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Interest of HIV-1 Next-Generation Sequencing in a diagnosis laboratory in France

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Background

The identification of the HIV subtype and the detection of resistance mutations are routinely sought in infected patients, before starting treatment as well as in the context of treatment modifications for escape or switch. The scientific mobilization due to the emergence of SARS-CoV-2 has brought the rapid development and access of many laboratories to NGS sequencing solutions. In the context of the HIV activity, Sanger sequencing methods are preferentially used routinely in France.

Objective

The objective of this study consists in the analyse the interest of HIV NGS sequencing, targeted , protease (PR), reverse transcriptase (RT), integrase (INT) and V3 loop or of the complete genome at the virology department of the University Hospital of Caen and the contribution of NGS compared to Sanger sequencing in a routine practice

Samples and Methods

Plasma and blood positives for HIV were included

Nucleic acids extraction EZ1 Qiagen

-When VL > 1 000 cp/ml: 1mL sample
- When VL < 1 000 cp/ml: 2x1mL sample and concentration using Amicon Ultra 0,5

Target amplification *ViroSeq™ Assay RT-PR, INT from Abbott Molecular*

RT → PCR → Agarose gel → Cycle sequencing

Target amplification *DeepChek® Assay RT, PR, INT from Advanced Biological Laboratories*

RT-PCR → Nested PCR → Agarose gel

Whole genome amplification *DeepChek® Assay from Advanced Biological Laboratories*

PCR: 5 fragments → Nested PCR → Agarose gel

Sanger Sequencing

ABI 3130 Applied Biosystems

Electropherograms are displayed for the four primer segments used to assemble the consensus sequence.

One by one identification of mutations

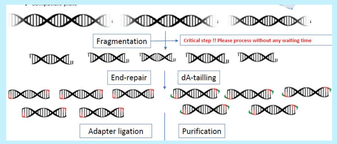
Double check of sequences alignements by 2 technicians

Data analysis

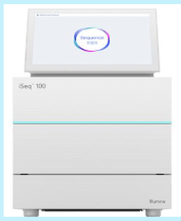
SmartGene

Manually added Fasta files

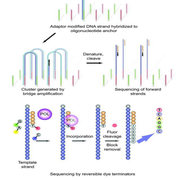
Library Preparation



Sequencing



iSeq100 Illumina



2 Fastq files / sample

Data analysis

DeepChek

ABL for NGS HIV analysis

Results

Samples caractreristics

- Prospectively include from **05/01/2021 to 03/31/2022**
- 94 samples from HIV patients were included
- HIV subtype : B,C, D, A1, F2, CRF01 and CRF02

94 samples

78 plasma
ARN Viral load: 100 and 4 656 805 copies/ml (range 2 to 6.6 log)

16 whole blood
ADN Viral load:50 and 3227 copies/million cells (range 1.7 to 3.5 log)

NGS ABL DeepChekAnalysis
68 plasmas succesfully amplified and analysed for 3 targets (RT, PR and INT)

Sanger Abbot Viroseq™ and SmartGene
55 plasmas succesfully amplified and analaysed

NGS ABL DeepChekAnalysis
12 whole blood succesfully amplified and analysed for 3 targets (RT, PR and INT)

Sanger Abbot Viroseq™ and SmartGene
10 whole bloods succesfully amplified and analaysed

Whole genome* amplification *DeepChek® Assay
10 samples analyzed in complete genome for all 5 fragments

1 sample failes: VL 50 copies/mL

9 samples amplified

- 3 samples 5/5 fragments succesfully analysed (subtype B, C and CRF02)
- 2 samples → 3/5 fragments analysed
- 4 samples → 4/5 fragments analysed where the HIV subtype was nonB non typable, A-like and G

General consideration

From the extraction of the nucleic acids to the rendering of the results (12 samples/run), the technical time for the Sanger sequencing was 10 days with the intervention of 2 technicians for the proofreading of the chromatograms and of a biologist for the interpretation of the results.
NGS sequencing makes possible to carry out the analysis in 4 days with 1 technician for the technical realization and 1 biologist for the analysis and interpretation of the results.
The concordance of the virus subtype was 100% and perfect consistency was identified between the reports of resistance mutations from the sequences obtained by the 2 methods (Sanger and NGS ABL).
The sensitivity of NGS sequencing made possible the identification of minority populations with mutations having an impact on the treatment for 8 samples.

Conclusion

NGS should occupy a more prominent place in HIV resistance surveillance and clinical care. The decreasing costs, due to the COVID-19 pandemic, and its ability to reveal resistant minority variants of the virus and the study of their impact. The ability to reveal resistant minority variants and the study of their impact allow to quickly adapt the treatment. The complete genome analysis provides information on the new HIV targets, capsid/maturation inhibitors, and the detection of compensatory mutations that could explain certain escapes without the detection of resistance mutations in the targeted genes.