A EVOLUÇÃO TECNOLÓGICA DOS TESTES PARA QUANTIFICAÇÃO DA CARGA VIRAL E DO CD-4

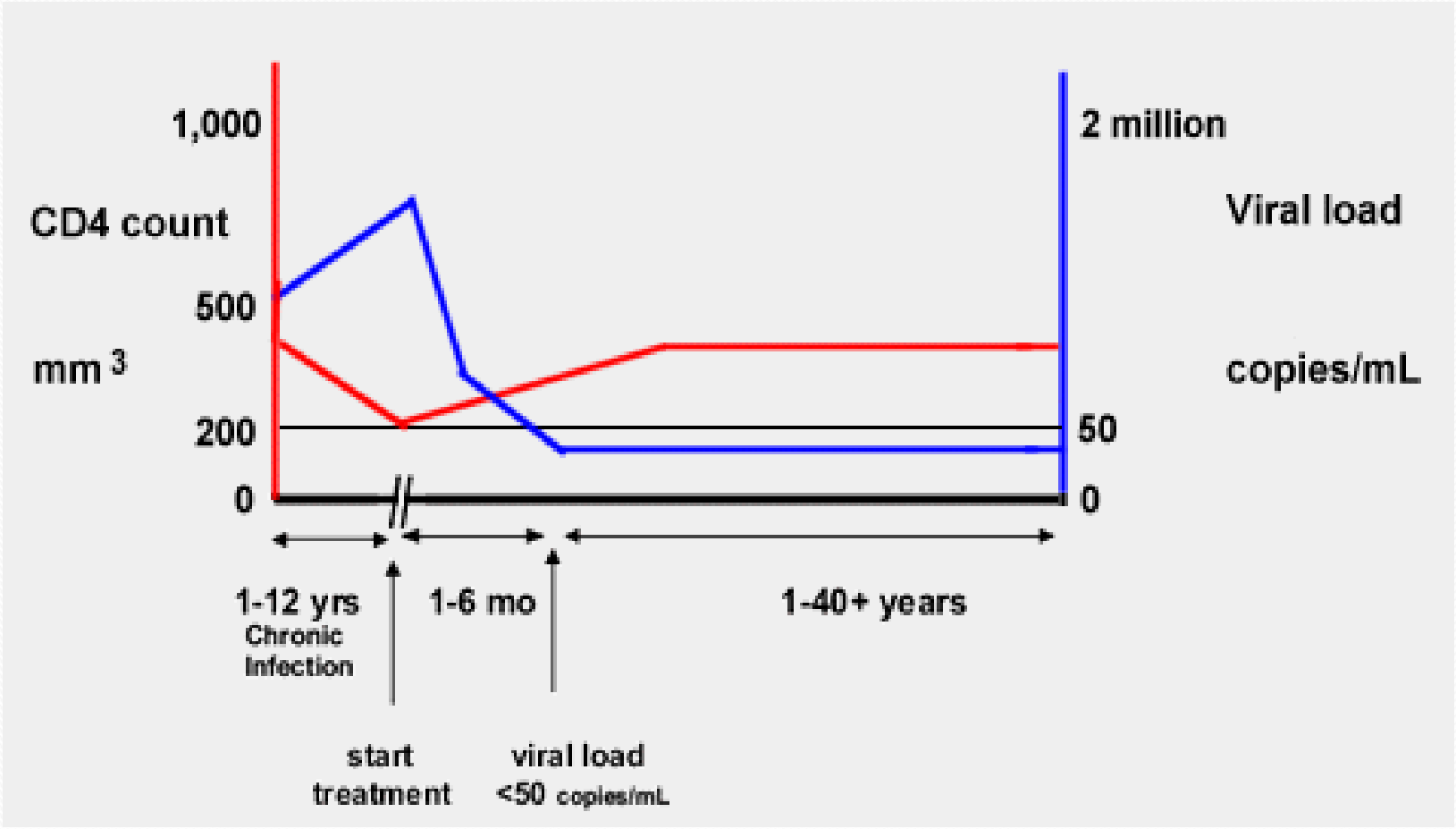
OUTUBRO 2016


Anos depois...
1973
2016

A importância da carga viral no monitoramento do tratamento com ARVs

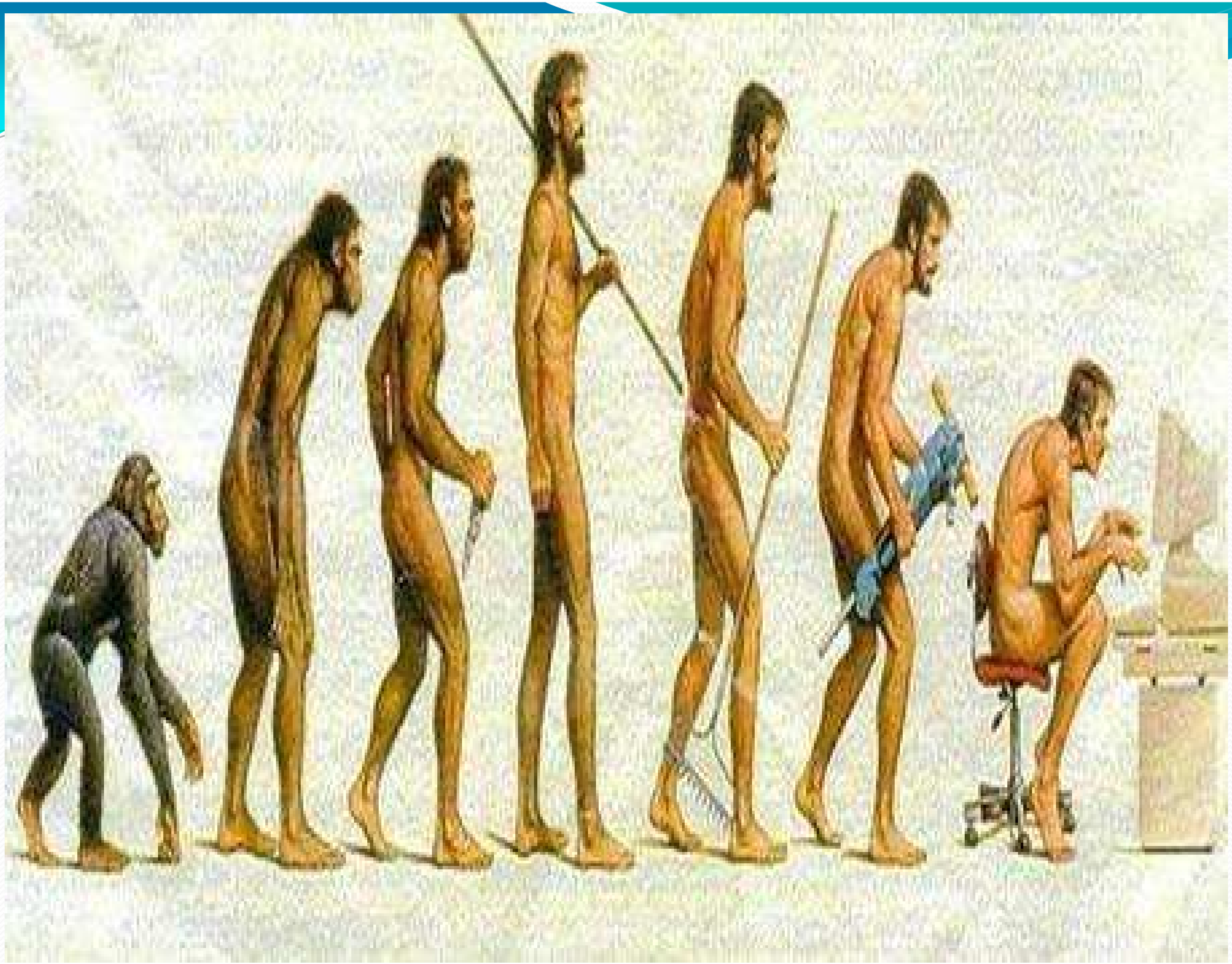
- CV É A CONCENTRAÇÃO DE VÍRUS NO PLASMA OU NO SANGUE TOTAL.
- PODE SER MEDIDA PARA VÁRIAS VIROSES, INCLUINDO O HIV- 1.
- A CV DO HIV INDICA A EXTENSÃO DA REPLICAÇÃO DO HIV NO CORPO.
- É UM EXCELENTE TERMÔMETRO DA REPLICAÇÃO DO HIV DENTRO DO SISTEMA IMUNE HUMANO.
- É UM BOM PARÂMETRO PARA MEDIR O POTENCIAL PATOGÊNICO DO HIV - QUANTO MAIS ALTA A CV MAIS RÁPIDA A PROGRESSÃO PARA AIDS.
- CV ALTA É ASSOCIADA COM MAIOR TRANSMISSÃO VERTICAL E SEXUAL

QUANDO A TERAPIA COMEÇA, A CV RESPONDE MUITO MAIS RÁPIDO DO QUE O CD4 OU OUTROS PARÂMETROS CLÍNICOS



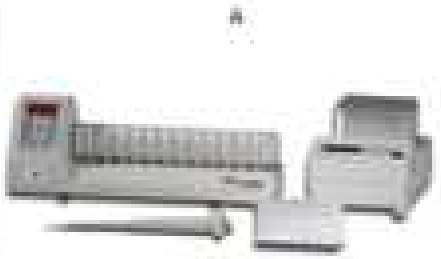
Carga Viral para o monitoramento da TARV

- **É recomendada OMS.**
- **O ponto de corte para definição de falha virológica é de 1000 copias/ml.**
- **A centralização dos exames em grandes centros é um problema devido as dificuldades para o transporte das amostras de plasma e para retorno dos resultados.**
- **A demora no resultado pode ser problemática, principalmente nos casos que exigem intervenção imediata. EX: Cerca de 40% dos resultados não chegam ao prontuário dos pacientes.**



EQUIPAMENTOS PARA CARGA VIRAL

VERSANT 440 MOLECULAR SYSTEM (SIEMENS).



NUCLISENS MINIMAG (A) E NUCLISENS EASYMAG (B). (BIOMÉRIEUX

QIASYMPHONY (B) ROTOR GENEQ. (QIAGEN

COBAS TAQMAN 96 E COBAS TAQMAN 48 ANALYSER (ROCHE

PCR REAL TIME ABBOTT


A
SAMBA
B
(i)
(ii)
(iii)

O SAMBA....

- J Clin Microbiol. 2014 Sep;52(9):3377-83. doi: 10.1128/JCM.00593-14. Epub 2014 Jul 16.
- **SAMBA HIV semiquantitative test, a new point-of-care viral-load-monitoring assay for resource-limited settings.**
- Ritchie AV[1], Ushiro-Lumb I[2], Edemaga D[3], Joshi HA[1], De Ruiter A[4], Szumilin E[3], Jendrulek I[4], McGuire M[3], Goel N[1], Sharma PI[1], Allain JP[5], Lee HH[6].
- **Abstract**
- Routine viral-load (VL) testing of HIV-infected individuals on antiretroviral therapy (ART) is used to monitor treatment efficacy. However, due to logistical challenges, implementation of VL has been difficult in resource-limited settings. The aim of this study was to evaluate the performance of the SAMBA semi-Q (simple amplification-based assay semiquantitative test for HIV-1) in London, Malawi, and Uganda. The SAMBA semi-Q can distinguish between patients with VLs above and below 1,000 copies/ml. The SAMBA semi-Q was validated with diluted clinical samples and blinded plasma samples collected from HIV-1-positive individuals. SAMBA semi-Q results were compared with results from the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test, v2.0. Testing of 96 2- to 10-fold dilutions of four samples containing HIV-1 subtype C as well as 488 samples from patients in the United Kingdom, Malawi, and Uganda yielded an overall accuracy for the SAMBA semi-Q of 99% (95% confidence interval [CI], 93.8 to 99.9%) and 96.9% (95% CI 94.9 to 98.3%), respectively, compared to to the Roche test. Analysis of VL data from patients in Malawi and Uganda showed that the SAMBA cutoff of 1,000 copies/ml appropriately distinguished treated from untreated individuals. Furthermore, analysis of the viral loads of 232 patients on ART in Malawi and Uganda revealed similar patterns for virological control, defined as either <1,000 copies/ml (SAMBA cutoff) or <5,000 copies/ml (WHO 2010 criterion; WHO, Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach, 2010). This study suggests that the SAMBA semi-Q has adequate concurrency with the gold standard measurements for viral load. This test can allow VL monitoring of patients on ART at the point of care in resource-limited settings.

GENEXPERT


GeneXpert.
Xpert® HIV-1 Viral Load
0303456789012345
12345678
Cepheid

Xpert HIV-1 Viral Load is a quantitative test that provides on-demand molecular testing.

Based on the GeneXpert technology, Xpert HIV-1 Viral Load automates the test process including RNA extraction, purification, reverse transcription and cDNA real time quantitation in one fully integrated cartridge.

Redefining Simple:

Easy

Run daily or on-demand

- No requirements for PCR room settings
- No daily maintenance or liquid waste management

Rapid

- 92 minutes run time with a viral load trend report*
- No batch, no delay
- 1 minute hands-on time

Flexible

- Compatible with any lab volume
- Providing up to 394 viral load results per 8 hours^
- Random access 24/7 availability
- Run multiple different tests on the same platform at any time
- Fixed cost per reportable result independent of daily volume

1. Transfer of 1ml plasma into HIV-1 viral load cartridge

2. Scan, insert cartridge and start test

3. Time to result **90 minutes** for **HIV-1 viral load, GeneXpert®**


GeneXpert
Xpert® HIV-1 Qual
Cepheid


GeneXpert
Xpert® HIV-1 Viral Load
Cepheid

EV0029

28th ECCMID
Amsterdam, Netherlands
9 – 12 April 2016
ESCMID

Xpert® HIV-1 Viral Load real-time RT-PCR (Cepheid) for rapid HIV-1 quantification

Aleksandra Maleska[1, 2], Anne Marie Mondain[1], Laure Ottomani[1] and Jacques Ducos[1]

[1]Virology Laboratory, Montpellier University Hospital; [2] Institut for Regenerative Medicine and Biotherapy, Laboratory of Clinical Biochemistry, Montpellier, France

Background

Transmission of Human Immunodeficiency Virus 1 (HIV-1) with accidental exposure to blood or contaminated body fluids was notified in previous studies. Since then, obligatory serology screening of HIV-1 was implemented for source patient's blood. The guidelines recommend processing to HIV-1 viral load testing if possible, leading to a complex lab organisation.

Xpert HIV-1 Viral Load, is a quantitative assay for monitoring of HIV-1 viremia in individuals treated with an antiretroviral therapy. With a limit of detection below 20 copies/ml and a time to results of 90 minutes, this real-time RT-PCR seems well suited for application in rapid measurement of HIV-1 viral load in urgent sample screening, although not validated for it.

Xpert HIV-1 Viral Load workflow

- **Aims: Evaluate and validate the use of Xpert HIV-1 Viral Load for rapid quantification of HIV-1 viremia.**

We therefore evaluated this assay following the international quality requirements (NF EN ISO 15189).

Material & Methods

HIV-1 viral load was assessed by real-time RT-PCR on Cobas AmpliPrep/Cobas TaqMan HIV test (CAP/CTM, Roche Diagnostics, Indianapolis) using raw plasma samples and compared to GeneXpert results (Cepheid, Sunnyvale) on either raw or diluted (1/2 or 1/3) plasma samples depending on the volume of leftover plasma available.

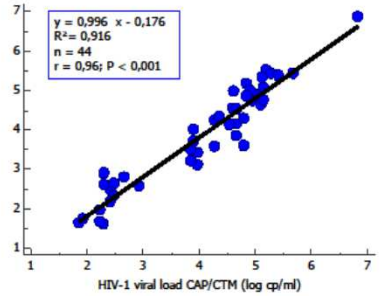
Xpert HIV-1 Viral Load method was evaluated following the European quality recommendations (ISO 15189).

- Precision of assay:
  - Repeatability
  - Reproducibility
  - Inter modules (units) variability
- Accuracy (3 external quality controls - EQC).
- Results of Xpert HIV-1 Viral Load were compared to the CAP/CTM HIV-1 test by means of linear regression and Bland-Altman difference plot.

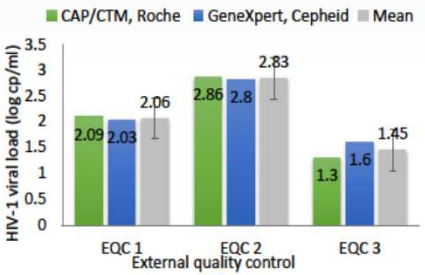
Results

	Repeatability	Reproducibility	Inter-units variability
Replicates (n)	27	28	24 (6x4 units)
Mean (log cp/ml)	2.76	2.6	2.71
Standard Deviation	0.06	0.1	0.06
CV (of lognormal distribution)	**13.80%**	**23.35%**	**6.90%**

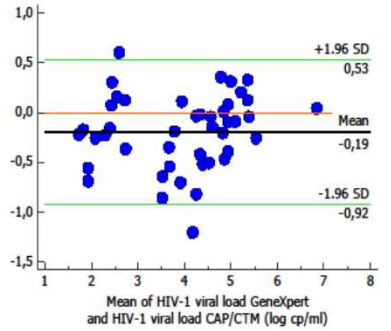
- Precision : data shows an excellent repeatability and low inter assay variability of Xpert HIV-1 viral load.
- HIV-1 has being quantified within 44/48 samples, while 4/48 specimens have an HIV-1 viral load below the limit of quantification (< 40 cp/ml).



- Correlation anlysis show a substantial correlation between the two methods according to the HIV-1 viral load of 1.64 to 6.87 log cp/ml.



- Accuracy was evaluated using EQC assessments analyzed on each assay. Xpert HIV-1 viral load was lower CAP/CTM with a mean difference of −0.03 log cp/ml.



- Concordance between GeneXpert and CAP/CTM HIV-1 test with mean difference of −0.19 log cp/ml from Bland–Altman plot.

Conclusion

Cepheid's new tool demonstrates excellent performances for the management and measurement of HIV-1 viral load. This assay is ideally suited for urgent samples and daily routine testing, thanks to the advantages of random access and very fast results.

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Sangue Seco para CV

- Foi usado no passado como alternativa para testagem em locais de acesso remoto.
- A coleta de amostra por punção digital é problemática devido a variação do volume de sangue colocado nos círculos do papel filtro.
- Para CV isso pode ser crítico!
- A OMS avaliou o desempenho desse tipo de amostra e considerou a sensibilidade e especificidade > 85% aceitável.

Table 4.12. Performance of assay type using DBS compared to plasma using a viral load threshold of 1000 copies/mL

>85% Sensitivity and Specificity

Failure	Abbott RealTime	Biocentric Charge Virale	bioMerieux Nucleisens	Roche TaqMan FVE	Roche TaqMan SPEX	Siemens kPCR
Sensitivity[a] (95% confidence interval [CI])	95% (82–99%)	95% (71–99%)	84% (79–89%)	85% (77–91%)	99% (97–100%)	91% (69–98%)
Specificity[a] (95% CI)	92% (79–97%)	55% (35–74%)	95% (86–98%)	94% (85–98%)	44% (18–74%)	88% (75–94%)

[a] Pooled estimates of sensitivity and specificity based on published data up to June 2015 *(395)*.

Evaluation of Alere Q Quant Cartridge

Amilcar Tanuri
Orlando C Ferreira Jr
LVM-UFRJ

ALERE-Q

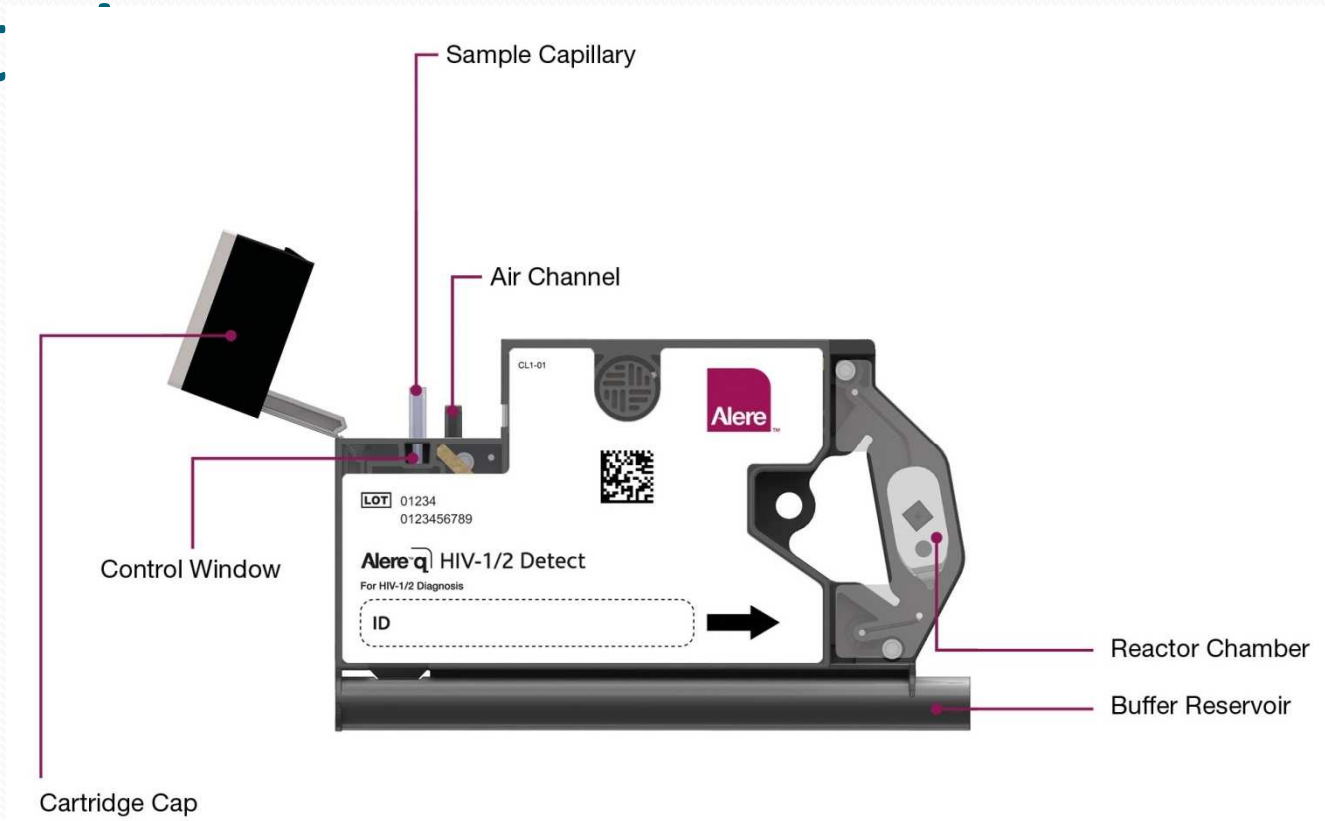
gy

QUALITATIVO E QUANTITATIVO

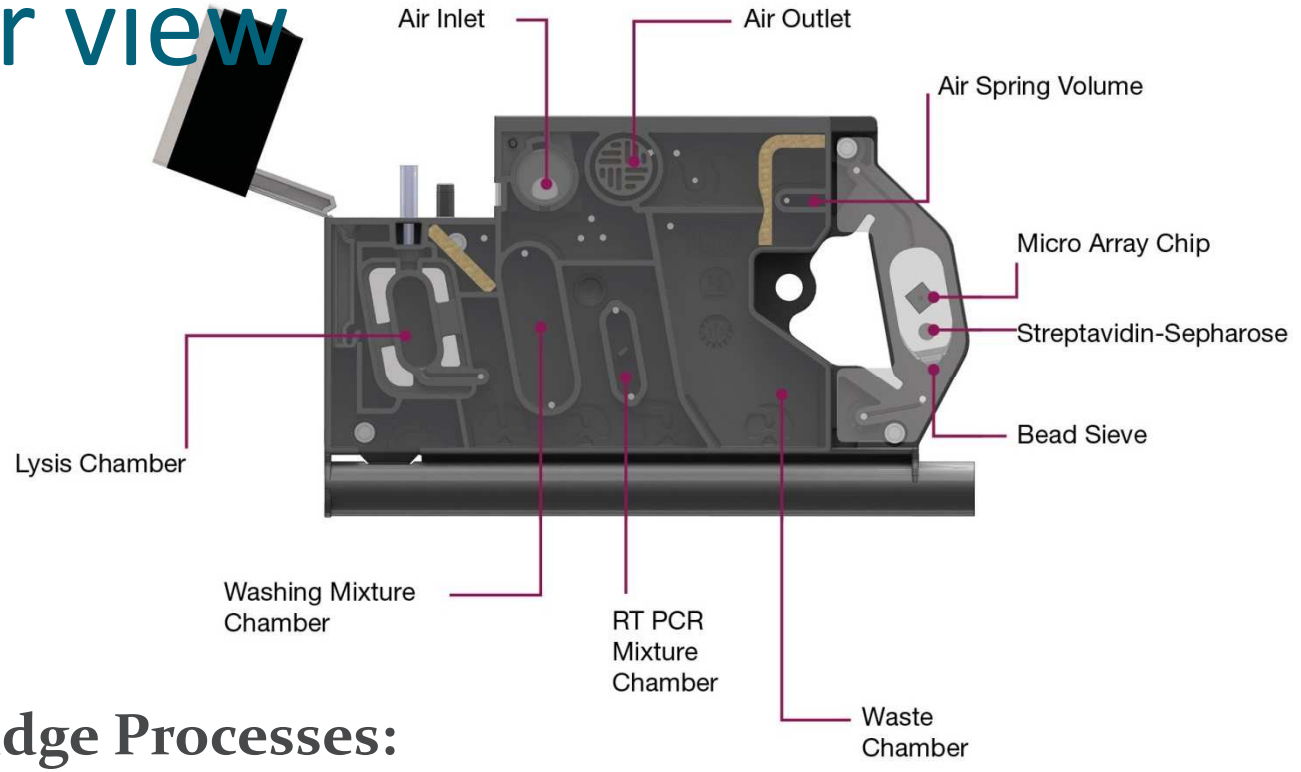
O cartucho contêm todos os reagentes necessários para fazer o teste

Connectivity

Alere™ q HIV 1/2 Detect Cartridge: front



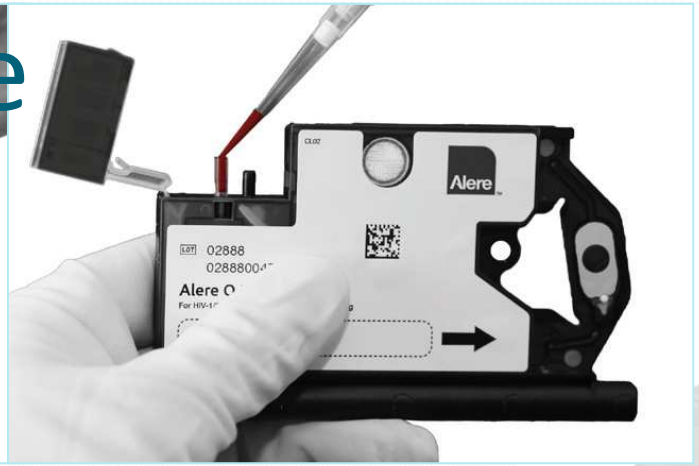
Alere q HIV 1/2 Detect Cartridge: clear view

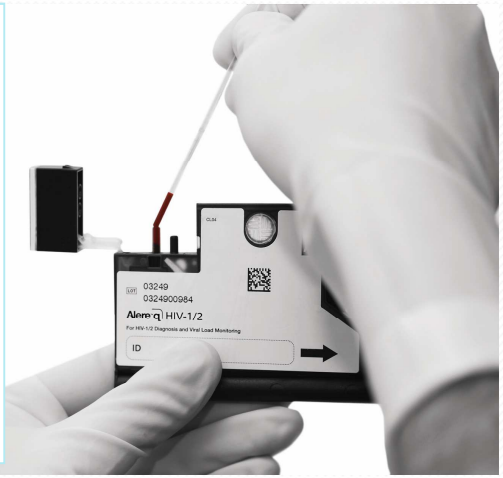


In Cartridge Processes:

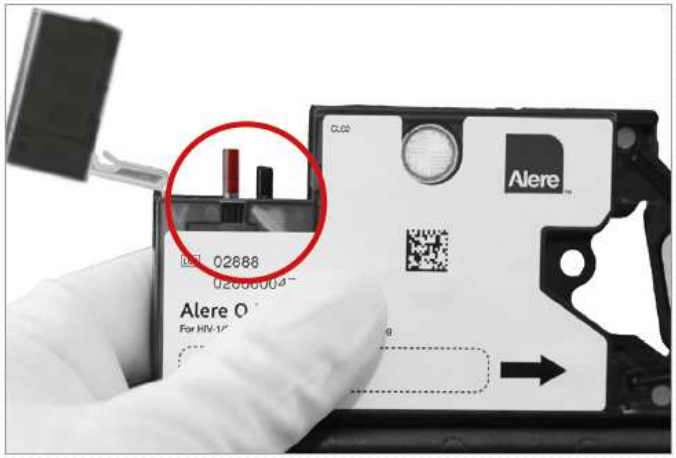
- Handling and Processing
- RNA Isolation
- Reverse Transcription and Amplification
- Detection and Quantification

Alere™ q HIV 1/2 Detect – Filling the Cartridge

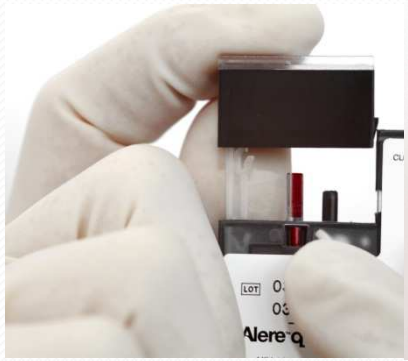




Fill the cartridge with sample (capillary/EDTA venous whole blood or plasma)



Check for sufficient sample loading



Close the cartridge cap

POC VL is a important technology for the availability of VL in Low Income Countries (LIC)

- Alere™ q HIV 1/2 Detect is a interesting device to use in LIC
- The results are given in 45 mim and one machine can run 12 samples per 8hs working days.
- The more important is that the result is available for the health professional during the patient visit and allow in time decision.

Evaluation of Alere™ q HIV 1/2 Detect in Plasma Specimens

- We organize a evaluation of Alere Q cartridge trying to compare the results obtained in Abbott M2000 technology.
- We used 537 samples from patients using two public laboratories in Brazil.
- Samples were selected from different virus load range.
- Samples were run in parallel and analyzed using a Alere™ q HIV 1/2 Detect using 25 ul of left over plasma volume.
- A subset of samples were run in a new 50 ul AlereQ Quantitative Cartridges.
- IRB Approval #

Discordâncias Clinicamente irrelevantes
Abbott ≠ Alere

Log CV	Abbott	Alere																
		ND	NQ	1,6-<2,0	2,0-<2,7	2,7-<3,0	3,0-<3,3	3,3-<3,7	3,7-<3,85	3,85-<4,0	4,0-<5,0	5,0-<6,0	≥6,0	N	%	N	NP	%
ND	99	98	0	0	0	1	0	0	0	0	0	0	0	--	--	--	--	--
NQ	55	52	0	0	0	2	1	0	0	0	0	0	0	--	--	--	--	--
1,6-<2,0	35	26	0	0	0	6	3	0	0	0	0	0	0	--	--	--	--	--
2,0-<2,7	82	27	0	0	0	12	20	20	2	1	0	0	0	--	--	--	--	--
2,7-<3,0	23	2	0	0	0	0	5	14	2	0	0	0	0	--	--	--	--	--
3,0-<3,3	43	4	0	0	0	3	5	8	10	6	7	0	0	31	72,0	969	2,0	1,4
3,3-<3,7	36	1	0	0	0	0	1	1	4	6	23	0	0	29	80,5	1.327	2,7	2,2
3,7-<3,85	10	1	0	0	0	0	0	0	0	0	9	0	0	9	90,0	495	1,0	0,9
3,85-<4,0	21	0	0	0	0	0	0	0	0	2	19	0	0	0	0	520	1,1	0
4,0-<5,0	97	0	0	0	0	0	0	1	1	1	52	42	0	2	2,1	3.278	6,8	0,1
5,0-<6,0	27	0	0	0	0	0	0	0	0	0	1	22	4	0	0	1.592	3,3	0
≥6,0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	58	0,1	0
	531	211	0	0	0	24	35	44	19	16	111	64	6	71	13.3	48.509		4,6

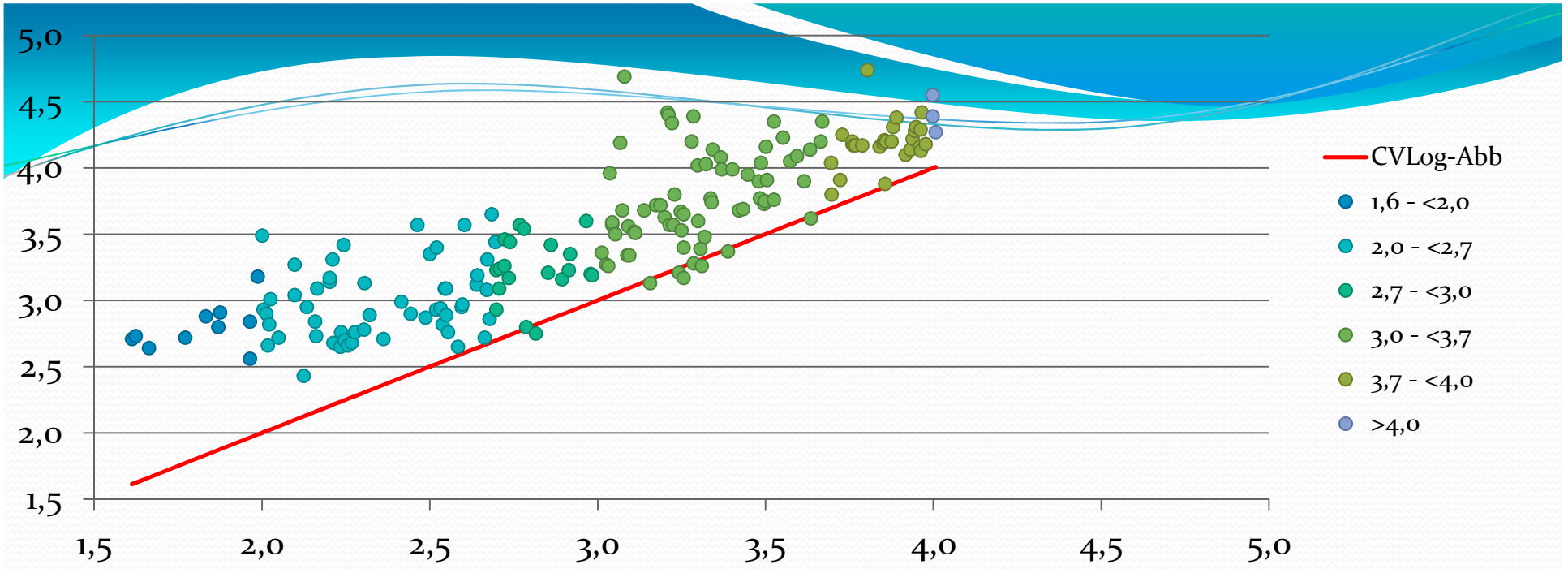
Analysis of 50 ul ™ q HIV 1/2 Quantitave Cartridges

- A new cartridge with 50ul capacity was developed to increase the sensitivity of the measurament and lowering the LOD.
- The new 50 ul Quantitave Cartridges has a provisory LOG arround 850-750 copies/ml.

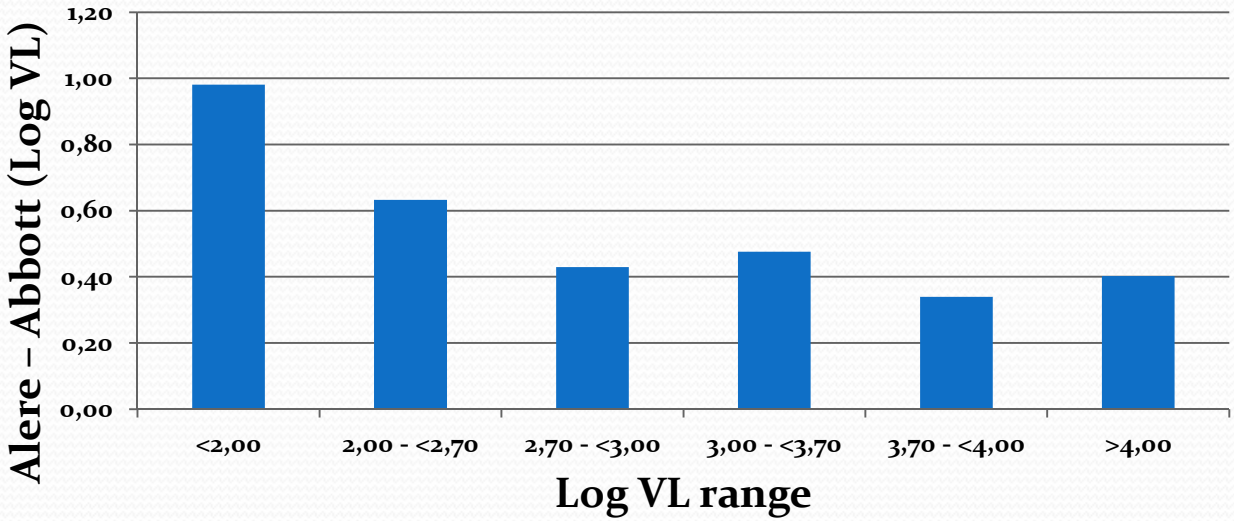
New evaluation of the 50 ul Cartridge

- Using a subset (n= 227) of the 537 plasma collection previously run in Alere™ q HIV 1/2 Detect in Plasma Specimens we run a new evaluation
- The results were analyzed focusing in false positives and negatives samples identified using Alere™ q HIV 1/2 Detect 25ul Cartridges.

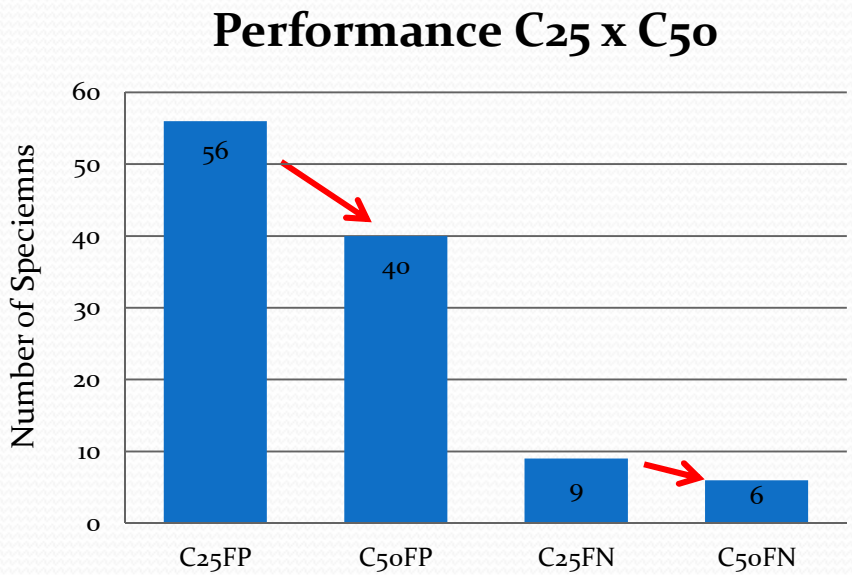

50 ul HIV VL cartridge
Alere
05390
0539000114



Average difference btwn Alere and Abbott by VL range



50 Cartridges yealded less FP samples in the reanalysis



Using the 50ul Cartridges we could correctly classified 15 samples being < 1000 copies/ml out of the 45 samples miss classified by the 25 ul Alere™ q HIV 1/2 Detect cartridges

CONTAGEM DE CÉLULAS T CD-4

BD FACSCount™ system

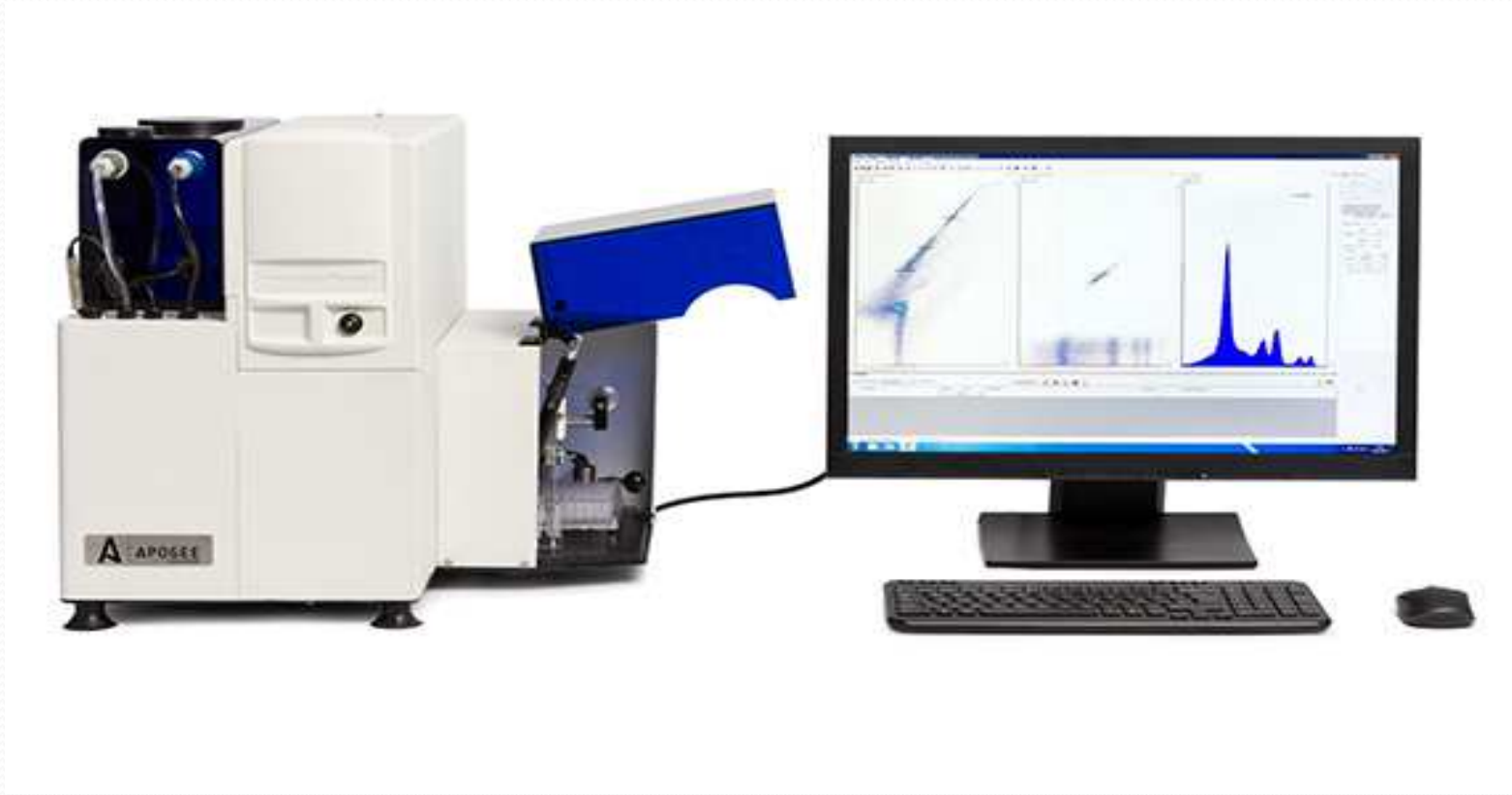
SYSMEX UF-1000i É um equipamento que realiza a análise da urina por citometria de fluxo fluorescente, com uso de um marcador (polimetina)
de DNA / RNA e luz laser. É totalmente automatizado,

O aparelho Pentra DX 120 da Horiba. Os métodos de medição são: Citoquímica, impedância, absorbância, citometria de fluxo e fluorometria. O analisador conta com 49 parâmetros, preparador de lâminas integrado e sistema prático de validação.

O aparelho Pentra DX 120 da Horiba. Os métodos de medição são: Citoquímica, impedância, absorbância, citometria de fluxo e fluorometria. O analisador conta com 49 parâmetros, preparador de lâminas integrado e sistema prático de validação.

Citômetro de fluxo A50-Micro

Apogee Flow Systems: Projetado para análise de micropartículas, o citômetro de fluxo A50-Micro é um equipamento de alta sensibilidade, que oferece a melhor resolução para partículas submicrométricas. Este citômetro é ideal para análise de microvesículas e detecção de bactérias em vários tipos de amostras.




BD FACSCanto II

Pima analyser

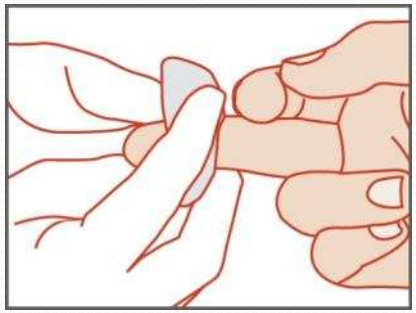
Pima Analyser -Features

- Absolute **CD4** count in **20 minutes**
- Mains A/C & Battery powered
- Portable & Robust
- Embedded software
- No External calibration
- On-board data archive

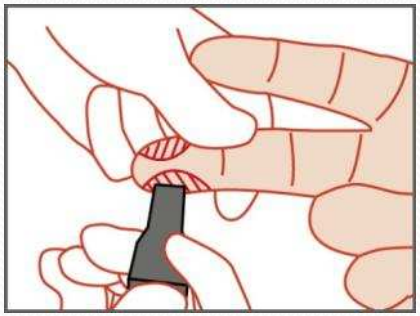
The Pima Test

Fingerstick Sample Collection

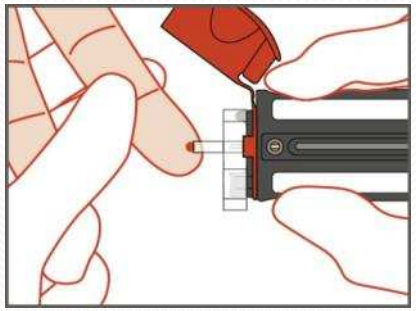
Sample is loaded into the Pima CD4 cartridge



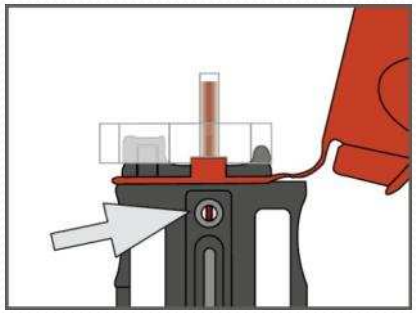
Select Finger & Clean



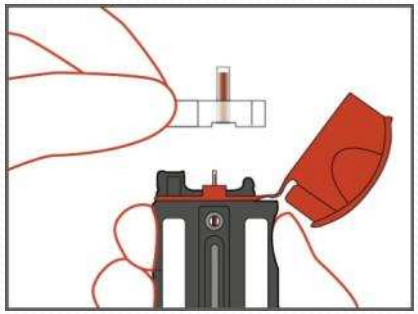
Lance finger



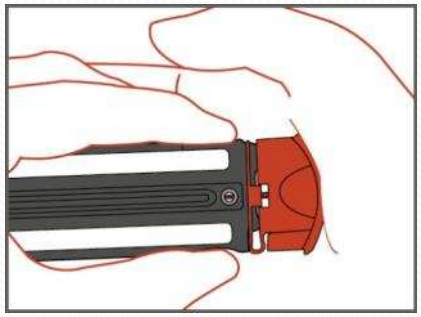
Collect Sample



Check fill



Remove Sample Collector



Cap Cartridge

The Pima Test

Printing Results

Results are stored on the analyser and can be printed using the Pima USB printer

PIMA Test Report

PIMA CD4

Sample:	96658
CD3+CD4+:	435c/µl
Result Date:	2009-07-15
Start Time:	12:21
Operator:	KLAB
Test ID:	243
Device:	PIMA-D-000029
Software:	0.37h

QC

Barcode:	pass
Expiry Date:	pass
Volume:	pass
Device:	pass
Reagent:	pass

Signature

OBRIGADA!

miriam.franchini@gmail.com