Next Generation Sequencing for HIV virus genotyping and detection of antiviral resistance

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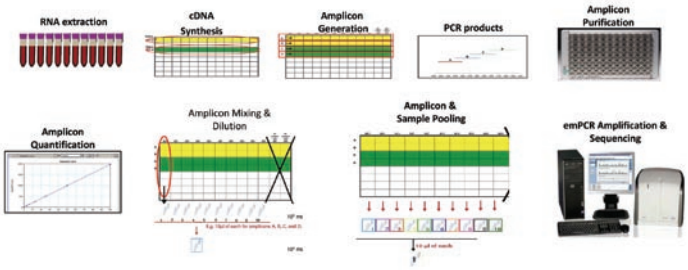
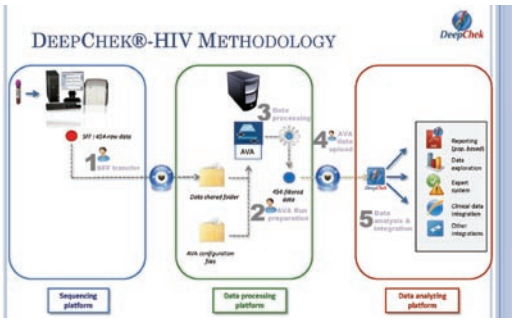
Background

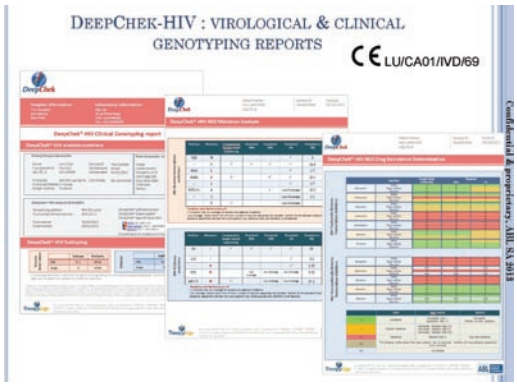
Sanger based HIV sequencing is currently the method of choice for the identification and follow-up of HIV drug resistance. To assess the utility of next generation sequencing (NGS) platform for the clinical HIV diagnostic laboratory, we have analyzed results obtained by Roche GS Junior (GSJ) and compared the mutations load and the prediction of HIV resistance using GS Junior with results derived by conventional sequencing on the AB3130 platform obtained by Roche GS junior (GSJ) and ABL DeepChek-HIV IT solution.

Materials and Methods

Amplicon Sequencing of HIV Reverse Transcriptase and Protease using a collaboration Yellow Plate from Roche Diagnostics







Results

Table 1. Comparison of HIV-1 subtypes detected by NGS and conventional sequencing

Patient	AB3130 RT	AB3130 Protease	454-DeepChek RT	454-DeepChek Protease
1	B	B	B	B
2	NA	NA	NA	NA
3	B	B	B	B
4	B	B	NA	B
5	B	B	B	B
6	B	D	B	B
7	29-BF	B	B	B
8	D	D	D	D
9	B	B	B	B
10	C	C	B	B

Table 2. Comparison of mutations detected by NGS and conventional sequencing

Patient	Viral Load Log/ Copies	AB3130	454-Deep-Chek	Additional Mut (%, Load)	Additional Resistance at 1% level
1	3.86			N/A	N/A
2	2.79	N/A	N/A		
3	2.65			N/A	N/A
4	2.95			PR (20%): D30N, M46I, G73S	Atazanavir/r (I) Fosamprenavir/r (I) Indinavir/r (I) Nelfinavir (R)
5	4.34			RT (1%): V118I RT (1%): G74I	**Abacavir (R) Didanosine (R)**
6	4.72			RT(1%): V106I PR (1%): M36I	**Efavirenz (I) Nevirapine (I)**
7	5.04			N/A	**N/A**
8	2.97			RT (Sanger): F227L	**N/A**
9	5.78			RT(1%): T215A RT(1%):K219Q	
10	3.14	Partial RT	Partial RT	RT(1%): K65R	**Abacavir (I) Tenofovir (I)**

*I, Low-level/Intermediate resistance Intermediate resistance; *R, High-level resistance

Conclusion

In our laboratory, NGS appears to be an effective new strategy for HIV-1 genotyping and detection of drug resistance. NGS was able to genotype HIV-1 accurately when compared to conventional sequencing and detected additional resistance-conferring mutations not detected by conventional Sanger sequencing. Samples with low viral loads were not reliable for either conventional or NGS sequencing. More sensitive detection and quantification of resistance mutations could have important clinical implications for the subsequent choice of antiviral treatment.