ABL received CE-IVD Mark for its "DeepChek® Assay Whole Genome HIV-1 Genotyping"

DEEPCHEK®-HIV ASSAYS & SOFTWARE

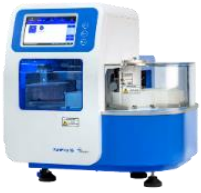
(RT/PR – INT – Whole Genome: RUO & CE IVD)

A unique and complete portfolio for HIV genotyping through SANGER & Next Generation (NGS) sequencing

- We are pleased to announce that our DeepChek® Assay Whole Genome HIV-1 Genotyping, intended to be used for HIV-1 genotyping through NGS sequencing, is now CE-IVD marked.
- This assay completes a unique line of products dedicated to HIV-1 Genotyping and resistance determination through SANGER and NGS sequencing.

- RT / PROT (CE-IVD & RUO)
- INT (CE-IVD & RUO)
- V3 Loop (RUO)
- Whole Genome (CE-IVD & RUO)

Overview of a typical workflow



RNA extraction

PCR

SANGER sequencing

NGS sequencing

Data analysis

Instruments available through reagent rental

Highlights on the DeepChek® technology

ROBUST
CE-IVD / RUO (with analytical data)

FLEXIBLE
For low to high throughput
1-384 samples in one run

FAST
~1-2 days (SANGER)
~2-4 days (NGS)

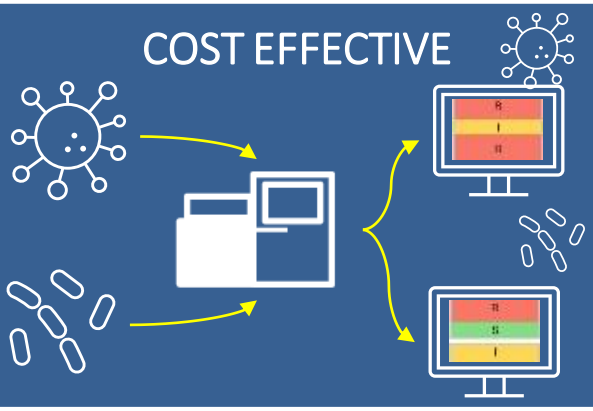
STANDARDIZED
Kits, Software, support, Integration with LIMS/HIS, Automation

COMPREHENSIVE
Genotyping, Drug Resistance, Tropism, Reporting, Storage…

SECURED
Healthcare Cloud Access, Local installation

POOLING CAPACITY
Pool several DeepChek® Libraries (samples) from different applications in the same NGS run

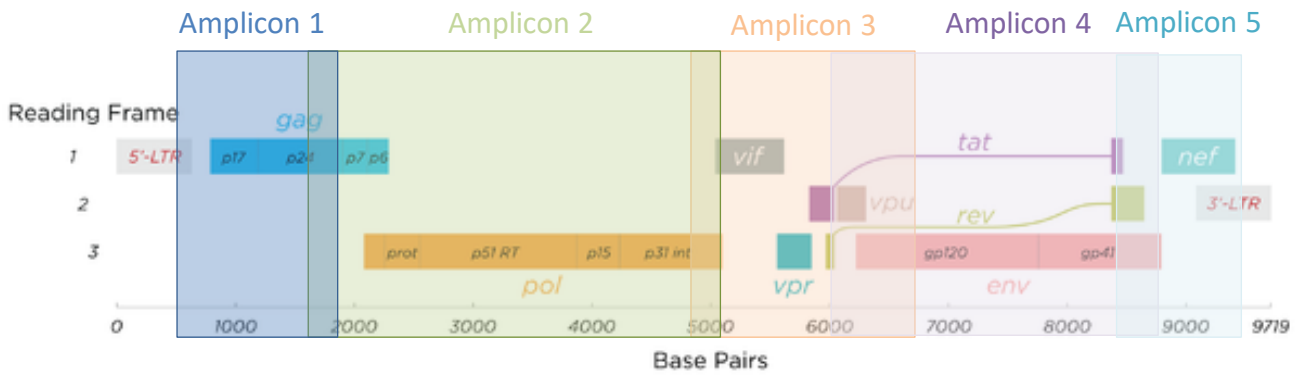
COST EFFECTIVE



- HIV
- SC2
- TB
- HCV
- HBV
- CMV
- HSV
- 16s RNA
- HPV
- BKV
- …

DeepChek® Assay Whole Genome HIV-1 Genotyping

- Multiplex 2-PCR steps test using extracted RNA from HIV-1 positive patients followed by NGS
- Used for amplifying whole-genome of the human immunodeficiency virus type one (HIV-1) for the determination of antiviral resistance associated mutations in HIV-1 targeted genes including new entry & capside inhibitors
- Inclusivity: excellent coverage of all representative HIV-1 subtypes from M group strains (A1, A2, B, C, D, F1, F2, G, H, J, K and L)
- Cross-reactivity: The assay reacted (i.e., good agarose gel band) for some of the tested microorganisms commonly found in infectious diseases specimens. After NGS and data analysis, no reads mapped with HIV-1
- LoD: 25'000 cp/mL
- Validated with Roche MagNA Pure 24 instrument and reagents, Thermo Fisher Scientific ProFlex PCR System; (model 3 x 32) and Illumina iSeq-100 instrument (SBS chemistry, iSeq-100 Flow Cell) with a run of 24 samples
- Shall be compatible with other magnetic-beads RNA isolation instruments and reagents, any PCR thermal cycler with ramp rate of ≥ 1°C/s and NGS instruments equivalent to SBS chemistry
- Shall be compatible with run of 48 / 96 and 384 samples to reach assay cut-off (minimal median total coverage of 1000 reads for the amplicons and a Phred Quality Score Q30>80% (or equivalent))
- Push-button data analysis validated with the use of the ABL DeepChek® software (Whole genome HIV-1 (REF S-22-05 (WHM)). Shall be compatible with other downstream NGS analysis software tailored to HIV-1.
- Same thermal cycling program in five (5) distinct wells
- Coverage : ~ 9600 bp
- Number of amplicons: 5x ~700 to 3500 bp



HIV-1 gene associated with antiviral resistance when using the DeepChek® Assay Whole Genome HIV-1 Genotyping

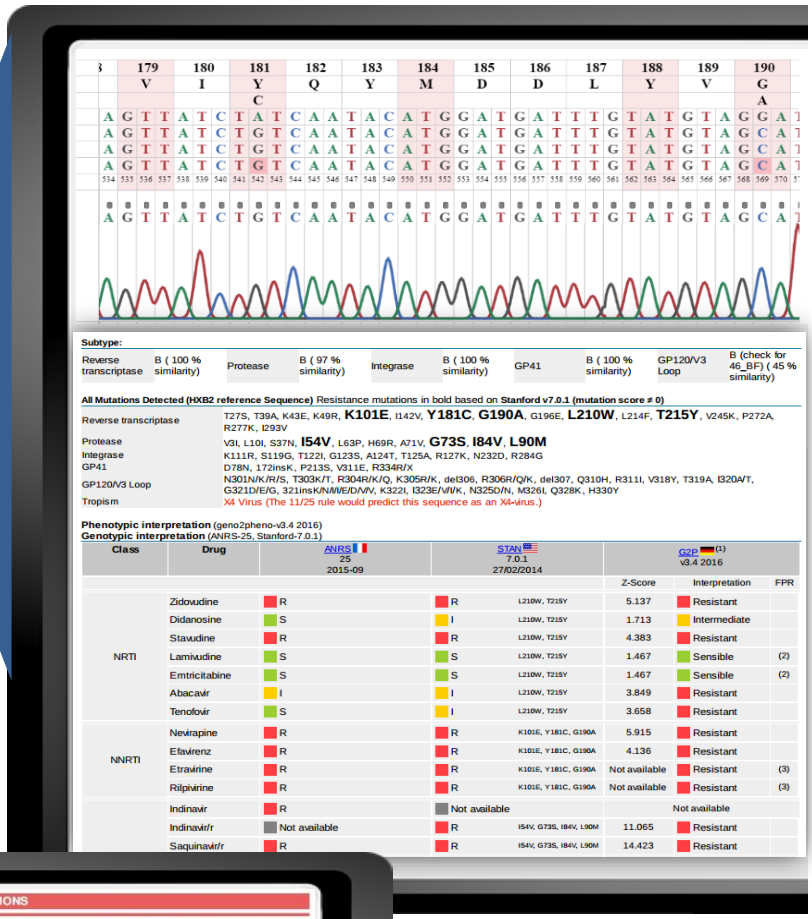
Anti-HIV drug class	HIV-1 gene target	Related drugs	Assay fragment #
Capsid inhibitors	*gag*	lenacapavir	1
Nucleoside reverse transcriptase inhibitors (NRTIs)	*reverse transcriptase*	zidovudine, lamivudine, emtricitabine,abacavir, tenofovir disoproxil fumarate, tenofovir alafenamide , islatravir, didanosine and stavudine	2
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	*reverse transcriptase*	efavirenz, nevirapine, etravirine, rilpivirine and doravirine	2
Protease inhibitors (PIs)	*protease*	lopinavir, atazanavir, darunavir, ritonavir, indinavir, saquinavir, nelfinavir, fosamprenavir and tipranavir	2
Integrase inhibitors (IIs)	*integrase*	raltegravir, elvitegravir, dolutegravir, cabotegravir, bictegravir	2
n.a	*vif, vpr, vpu (accessory proteins)*	enfuvirtide	3
Fusion inhibitors	*gp41*	Fostemsavir	4
Post-attachment inhibitors	*gp120*	n.a	4
n.a	*nef (accessory protein)*	n.a	5

Reporting

CE IVD

SANGER

ViroScore®



Subtype:									
Reverse transcriptase	B (100 % similarity)	Protease	B (97 % similarity)	Integrase	B (100 % similarity)	GP41	B (100 % similarity)	GP120/V3 Loop	B (check for 46_BF) (45 % similarity)

All Mutations Detected (HXB2 reference Sequence) Resistance mutations in bold based on **Stanford v7.0.1 (mutation score ≠ 0)**

Reverse transcriptase	T27S, T39A, K43E, K49R, **K101E**, I142V, **Y181C**, **G190A**, G196E, **L210W**, L214F, **T215Y**, V245K, P272A, R277K, I293V
Protease	V3I, L10I, S37N, **I54V**, L63P, H69R, A71V, **G73S**, **I84V**, **L90M**
Integrase	K111R, S119G, T122I, G123S, A124T, T125A, R127K, N232D, R284G
GP41	D78N, 172insK, P213S, V311E, R334R/X
GP120/V3 Loop	N301N/K/R/S, T303K/T, R304R/K/Q, K305R/K, del306, R306R/Q/K, del307, Q310H, R311I, V318Y, T319A, I320A/T, G321D/E/G, 321insK/N/M/E/D/V/V, K322I, I323E/V/I/K, N325D/N, M326I, Q328K, H330Y
Tropism	X4 Virus (The 11/25 rule would predict this sequence as an X4-virus.)

Phenotypic interpretation (geno2pheno-v3.4 2016)
Genotypic interpretation (ANRS-25, Stanford-7.0.1)

Class	Drug	ANRS 25 2015-09	STAN 7.0.1 27/02/2014		G2P (1) v3.4 2016		
					Z-Score	Interpretation	FPR
NRTI	Zidovudine	R	R	L210W, T215Y	5.137	Resistant	
	Didanosine	S	I	L210W, T215Y	1.713	Intermediate	
	Stavudine	R	R	L210W, T215Y	4.383	Resistant	
	Lamivudine	S	S	L210W, T215Y	1.467	Sensible	(2)
	Emtricitabine	S	S	L210W, T215Y	1.467	Sensible	(2)
	Abacavir	I	I	L210W, T215Y	3.849	Resistant	
	Tenofovir	S	I	L210W, T215Y	3.658	Resistant	
NNRTI	Nevirapine	R	R	K101E, Y181C, G190A	5.915	Resistant	
	Efavirenz	R	R	K101E, Y181C, G190A	4.136	Resistant	
	Etravirine	R	R	K101E, Y181C, G190A	Not available	Resistant	(3)
	Rilpivirine	R	R	K101E, Y181C, G190A	Not available	Resistant	(3)
	Indinavir	R	Not available		Not available		
	Indinavir/r	Not available	R	I54V, G73S, I84V, L90M	11.065	Resistant	
	Saquinavir/r	R	R	I54V, G73S, I84V, L90M	14.423	Resistant	

CE IVD

NGS

DeepChek®

SUBTYPING

PROT	B (94.95%)	RT	B (95.83%)
INT	B (95.53%)		

COVERED POSITIONS

PROT	1-99	RT	1-327
INT	32-289		

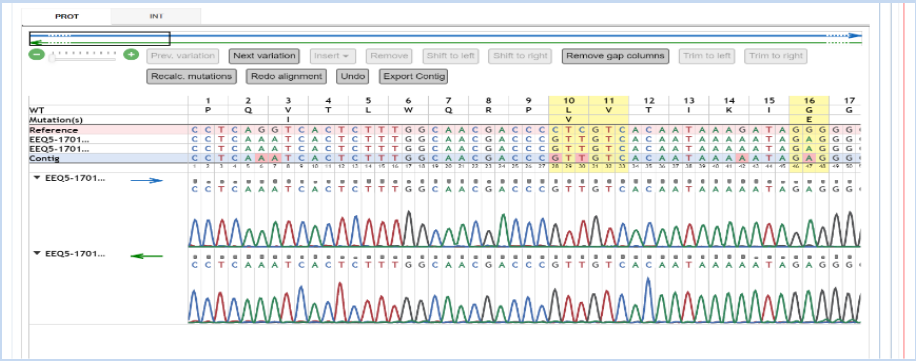
[RT, PROT, INT] : Subtype B K03455 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool). Subtyping determination performed through homology testing of a 20% consensus sequence generated from all the reads mapped to this particular region and compared to an updated set of reference sequences.

ANTIRETROVIRAL DRUG		INTERPRETATION	DEEPCHEK® HIV (Stanford 8.8)	
	Generic name	Assessment	Resistance mutations >20.00%	Resistance mutations between >3% and <20.00%
PI	Atazanavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Darunavir/r	I	I54L (78.56%)	
	Fosamprenavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Indinavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Lopinavir/r	I	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Nelfinavir	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Saquinavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)	
	Tipranavir/r	S	I54L (78.56%)	
NRTI	Abacavir	I	D67N (96.96%), M184V (99.35%)	
	Didanosine	I	D67N (96.96%), M184V (99.35%)	
	Emtricitabine	R	M184V (99.35%)	
	Lamivudine	R	M184V (99.35%)	
	Stavudine	S	D67N (96.96%), M184V (99.35%)	
	Tenofovir	S	D67N (96.96%), M184V (99.35%)	
	Zidovudine	S	D67N (96.96%), M184V (99.35%)	
NNRTI	Doravirine	S		
	Efavirenz	S		
	Etravirine	S		
	Nevirapine	~~S~~		
	Rilpivirine	S		
INI	Bictegravir	I	G140S (99.66%), Q148H (99.33%)	
	Dolutegravir	I	G140S (99.66%), Q148H (99.33%)	
	Elvitegravir	R	G140S (99.66%), Q148H (99.33%)	
	Raltegravir	R	G140S (99.66%), Q148H (99.33%)	

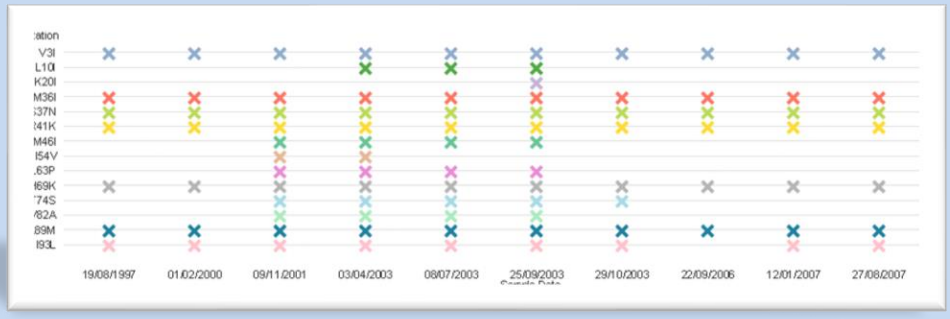
S — Susceptible (S) / Potential low-level resistance (PLLR)
I — Low-level resistance (LLR) / Intermediate resistant (IR)
R — High-level resistance (HLR)

Key features

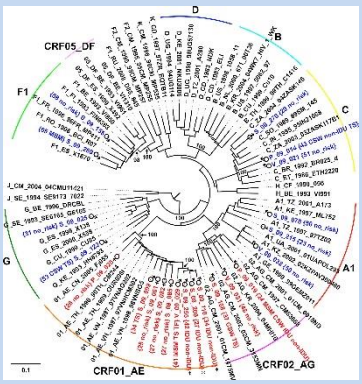
EMBEDDED CHROMATOGRAM EDITOR



SINGLE or CUMULATIVE GENOTYPING



SUBTYPING DRUG RESISTANCE ASSESSMENT



Drug	ANRS 31 12-2020		STAN 9.0 03-2021	
Zidovudine	R	D67N, L210W, T215Y, K219Q	R	D67N, L210W, T215Y, K219Q
Didanosine	Not available		R	D67N, T69D, L210W, T215Y, K219Q
Stavudine	Not available		R	D67N, T69D, L210W, T215Y, K219Q
Lamivudine	S		S	L210W, T215Y
Emtricitabine	S		S	L210W, T215Y
Abacavir	R	D67N, L210W, T215Y	I	D67N, L210W, T215Y, K219Q
Tenofovir	R	E44D, D67N, T69D, L210W, T215Y	I	D67N, L210W, T215Y, K219Q

		Algorithm	Sanger based sequencing	15.00%	10.00%	5.00%	3.00%
Abacavir	ANRS		I	I	I	I	I
	Grade		I	I	I	I	I
	Rega institute		S	S	S	S	S
	Stanford		LLR	LLR	LLR	LLR	LLR
Didanosine	ANRS		NA	NA	NA	NA	NA
	Grade		I	I	I	I	I
	Rega institute		S	S	S	S	S
	Stanford		LLR	LLR	LLR	LLR	LLR
Emtricitabine	ANRS		R	R	R	R	R
	Grade		R	R	R	R	R
	Rega institute		R	R	R	R	R
	Stanford		HLR	HLR	HLR	HLR	HLR

- HIVDB/STANFORD
- ANRS
- REGA
- GENO2PHENO
- HIV-GRADE
- RIS
- RENAGENO

Other features

Technology

- Web-based
- Database included
- Local or Cloud (+HDS) hosting
- Unlimited user accounts
- Unlimited analyses

Security

- Data access restriction
- Logging of user accesses
- Encrypted database
- Reports validation
- Quality control

Main features

- SANGER & NGS
- Subtyping & virtual-phenotyping
- Drug resistance
- GSS & regiment ranking
- Tropism
- Export
- Batch mode analysis

Services

- Guidelines updates
- Annual upgrades (versions)
- Historical data import
- LIMS integration
- HIS integration
- Support
- Trainings
- Report customization

References

DeepChek® CE-IVD Assays

PR / RT Genotyping and Drug Resistance V1 (CE-IVD)	121A24
INT Genotyping and Drug Resistance V1 (CE-IVD)	122A24
DeepChek® Assay **Whole Genome** HIV-1 Genotyping V1.x (24 tests) (CE-IVD)	170A24
PR / RT Sequencing V1 (CE-IVD) (accessory for 121A24) (Sanger)	125A24
INT Sequencing V1 (CE-IVD) (accessory for 122A24) (Sanger)	126A24

DeepChek® RUO Assays

PR / RT Genotyping and Drug Resistance V1 (RUO)	101B24
INT Genotyping and Drug Resistance V1 (RUO)	102C24
DeepChek® Assay **Whole Genome** HIV-1 Genotyping V1.x (24 tests) (RUO)	170A24
V3 Loop/Tropism Assay (RUO)	103A24

General Laboratory Products

DeepChek® SANGER SEQUENCING REACTION V1 (24rx/48rx)	123A24/123A48
DeepChek® NGS LIBRARY PREPARATION V1 (24 indexes)	116B24+124B24
DeepChek® NGS LIBRARY PREPARATION V1 (48 indexes)	116B48+124B48
DeepChek® NGS LIBRARY PREPARATION V1 (96 indexes)	116B96+124B96
DeepChek® NGS LIBRARY PREPARATION V1 (384 indexes)	116B384+124B384
DeepChek® NGS Clean-up beads (60mL)	N411-02
DeepChek® 96x0.2 mL wells plate (25 units)	B70501-01

Software – HIV module

DeepChek® (CE-IVD)	S-12-023 (HUS)
ViroScore® (CE-IVD)	S-09-014 (VSH)