Hospital, Milan, were included in the study. We focused our analysis on samples with high viremic values and we included 16 samples with low-level viral load (RNA 50-2000 copies/mL). Viral RNA was extracted from 500 µL of plasma samples using the QIASymphony SP equipment (QIAGEN), while LLV samples were previously concentrated by centrifugation of 1 mL of plasma at 24,000g for 2 h at 4ºc.
All samples were genotyped for routine clinical purpose by using ViroSeq® HIV-1 Genotyping System (Abbott Molecular) or an in-house assay for samples with LLV.
The DeepChek protocol was used according to the manufacturer instructions. For samples resulting negative in the first step, a nested-PCR protocol (“DeepChek Nested-PCR and Sequencing PR/RT Assay v2”) was performed. Protease and RT master mix must be prepared separately.
Resistance Testing was performed by Sanger Sequencing on 3130xl Genetic Analyzer (Applied Biosystems) as recommended by the manufacturer and analyzed with Viroscore Software provided.
Two samples were also sequenced by NGS technology with Ion PGM Systems platform (ThermoFisher Scientific) and resistance interpretation was performed with DeepChek Software.

Results

Amplification and sequencing were successful in all samples with viremia >1000 copies/mL (26/45) allowing to produce a GRT for each patient by Viroscore software. GRT is easy to perform and can be also customized allowing interpretation with different algorithms analyzing resistance associated mutations. Sequencing and drug resistance testing were also successful in 56% (9/16) of samples with a low-viral load. Three samples had an unknown viremia and two of them were successfully sequenced.
The introduction of this novel assay in laboratory routine would prevent labour intensive in-house assays also improving sample processing rates.
Data obtained from NGS sequencing on two samples were analyzed using DeepChek software (ABL) resulted in the identification of an additional resistance mutation (RT A62V) compared to Sanger, present in 1.04% of the sequenced genomes.

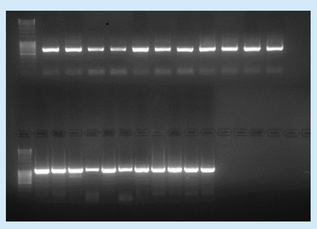

Figure 1: Detection of amplification product after PCR for samples with viral load >50 copies/mL (above: PR gene; below: RT gene).

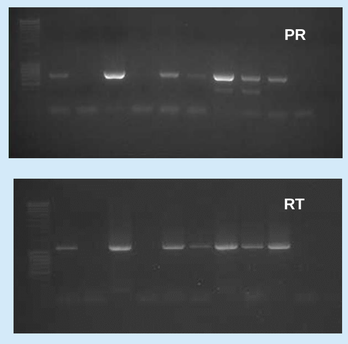

Figure 2: Amplification products after PCR for samples with low-viral load. No detectable amplification for Sample 2 and 4.

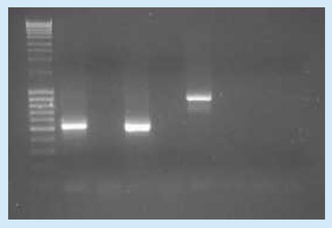

Figure 3: Amplification products of Samples 2 and 4 after execution of Nested-PCR (No detectable amplification for RT gene of Sample 4).

Successful sequencing	PCR	Nested-PCR	PCR + Nested-PCR		
n.	PR+RT			PR	RT
26	19	3	2	1	1
9	4	1	1	2	1
2	2				

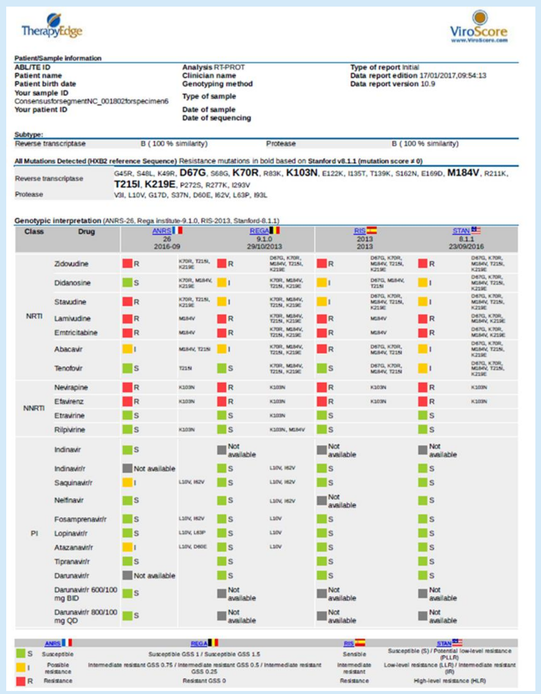

Figure 4: GRT Report with Viroscore software.

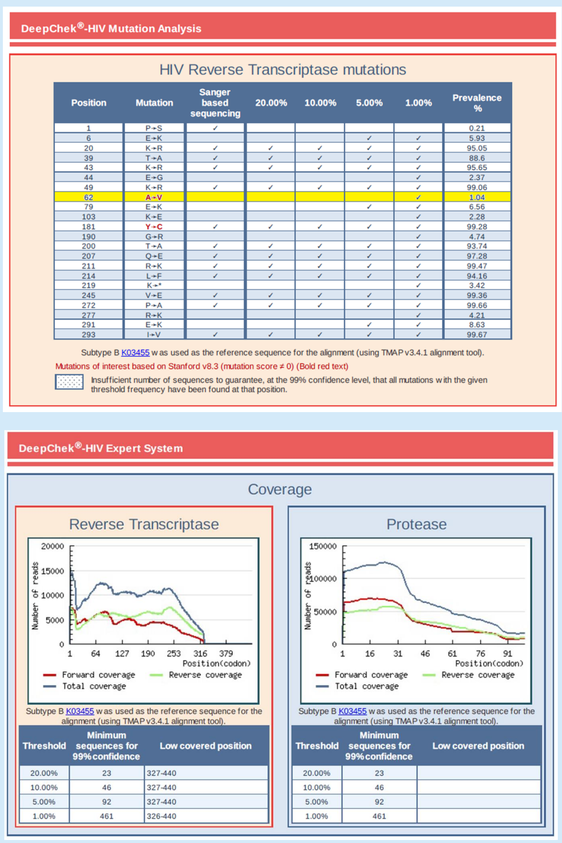

Figure 5: GRT Report using DeepChek software.

Conclusions

Our results demonstrate that DeepChek kit for performing GRT is reliable, with a high success rate also in samples with low viral load.
The analysis software Viroscore provided is easy-to-use and decreases the time of sequence analysis as well as the creation of GRT report.
DeepChek software represent an interesting analysis tool for easy implementation of ultra deep sequencing methods in virological diagnostics.

References

1. Pou C., et al., *Improved prediction of salvage antiretroviral therapy outcomes using ultrasensitive HIV-1 drug resistance testing.* Clin Infect Dis. 2014 Aug 15;59(4):578-88.
2. Mohamed S., et al., *Comparison of ultra-deep versus Sanger sequencing detection of minority mutations on the HIV-1 drug resistance interpretations after virological failure.* AIDS 2014, Jun 1;28(9):1315-24.