ABL Advanced Biological Laboratories

Improving Disease Management

DEEPCHEK®-HIV ASSAYS & SOFTWARE

End-to end Solutions for HIV Genotyping for Drug Resistance Assessment (**SANGER & NGS**)

PRESENTATION

Confidential & Proprietary ABL
Do Not Distribute – Strictly Private and Confidential

Presentation of ABL

About us

Advanced Biological Laboratories (ABL), S.A., is a diagnostic and medical software company founded in 2000 as a spin-off from LIH (Luxembourg)

ABL's mission is to develop, manufacture and commercialize proprietary end-to-end diagnostic solutions for infectious diseases and oncology, in recurrent chronic diseases

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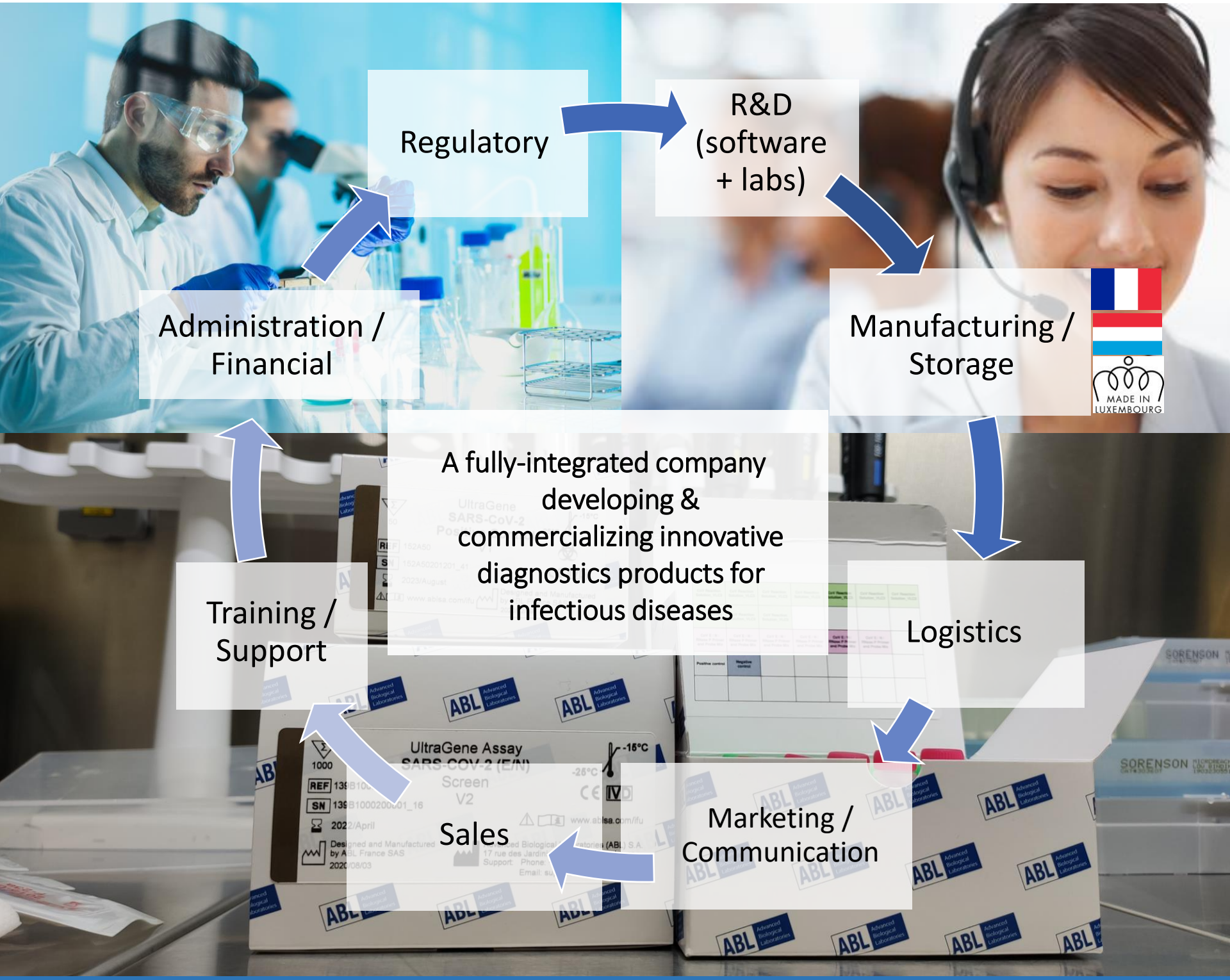
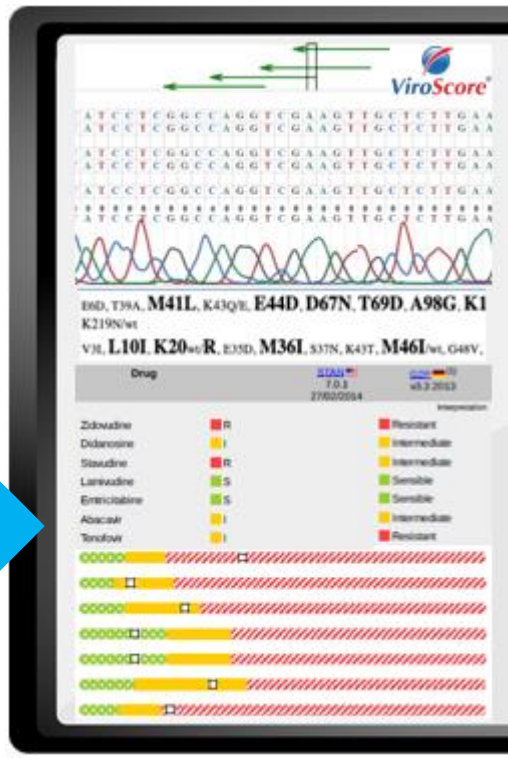
The group is a 100% privately owned company, with offices in Luxembourg, France, Spain, South Africa and United States

An international, fully-integrated Diagnostics company



Presentation of ABL Genotyping solutions

DeepChek®



Workflow Overview

RNA extraction

PCR

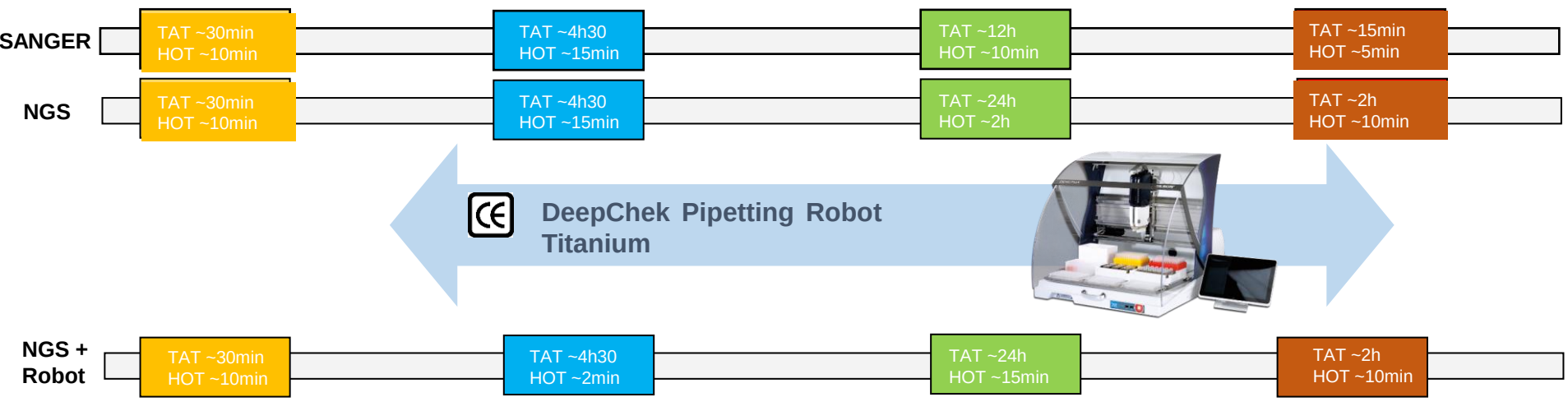
SANGER sequencing

NGS sequencing

Data analysis

Lab & data analysis automation

	SANGER	NGS
Turnaround time	~17h	~3-4 days
Hands-on-time	~40min	~1-3 h



Pipetting Robot

LIBRARY PREPARATION

Fragmentation plate set-up

Fragmentation reaction

End-repair & A-tailing plate set-up

End-repair & A-tailing reaction

Adaptor ligation set-up & reaction on-deck

AMPLIFICATION

SPRI clean-up & PCR mix distribution

PCR

LIBRARY CLEAN-UP

Centrifuge

Post PCR clean-up

NORMALIZATION

Quantification

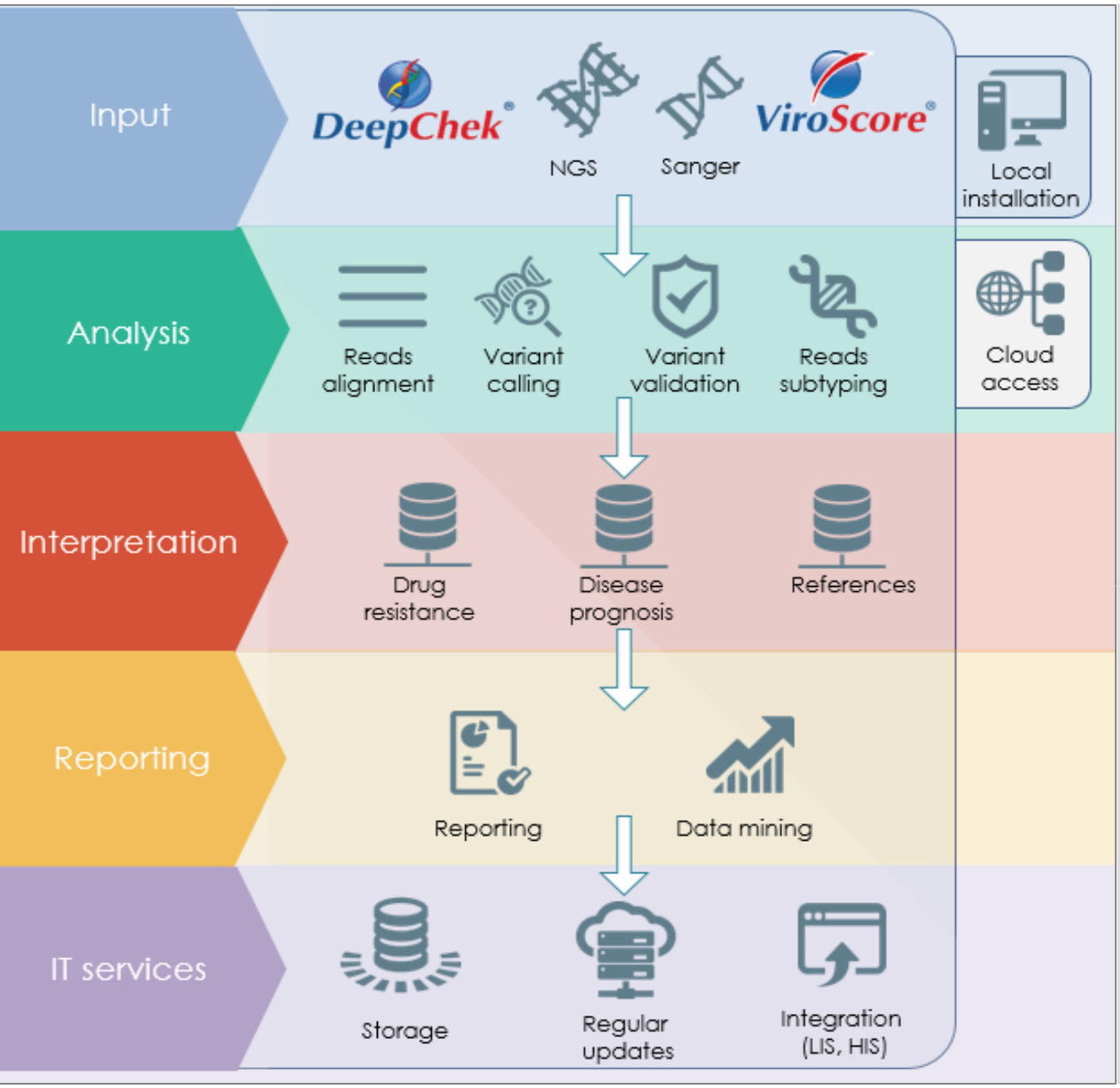
Normalization and Pooling in a single tube

Automated protocol is complet

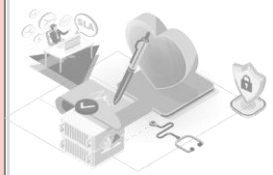
SEQUENCING

Proceed to sequencing on NGS Platform

Software workflow



Cloud



Secured Healthcare environment (**HDS**)

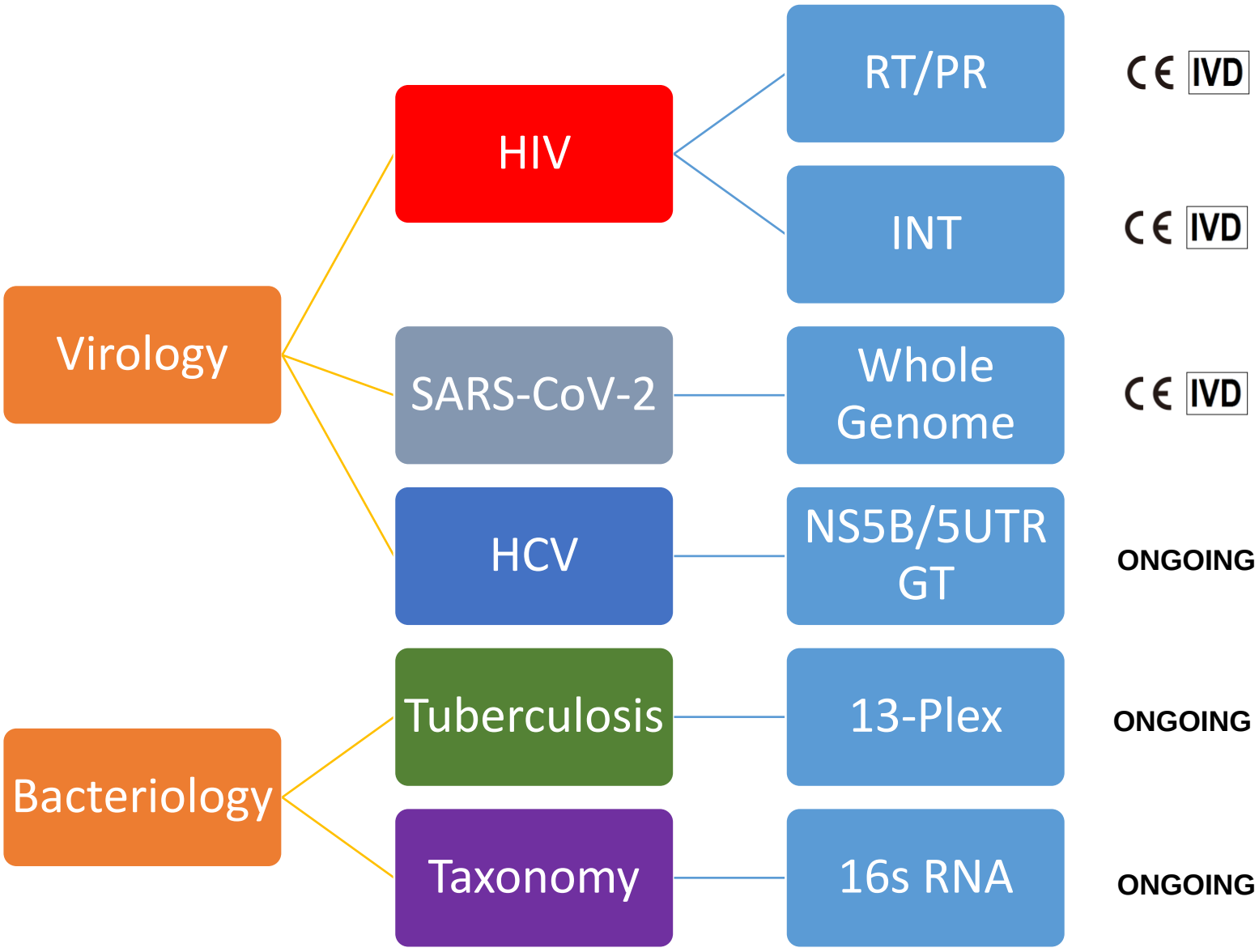
Nadis, HIV Ref Lab in Rouen…

Local Virtual Private Server

Local Hardware

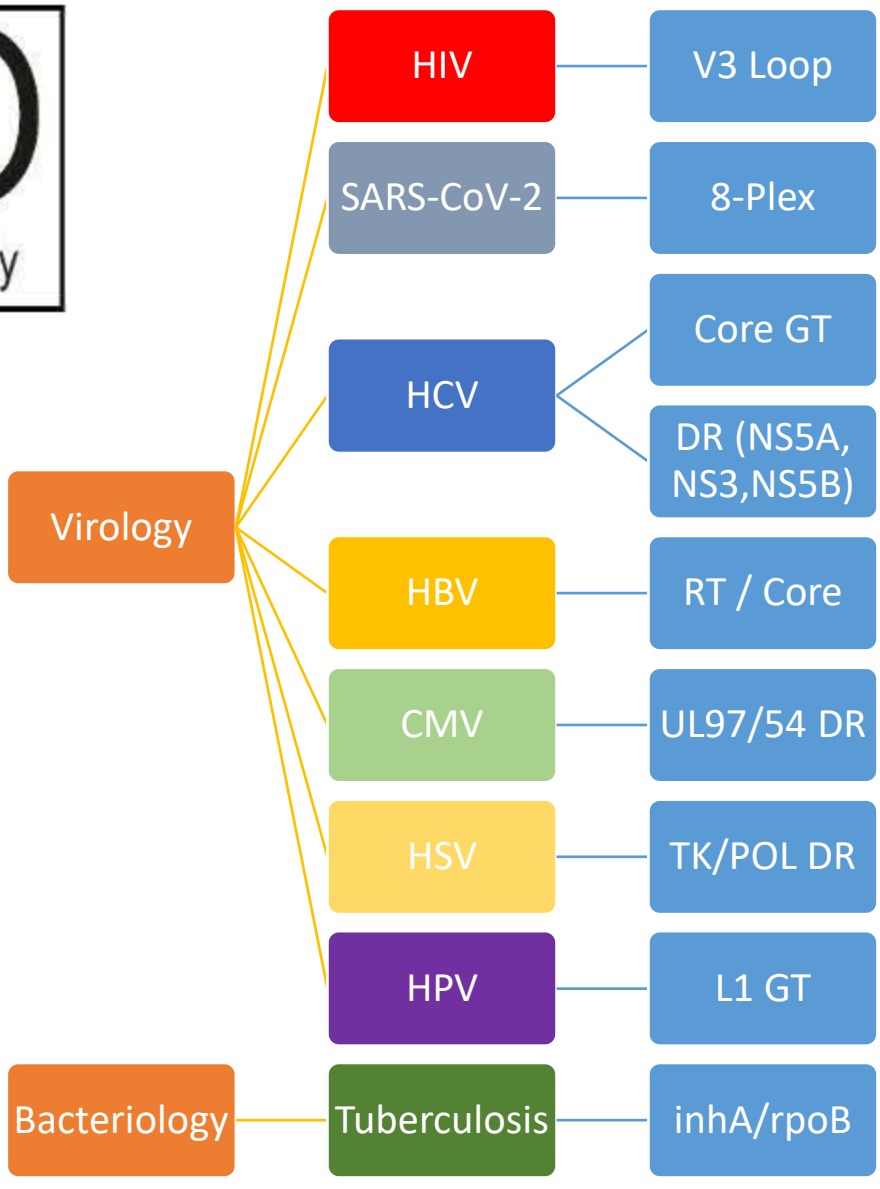
Regulated applications

CE FDA 510(k) US- FDA | Australian Government Department of Health Therapeutic Goods Administration TGA- Australia | ANVISA ANVISA-Brazi | CERTIFIED ISO 13485 MEDICAL DEVICES • QUALITY MANAGEMENT | All pharmaceutical sites certified ISO 15378 GMP compliant | CDSCO CDSCO | FDA US- FDA



Research use applications

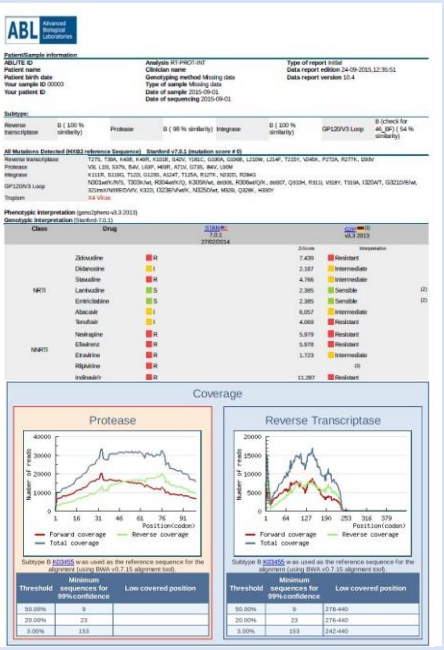
RUO
Research Use Only



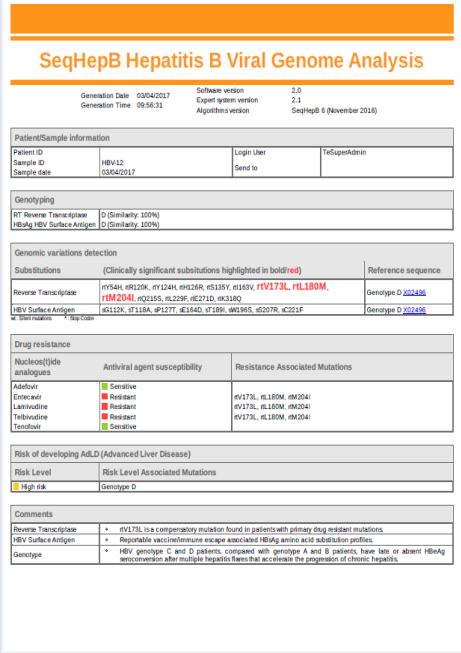
Examples of reports

GENOTYPING

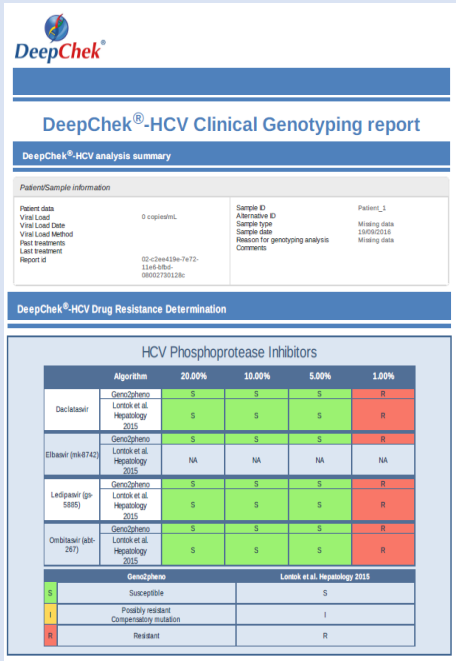
HIV



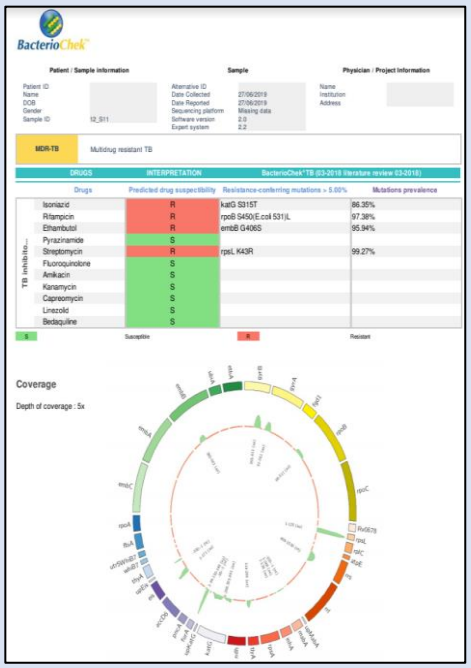
HBV



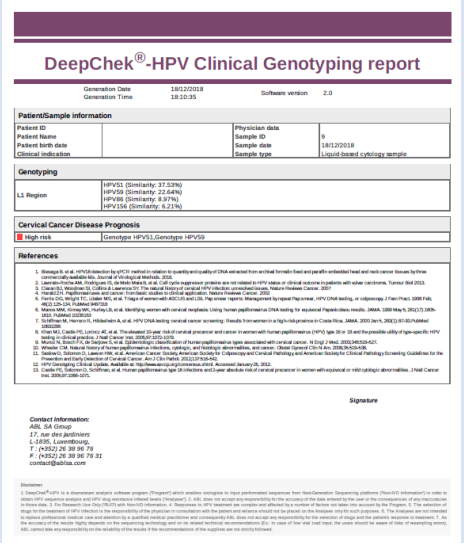
HCV



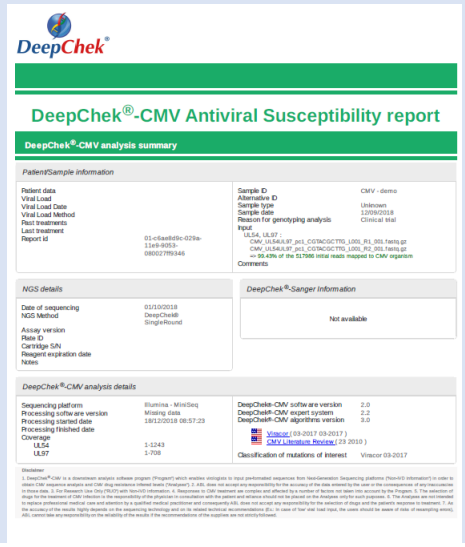
TB



HPV

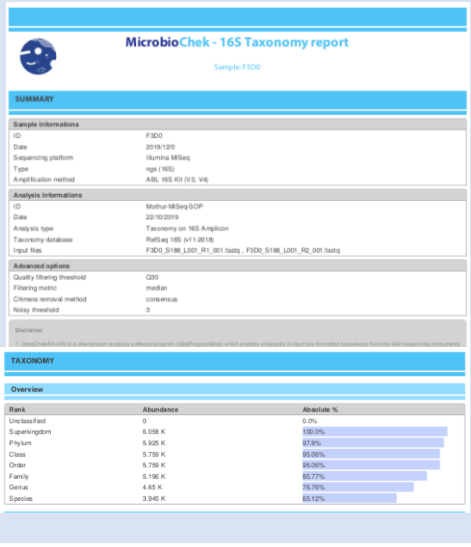


CMV



HSV

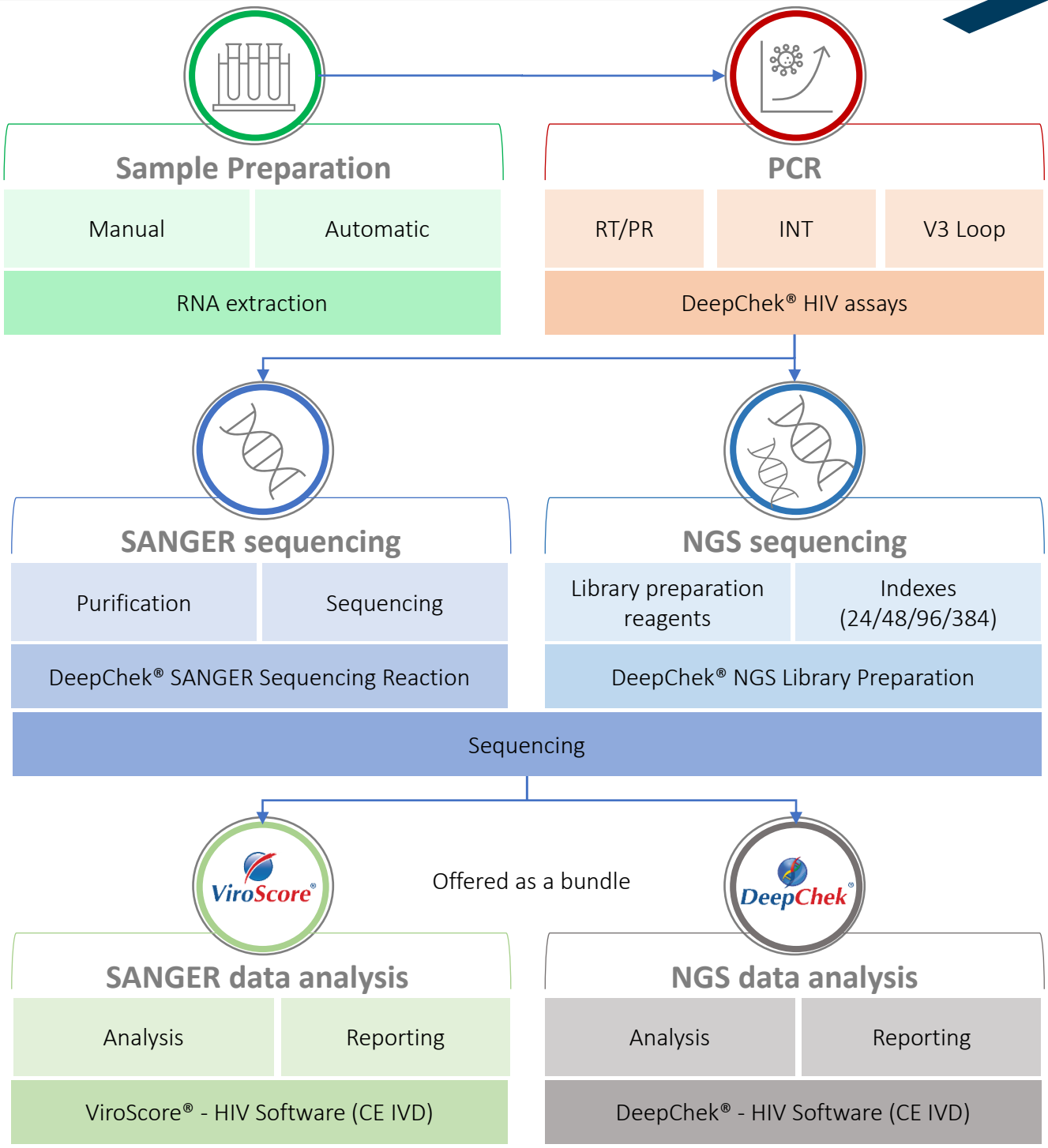
16s RNA



HIV Genotyping solutions

DEEPCHEK®-HIV ASSAYS & SOFTWARE
Workflow Overview

HIV



DEEPCHEK®- HIV ASSAYS
Highlights

HIV

- **LoD**: 1000 copies/mL (down to ~50-100 copied/mL)
- **High analytical reproducibility** and **repeatability** were evidenced by Percent Agreement being 100%
- **Highly specific** –pure & CRF strains
- From sample to drug resistance in ~17 hours for SANGER & 3-4 daya for NGS for 24 - 96 samples

DeepChek® Assay	Downstream sequencing instrument used with DeepChek® Assay	Device 2 used for agreement concordance	Nb. of samples tested	Concordance (%)
PR/RT + INT	Illumina MiSeq	Abbott® Dx - ViroSeq® HIV-1 Genotyping PR/RT + INT (Sanger)	23	100%
PR/RT + INT	Illumina MiSeq	Vela Dx - Sentosa® HIV-1 Genotyping PR/RT/INT (NGS)	18	100%

100% agreement with annual ANRS quality control programs

100% agreement with annual QCMD panel (RT+PR & INT) studies

DEEPCHEK®-HIV ASSAYS & SOFTWARE
ABL Plan for IVDR

HIV

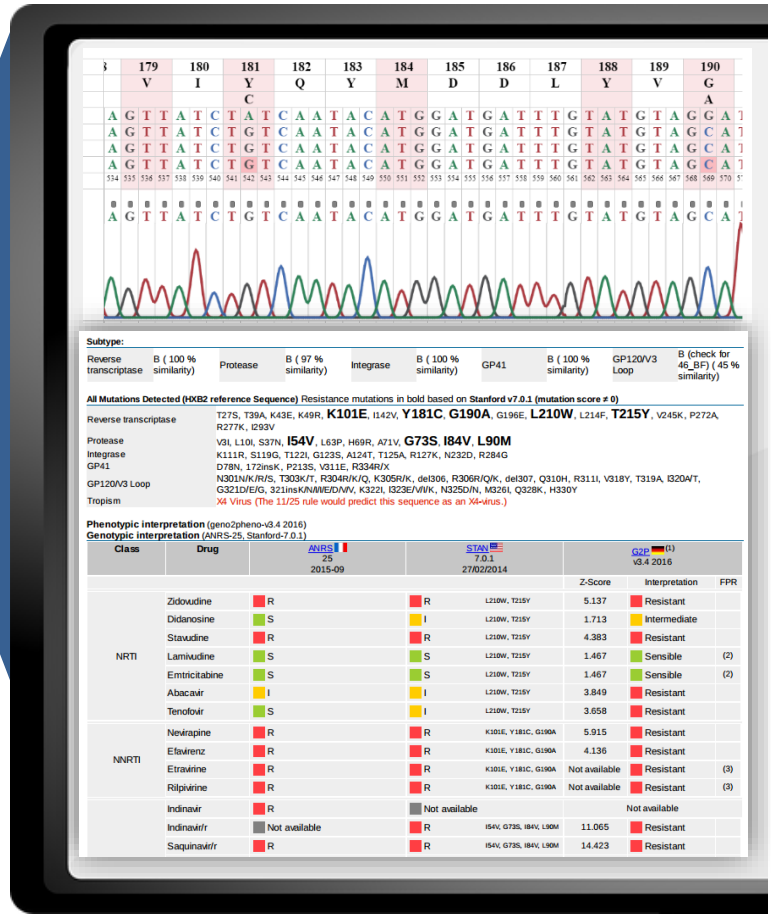
- Production of the IVD kits is already ISO 9001 certified
- Full design, development, production and support of IVD medical devices process is compliant with the ISO 13485:2016
- Since 2019, the Quality Management System (QMS) is developed to be meet the essential requirements of the new EU regulation for IVD medical devices (2017/746) for Class C and D products (highest classification)
- The Compliance team is coached and supported externally by a german group of experts in medical devices (reagents, instruments and software) to be ready for the certification
- Contacts with notified bodies to review and certify each IVD product according to the new EU regulation (ISO 13485 and technical file)
- ABL has a regulatory strategy for each product to be compliant with the new EU regulation, including the transition period

DEEPCHEK®-HIV ASSAYS & SOFTWARE
Reporting

HIV

SANGER

ViroScore® CE IVD



	179	180	181	182	183	184	185	186	187	188	189	190
	V	I	Y	Q	Y	M	D	D	L	Y	V	G
			C									A

Subtype:

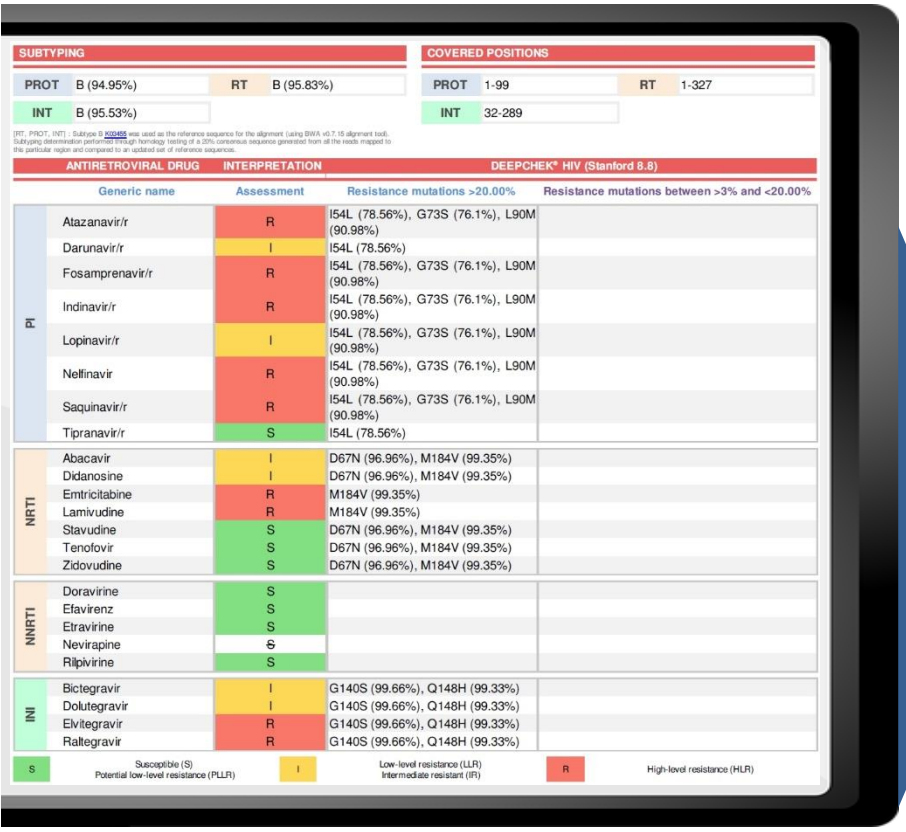
Reverse transcriptase	B (100 % similarity)	Protease	B (97 % similarity)	Integrase	B (100 % similarity)	GP41	B (100 % similarity)	GP120/V3 Loop	B (check for 46_BF) (45 % similarity)	

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/N/V, K322I, I323E/V/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	



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 25% 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)

NGS

DeepChek® CE IVD

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

Technology	Security	Main features	Additional features	Services
•Web-based (browser only) •Software & database •Local or Cloud (+HDS) hosting •Unlimited user accounts •Unlimited analyses	•Data access restriction (pools, read-only mode…) •Logging of user accesses •Encrypted database •Reports validation	•SANGER data management (AB1, FASTA) with embedded chromatogram editor •NGS data management (FASTQ) with dedicated pipeline •Genotyping (per sample & cumulative) •Subtyping •Virtual-phenotyping •Drug resistance through up-to-date guidelines •GSS determination and regiment ranking •Tropism •Reporting & labelling	•Report customization •Contamination check •Quality control •Export (reports, FASTA, XML…) •Batch mode analysis •Data mining	•Constant updates •Annual upgrades (versions) •Historical data import •LIMS integration •HIS integration •Support •Trainings

A WEB-BASED SYSTEM

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

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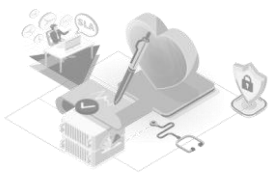
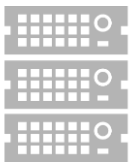
SYSTEM AVAILABILITY

Cloud

Local Virtual Private Server



Secured Healthcare environment (HDS)



Local Hardware

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

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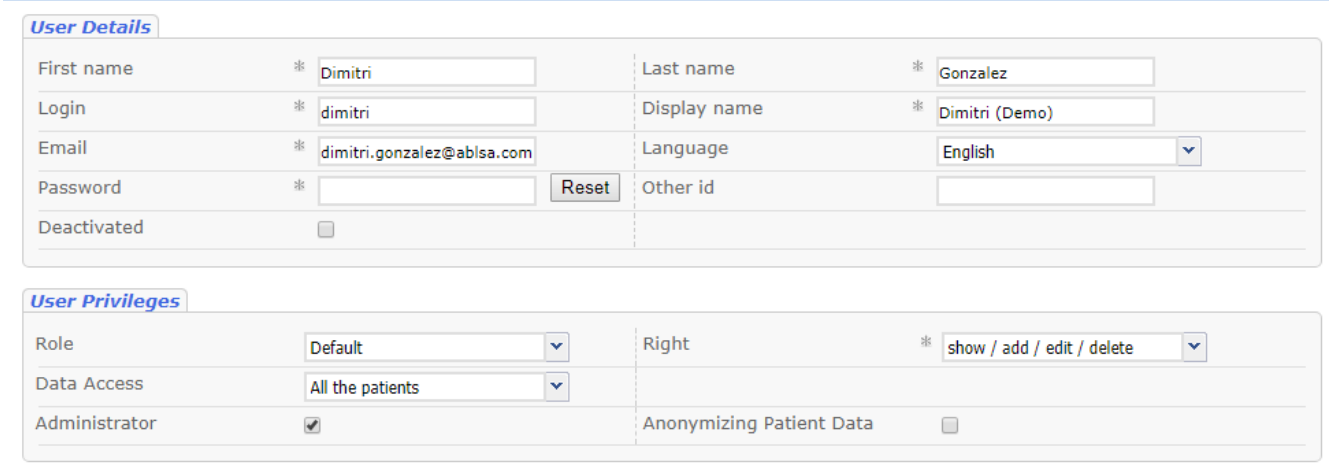
SECURED DATA ACCESS

MULTI-SITE COLLABORATIVE ACCESS POSSIBLE



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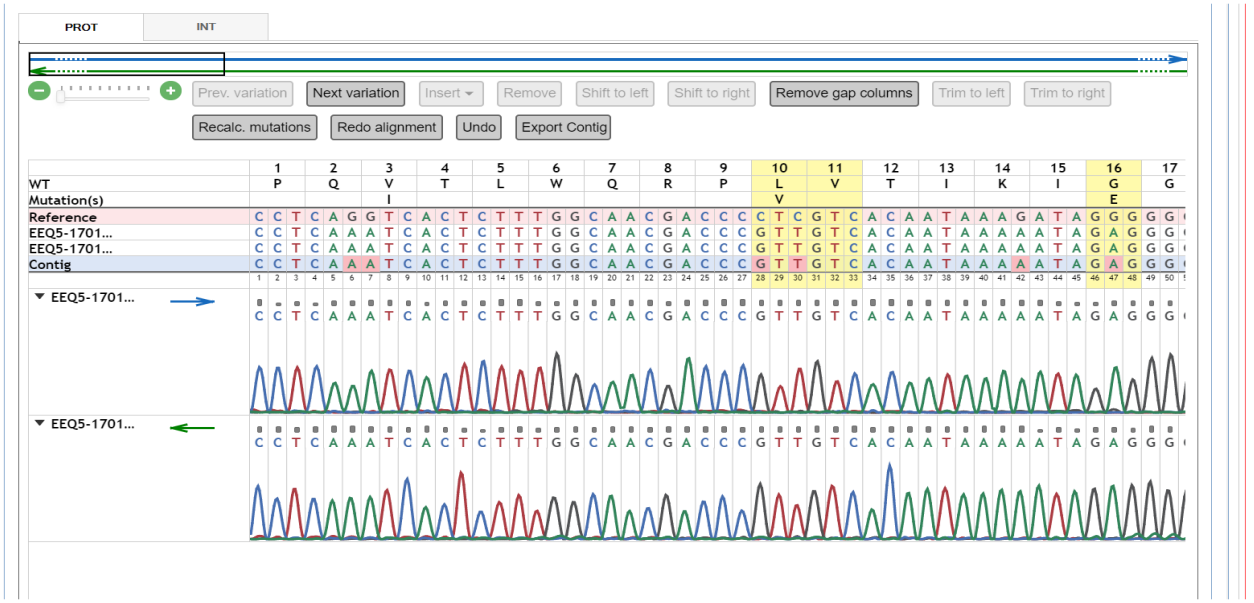
EMBEDDED CHROMATOGRAM EDITOR



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SINGLE or CUMULATIVE GENOTYPING

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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SUBTYPING

COMET

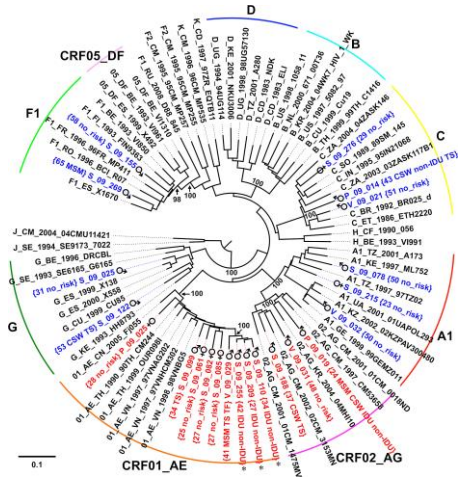
COMET HIV-1

(COntext-based Modeling for Expeditious Typing)

LUXEMBOURG INSTITUTE OF HEALTH

RESEARCH DEDICATED TO LIFE

PHYLOGENETIC ANALYSIS



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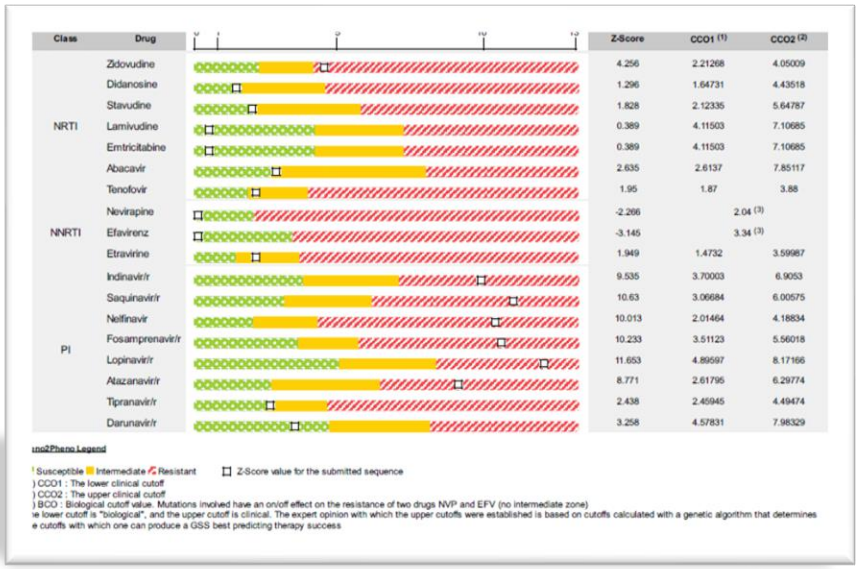
VIRTUAL-PHENOTYPING



Class	Drug	Z-Score	CCO1 (1)	CCO2 (2)
NRTI	Zidovudine	4.256	2.21268	4.05009
	Didanosine	1.296	1.64731	4.43518
	Stavudine	1.828	2.12335	5.64787
	Lamivudine	0.389	4.11503	7.10685
	Emtricitabine	0.389	4.11503	7.10685
	Abacavir	2.635	2.6137	7.85117
	Tenofovir	1.95	1.87	3.88
NNRTI	Nevirapine	-2.266		2.04 (3)
	Efavirenz	-3.145		3.34 (3)
	Etravirine	1.949	1.4732	3.59987
PI	Indinavir/r	9.535	3.70003	6.9053
	Saquinavir/r	10.63	3.06684	6.00575
	Nelfinavir	10.013	2.01464	4.18834
	Fosamprenavir/r	10.233	3.51123	5.56018
	Lopinavir/r	11.653	4.89597	8.17166
	Atazanavir/r	8.771	2.61795	6.29774
	Tipranavir/r	2.438	2.45945	4.49474
	Darunavir/r	3.258	4.57831	7.98329

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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DRUG RESISTANCE ASSESSMENT

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

SANGER

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

NGS

	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

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GSS & REGIMEN RANKING

Recommendation	Combination Regimen	Algorithm	Sanger based sequencing	15.00% ↓	10.00%	5.00%	3.00%
AI	Emtricitabine Tenofovir Dolutegravir	ANRS	1 / 3	1 / 3 *	1 / 3 *	1 / 3 *	1 / 3 *
		Grade	0.5 / 3	0.5 / 3 *	0.5 / 3 *	0.5 / 3 *	0.5 / 3 *
		Rega institute	1 / 3	2 / 3	2 / 3	2 / 3	2 / 3
		Stanford↓	1 / 3	2 / 3	2 / 3	2 / 3	2 / 3
AI	Emtricitabine Tenofovir Atazanavir/r	ANRS	2 / 3	2 / 3	2 / 3	2 / 3	2 / 3
		Grade	1.5 / 3	1.5 / 3	1.5 / 3	1.5 / 3	1.5 / 3
		Rega institute	2 / 3	2 / 3	2 / 3	2 / 3	2 / 3
		Stanford↓	2 / 3	2 / 3	2 / 3	2 / 3	2 / 3
AI	Lamivudine Tenofovir Atazanavir/r	ANRS	2 / 3	2 / 3	2 / 3	2 / 3	2 / 3
		Grade	1.5 / 3	1.5 / 3	1.5 / 3	1.5 / 3	1.5 / 3
		Rega institute	2 / 3	2 / 3	2 / 3	2 / 3	2 / 3
		Stanford↓	2 / 3	1 / 3	2 / 3	2 / 3	2 / 3

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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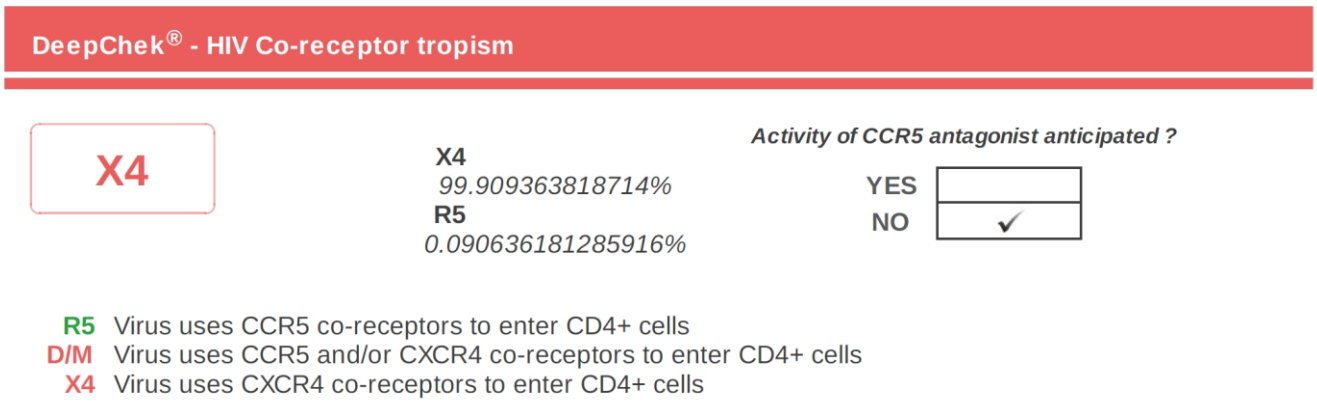
TROPISM DETERMINATION



VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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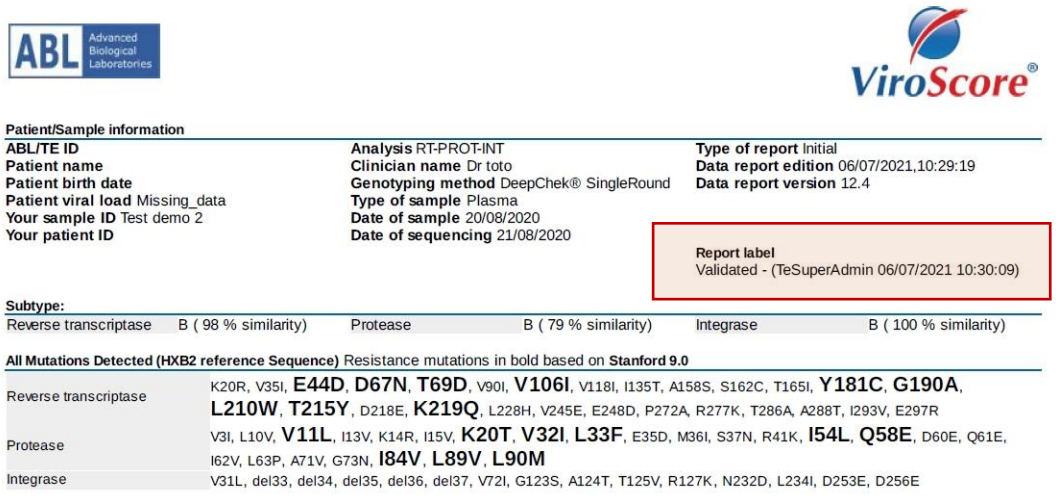
REPORT VALIDATION



Patient/Sample information

ABL/TE ID	**Analysis** RT-PROT-INT	**Type of report** Initial
Patient name	**Clinician name** Dr toto	**Data report edition** 06/07/2021,10:29:19
Patient birth date	**Genotyping method** DeepChek® SingleRound	**Data report version** 12.4
Patient viral load Missing_data	**Type of sample** Plasma	
Your sample ID Test demo 2	**Date of sample** 20/08/2020	
Your patient ID	**Date of sequencing** 21/08/2020	

Report label
Validated - (TeSuperAdmin 06/07/2021 10:30:09)

Subtype:

Reverse transcriptase	B (98 % similarity)	Protease	B (79 % similarity)	Integrase	B (100 % similarity)

All Mutations Detected (HXB2 reference Sequence) Resistance mutations in bold based on **Stanford 9.0**

Reverse transcriptase	K20R, V35I, **E44D**, **D67N**, **T69D**, V90I, **V106I**, V118I, I135T, A158S, S162C, T165I, **Y181C**, **G190A**, **L210W**, **T215Y**, D218E, **K219Q**, L228H, V245E, E248D, P272A, R277K, T286A, A288T, I293V, E297R
Protease	V3I, L10V, **V11L**, I13V, K14R, I15V, **K20T**, **V32I**, **L33F**, E35D, M36I, S37N, R41K, **I54L**, **Q58E**, D60E, Q61E, I62V, L63P, A71V, G73N, **I84V**, **L89V**, **L90M**
Integrase	V31L, del33, del34, del35, del36, del37, V72I, G123S, A124T, T125V, R127K, N232D, L234I, D253E, D256E

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HIV

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CONTAMINATION CHECK

Screening for similar strains

Region	Similar strains			
Reverse transcriptase	No significant homology detected compared to the strains stored in the database.			
Protease	No significant homology detected compared to the strains stored in the database.			
	Sample ID	**Sample date**	**Date performed**	**Identities**
Integrase	1	10/02/2021	04/03/2021 13:28:31	96.40%
	2	26/09/2019	24/10/2019 10:56:31	96.15%
	3	19/05/2021	07/06/2021 15:41:50	95.87%
	4	26/03/2020	19/10/2020 13:24:58	95.76%
	5	22/07/2020	16/10/2020 13:44:56	95.88%
	6	03/01/2020	27/01/2020 11:18:17	96.10%
	7	08/02/2021	15/03/2021 11:17:11	96.24%
	8	06/02/2020	03/03/2020 16:33:41	95.62%
	9	27/05/2021	18/06/2021 11:56:32	95.76%
	10	18/05/2021	07/06/2021 11:45:27	95.61%
		14/04/2021	23/06/2021 10:56:38	95.23%

Only the 10 first matches scored with an identity > 95% are displayed.

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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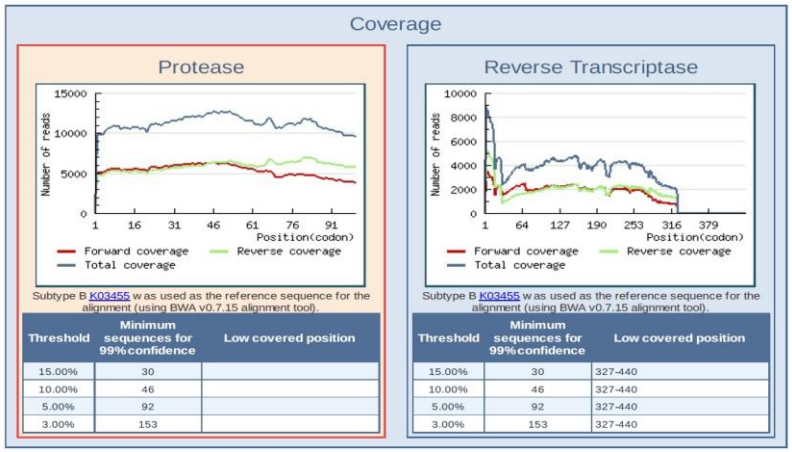
QUALITY CONTROL

SANGER

Sequence	Quality
REVERSE TRANSCRIPTASE	Good
PROTEASE	Good
INTEGRASE	Good
GP120/V3Loop	
GP41	

	RT	PROT	INT	GP120/V3Loop	GP41
Length (nt)	936 (Good)	297 (Good)	778 (Good)		
Stop codons	0	0	0		
Insertions	0	0	0		
Deletions	0	0	5		
Frame shift	0 (Good)	0 (Good)	1 (Good)		
Ambiguity (2 bases/nt)	0	0	0		
Ambiguity (3 bases/nt)	0 (Good)	0 (Good)	0 (Good)		
Ambiguity (4 bases/nt)	0	0	0		
Bad characters	0	0	0		
Minimal position	1	1	30		
Maximal position	312	99	289		
Ambiguity 2 / resistance codon	0	0	0		
Ambiguity 3 / resistance codon	0	0	0		
Ambiguity 4 / resistance codon	0	0	0		
Sequence ambiguity (%)	0.00	0.00	0.00		

NGS



Subtype B K03455 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
15.00%	30	
10.00%	46	
5.00%	92	
3.00%	153	

Subtype B K03455 was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool).

Threshold	Minimum sequences for 99% confidence	Low covered position
15.00%	30	327-440
10.00%	46	327-440
5.00%	92	327-440
3.00%	153	327-440

VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

HIV

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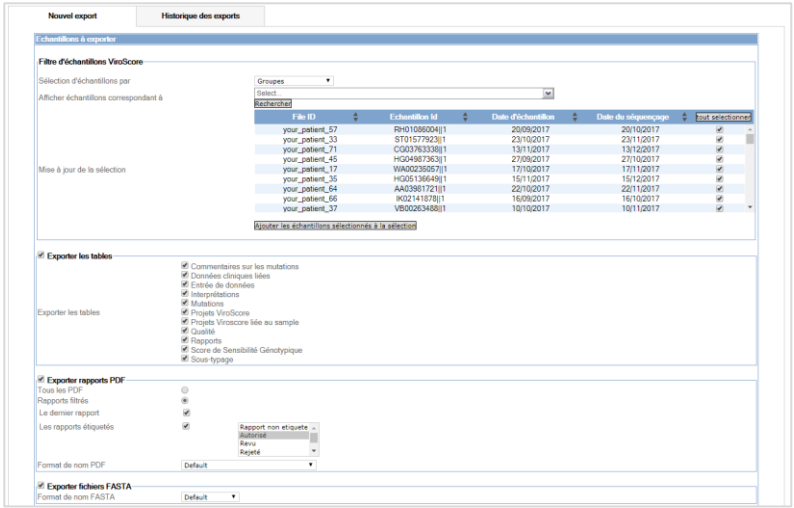
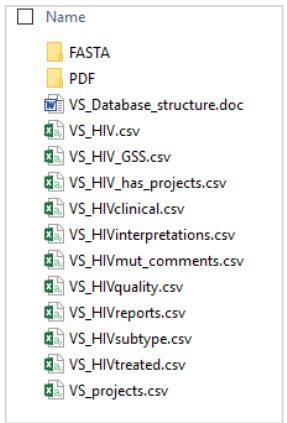
DATA EXPORT





VIROSCORE® & DEEPCHEK®- HIV SOFTWARE Highlights

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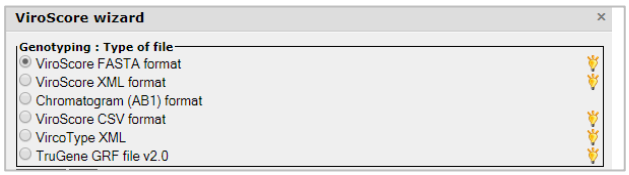
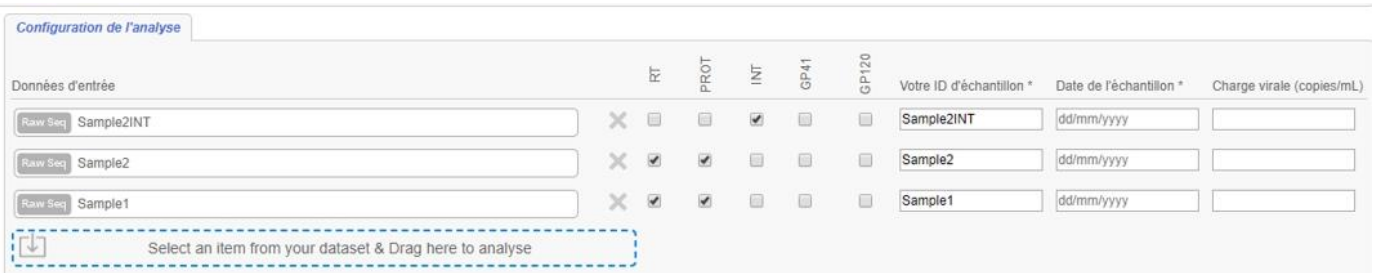
BATCH ANALYSIS LARGESCALE

SANGER



NGS



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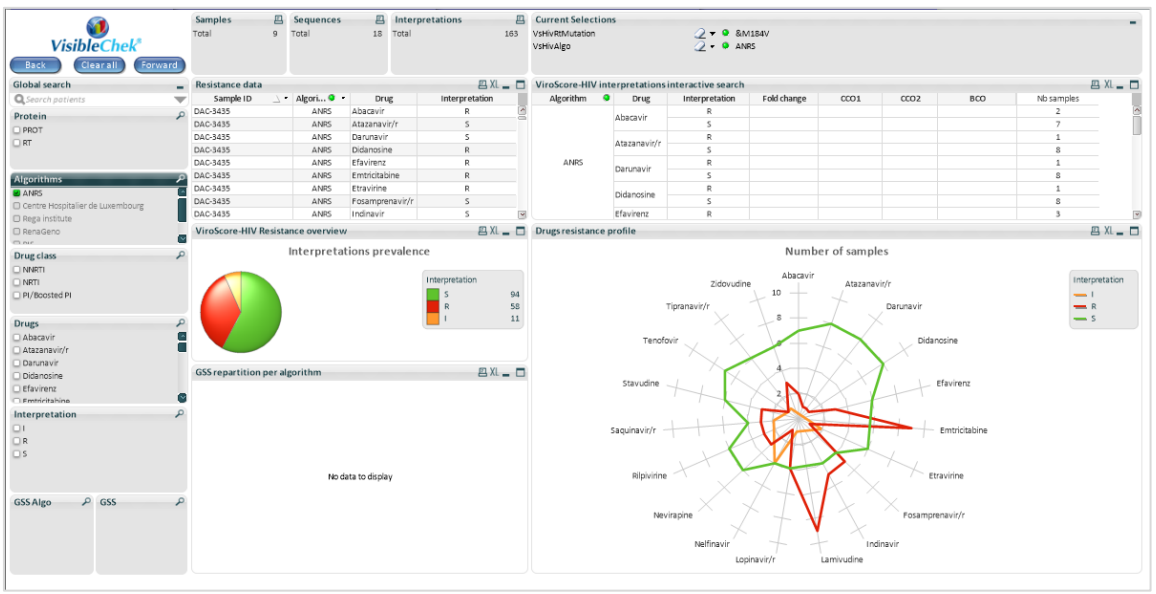
DATA MINING



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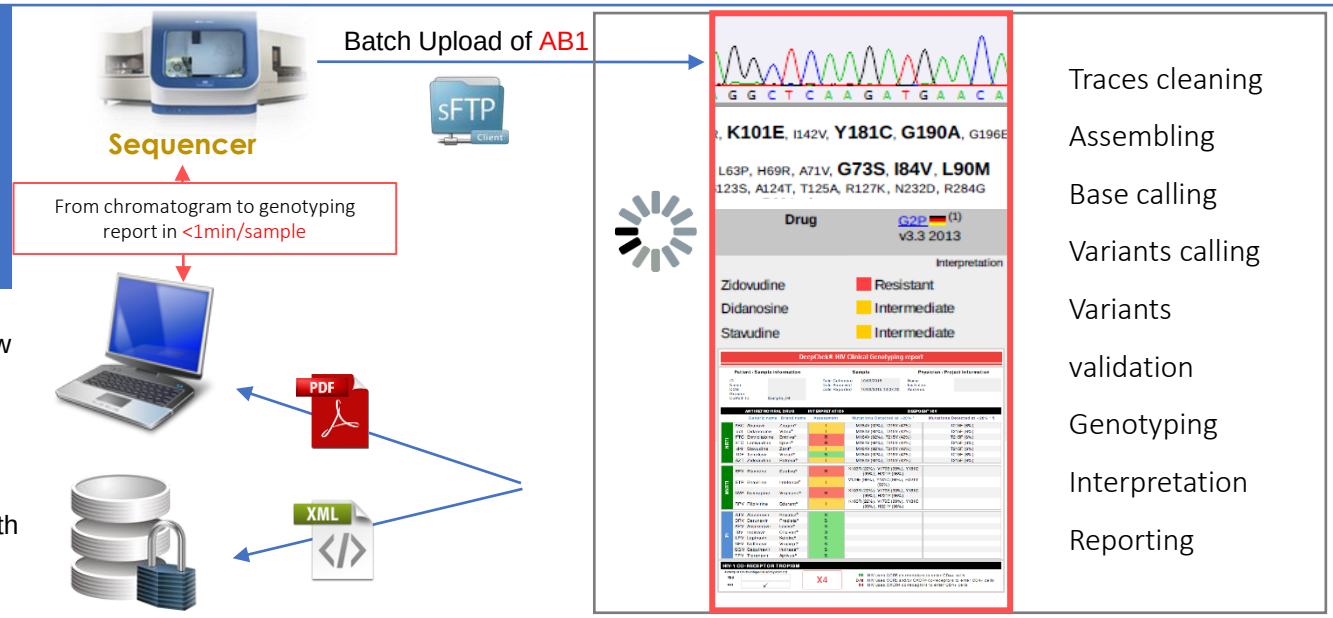
AUTOMATION + LIMS / HIS INTEGRATION



CONTACT

- Advanced Biological Laboratories S.A.
- 52- 54 Av du X Septembre, Luxembourg
- contact@ablsa.com
- https://www.ablsa.com
- TEL : (+352) 26 38 96 761
- FAX : (+352) 26 38 96 76 31

September 2021