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Pooling of microbiological samples to optimize testing: comparative analysis of three NGS platforms

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Introduction

Subtype and drug-resistance mutations were mostly assessed routinely using Capillary Electrophoresis (CE) sequencing which does not detect co-infection or minor variants (frequency below 15-20%). Next Generation Sequencing (NGS) has become the new standard for genotypic drug resistance testing. The SARS-CoV-2 pandemic implied a rapid development of laboratory testing solutions focused on SARS-CoV-2 detection and sequencing: many laboratories are now equipped to perform NGS. The objective of this study was to evaluate the performances of three NGS platforms using sample pooling for microbiological testing.

Methods

Positive human plasmas (HIV, HCV and HBV), positive human viral transport media (SARS-CoV-2), positive sputum (Mycobacterium Tuberculosis) and HIV-1 external controls were purified using MagNa Pure24 (Roche). A total of 16 clinical samples were tested: amplifications and libraries were performed using DeepChek assays (ABL) intended for target specific and whole genome sequencing and NGS library preparation. The NGS libraries were also converted using the MGIEasy Universal Library Conversion Kit (CAT N 1000004155 MGI). Libraries were sequenced (2x150bp) using two Illumina instruments (iSeq100 and MiSeq) and the DNBSEQ-G400 (MGI). Output sequences were compared to the interest pathogen reference genomes. The DeepChek software (ABL) was used for the analysis of subtypes, mutations and induced drug resistance for all pathogens. Statistical analysis was performed using Prism 9 software (v 9.5.1).

Results

The Q30 was 86%, 75% and 95% for iSeq100, MiSeq and DNBSEQ-G400, respectively. The median sequence number per sample was 102.488, 768.506 and 190.040 for iSeq100, MiSeq and DNBSEQ-G400, respectively. Only 0.25% of the MGI reads for each sample (randomly from each file and balanced over the 4 lanes) was used. Significant difference is observed for the percentage of reads mapped to the pathogen between DNBSEQ-G400/iSeq1000 and DNBSEQ-G400/MiSeq, ($p = 0.03$ and $p < 0.01$), respectively. No significant difference is observed with the total number of mutations of interest. All samples were accurately genotyped, and all mutations of interest were detected with the three NGS platforms.

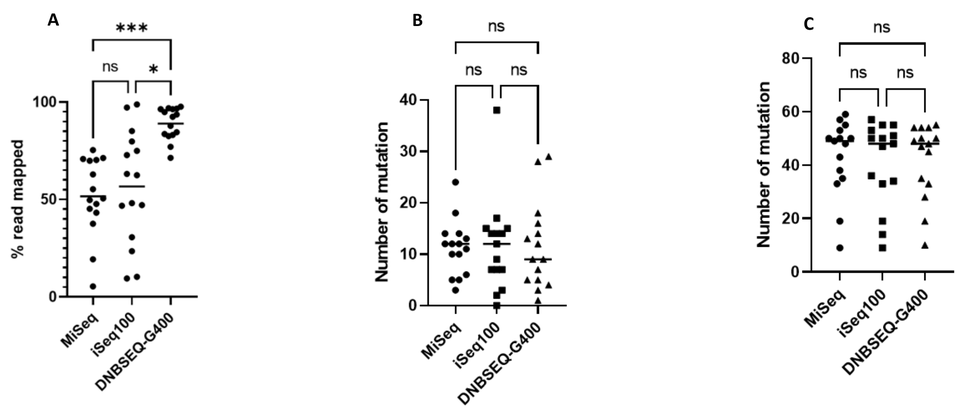

Figure 4: (A) % of read mapped to the pathogen, **(B)** TOTAL Minority Mutations: (>1% to <20%), **(C)** TOTAL Majority Mutations (>20% to <100%)

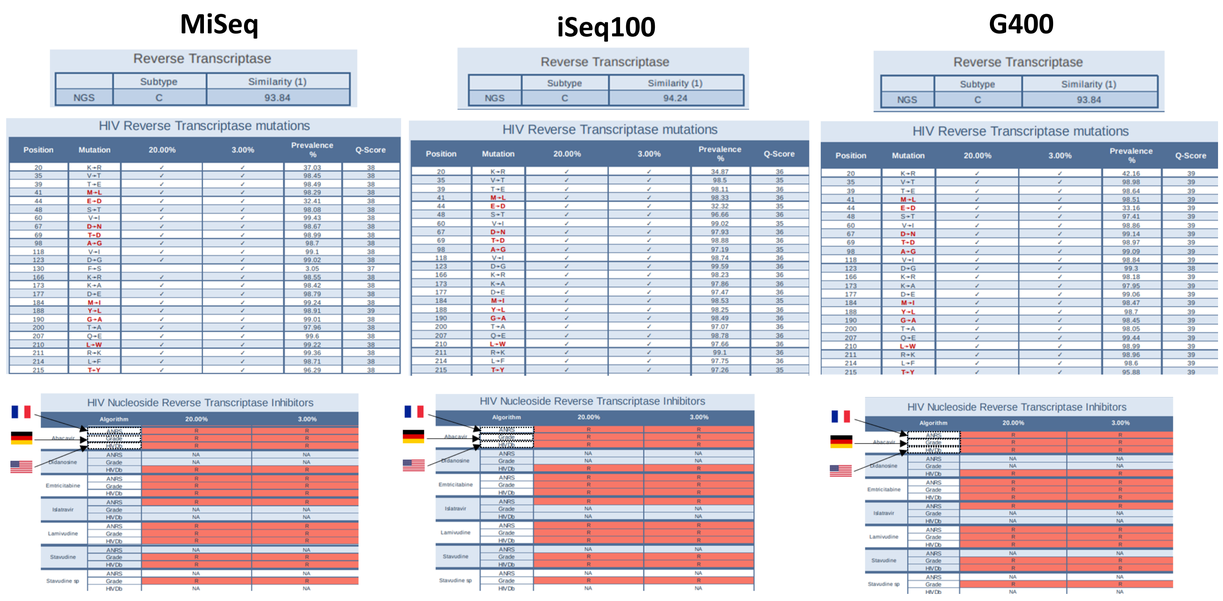

Figure 5: Comparison of the HIV-1 subtype and drug resistance mutation using DeepChek® software.

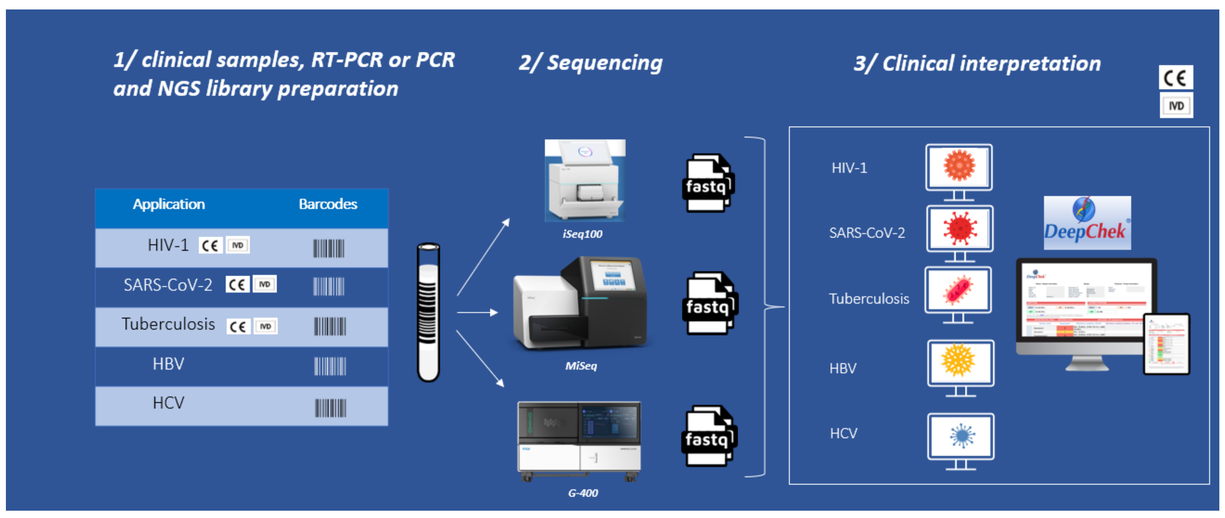

Figure 1: The workflow of pooling sequencing with DeepChek® assays.

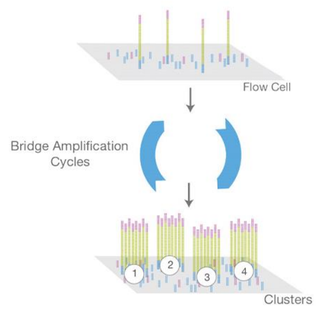

Figure 2: Sequencing by synthesis (SBS), Illumina.

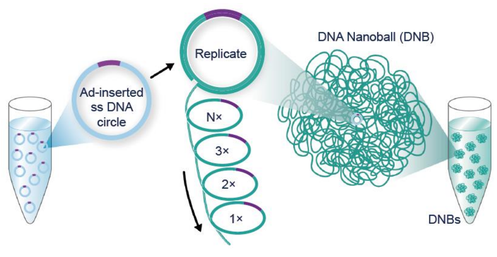

Figure 3: DNBSEQ™ DNA nanoballs sequencing, MGI.

Conclusions

This study is the first evaluation of sample pooling for microbiological testing using the DeepChek assays using NGS and analysed by an easy-to-use software. Equivalent results between iSeq100, MiSeq and DNBSEQ G400 were observed for all pathogens. The NGS should occupy a major place in microbiology applications testing for subtyping, mutation determination and analysis, and drug resistance surveillance. It should enable to reveal resistant minority variants or new mutations and study their impact. The used sequencing methods show an overall comparable quality: further head-to-head comparisons shall be conducted to better determine the use-case of each platform and include turn-around time, economics.