Novel End-to-end Sequencing Solutions for Sanger and Next Generation Sequencing (NGS) of HIV and Viral Hepatitis C (HCV).

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Introduction

- The management of patients infected with HIV or Viral Hepatitis C (HCV) relies on an accurate viral genomic profiling.
- Molecular assays combining reagents and powerful data analysis software are on demand by clinical diagnostics labs. We present the DeepChek® SingleRound RT-PCR and Sequencing HIV & HCV Assays (Fig. 1).

Methods

- Targeting key HIV and HCV regions (most discriminant subtyping and drug resistance positions are covered - Fig. 2) is the way virology applications are developed.
- The DeepChek® SingleRound RT-PCR and Sequencing Assays are agnostic of the Sanger or NGS platform.
- The ABL’s Assays are standardized (GMP manufacturing - Fig. 3).
- They embed all the reagents required for a robust viral sequences amplification.

Fig. 3: Assays manufacturing illustration.

- HIV-1 reverse transcriptase, protease and integrase amplicons, and NS5B amplicons have been generated (Fig. 4) from a panel of hundreds of well-characterized frozen clinical plasma samples from the Caribbean region, Brazil and Europe.

Fig. 4: The DeepChek® SingleRound RT-PCR and Sequencing Assays – Workflow overview.

Results

- Overall performance of the assays are shown in Figure 5.
- Amplicons were sequenced using two methodologies (Sanger sequencing with Big Dye kits on one hand and NGS with Illumina Nextera XT and Miseq® on the other hand).
- Sequences were analyzed with ViroScore® and DeepChek® technology respectively (Fig. 5 A & B); clinical genotyping reports (combining genotypes, mutations, and drug resistance assessment) were automatically generated.
- All results were in agreement with previous samples characterization.

Conclusions

- We developed an innovative and robust end-to-end solution which combines reagents and analysis software systems, directly compatible with diagnostics actionable interpretations for HIV and HCV infection disease management.

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