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

Methods

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- 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).



Fig. 1: The DeepChek® SingleRound RT-PCR and Sequencing Assay Technology.

- 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.



Fig. 2: The DeepChek® SingleRound RT-PCR and Sequencing Assays – Regions of interest for HIV (A) and HCV (B).



• 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

PERFORMANCES

- ☑ From 150uL to 1mL plasma
- ☑ 24 samples/kit
- \square <u>Specificity</u>: validated with most subtypes
- Sensibility: detection of low viral load using ultracentrifugation
- \square 12 months expiration date on an average
- ☑ No need for gel confirmation (soon)

SINGLEROUND RT-PCR

- \square Reduce risks of contamination
- ☑ No need for Nested-PCR (systematically)

Advanced Biological Laboratories e ID sample_2 nple Blood rP:ROTINT e dition 06-03-20 t version 10.4 scriptase C a Detected (HXB2 n	15, 15:06:13 Protease eference Sequence) Resistance n	C nutations in bold based on Sta	Type of report Corrected Your patient ID Genotyping method Homebrew Date of sample 2015-03-05 Date of sequencing 2015-03-05 Integrase C nford v7.9.1 (mutation score # 0)	GP120V3 Loop B (check for 46_BF)		Hospital info Test Hospital test address New-York	rmation	ABL Lab 36 av Vict 1411 Lux		tion		vsician details	
vorptase V35T, E36A, T39E, D67N, K70R, K1020, D123S, I135wtV/T/JA, A158wtS, S162C, K166R, K173A, Q174N, D177E, Q207E, R211K, L214F, K219E, V245Q, E291D, V220L (293V), P294T, Q34P, Q35D, R356K, M357R, Q359T, K366R, T377Q, K39R, T400A													
	Vol. L10F, T125, H3V, H5V, L19I, K20TiA, L33F, E35D, MoeL, S37D, R41K, I54V, I62V/Iwt, L63V, I64V, I66wiT, H69K, T74S, V82A, L89MI, L90M, I83L, 036F/imi D10E, S24M, D25E, V31L, V72L, L1011, T112V, G123S, A124T, R127K, K173R, K188R, H91L, V201L, K215N, T218L, N232D, L234L, D278A, S283G, R284G					DeepChek [®] -HIV Clinical Genotyping report							
юр	N301S/R/K/wt, T303wt/		Al241, K1276, K1266, K1666, H91L, V2011, K2166, 1216 006, R306K/w/Q, del307, Q310H, R311I, V318Y, T319A, I32			DeepChek®-H	IIV analysis	summary					
interpretation (g	eno2pheno-v3.3 2013) anford-7.0.1)					Patient/Sample in	nformation			Next Generatio	on Sequenc	ing (NGS) system	
Class	Drug		STAN ==(1) 7.0.1 27/02/2014 Z-Score	(22) (2) v3.3 2013		Name Your patient ID ABL/TE ID	John Doe Test123 DEV-99999	Sample ID Sample type Sample date		Assay Assay version Reagent Lot ID	1.0	he454-HIV 56767	
	Zidovudine	R	4.256	Resistant						Cartridge S/N	1		
	Didanosine	<mark>.</mark>	1.296	Sensible		Viral Load Viral Load Metho		mL Comments	My comments	Expiration date Test type		01/2020 Iotyping	
	Stavudine	<mark>.</mark>	1.828	Sensible		Sanger method				Notes	Gen	locybing	
NRTI	Lamivudine	S	0.389		0								
	Emtricitabine	S	0.389		0								
	Abacavir		2.635	Intermediate		DeepChek®-HIV a	inalysis informatio	n					
	Tenofovir	-	1.950	Intermediate		Sequencing plat	form	454 GS Junior	DeepChek	software version	n 1.1		
	Nevirapine	S	-2.266	Sensible		ProcessingSoftw		AVA v2.5.1		expert system	1.5		
NNRTI	Efavirenz	S	-3.145	Sensible				0.00.0000		algorithmsversion	ion 9.4		
	Etravirine	S	1.949	Intermediate		Date started Date finished		19/01/2012 19/01/2012	ANRS	(20 - 2011-10) institute (v8.0.1 - 09/02	2/2009.1		
	Rilpivirine Indinavir/r	R	9.535	Resistant		Date maneu			Stank	rd (6.1.1 - 30/11/2011)		
	Indinawr/r Saquinavir/r	R	9.535						Classificat	ion of mutations o	of interest: S	Stanford score <>0	
	Nelfinavir	R	10.013			DeepChek®-I	HIV Subtypi	ng					
	Fosamprenavir/r	R	10.233			Deepenek	ny Subtypi	-6					
PI	Lopinavir/r	R	11.653										
	Atazanavir/r	R	8.771	Resistant		se	Subtyp	e Similarity	e		Subtype	Similarity	
	Tipranavir/r	_ 1	2.438	Sensible		SON CLIP	B (*)	96.1%	Proteas	NGS	B (*)	99.1%	
	Darunavir/r	S	3.258	Sensible		NGS Saversite Saversite		95.6%	2	Sanger	В	98.7%	
	Raltegravir	S	susceptible (F	Predicted FC: 0.3)						_			
1	Elvitegravir	gravin Susceptible (Predicted FC: 0.9)					ation performed throug o an updated set of refe	h an homology testing o rence sequences.	of a 20% consensus sec	quence generated from	n all the reads	per	
	Dolutegravir	S	susceptible										
EI	Maraviroc	Not available	CCR5-antago to be effective	nists like Maraviroc (Celsentri/Selzentry) are not like (5)	y	 DeepChek*HIVIzs downstream analysis so 1 genotyping assays, TRUGENE*HIV-1 (Siema antisetrovinaldrugs based on the lavel of sen Diagnostic Use only with IVD information or w Into account by the Program. 5. The selection 	ens Healthcare Diagnostics Inc.) or Vir sh Nty of patient's HIV virus ("Analys Ith combination of IVD Information ar of drugs for the treatment of HIVInfe	25eq [®] HIV-1 (Abbott Laboratories) ("Π ε"). 2. AGL does not accept any respo d n on-IVD information. For research u ction is the responsibility of the physici	VD information") in order to obtain on sibility for the accuracy of the dat use only with non-IVD information al- tan in consultation with the patient.	HIV sequence analysis and HIV dru ts entered by the user or the const one 4. Resp onces to HIV treatment and reflance should not be placed o	g reclatance Interpreta equences of any Insoc are complex and affect on the Analyzes only for	ations to adapt accordingly patient's curacles in those data. 3. For in Vitro sted by a number of factors not taken r such purpose s. 6. The Analyses are	
			<u>stan</u>			not intended to replace professional medical	care and attention by a qualified medi	cal practitioner and consequently ABL	does not accept any responsibility fo	or the selection of drugs and the pat	fent's response to trea	atmant.	
Susceptible (S)/ Potential low-level resistance (PLLR) Low-level resistance (LLR) / intermediate resistant (IR)						<i>(</i>	content @ 2013 APL 5	A Portions protected u	ander 115 erreinen an	77655721 77655/075	77655007		
	Low-revel resistance (LLK) / intermediate resistant (IK) High-level resistance (HLR)					TherapyEdge 27	79970; European tradem	arks 007550528 and 00				ABL Biological	
_			ragenerer reasonce (fLR)			inte	ernational equivalent.						

& B): clinical genotyping reports (combining genotypes, mutations, and drug resistance assessment) were automatically generated.

• All results were in agreement with previous samples characterization.

☑ No Nested-PCR used with high viral load samples

☑ Not targeting the genes used for viral load determination (HIV & HCV)

☑ Better and shorter workflow (cost-effectiveness)

 \square Use of high fidelity enzymes

☑ Include more samples per run

Fig. 5: Performances and reporting for Sanger (A) and Next Generation Sequencing (B) overview.

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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