UFRJ LVM laboratório de virologia molecular

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Alere HIV Combo: a fourth generation rapid test

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Overview

- How fourth generation rapid tests can contribute to reduce window period:
  - Fiebig classification system for staging primary HIV infection
  - Specificity of antibody and antigen components;
  - Sensitivity of Ab component;
    - Using 3rd generation RT as benchmark
  - Sensitivity of Ag component
    - Defining LoD for antigen and viral load detection
    - Using HIV early seroconversion panels
  - "Rapid" confirmatory algorithm for HIV fourth generation rapid test
    - Early Diagnosis and immediate linkage to care
  - How and when to use the HIV fourth generation RT

Staging primary HIV infection:

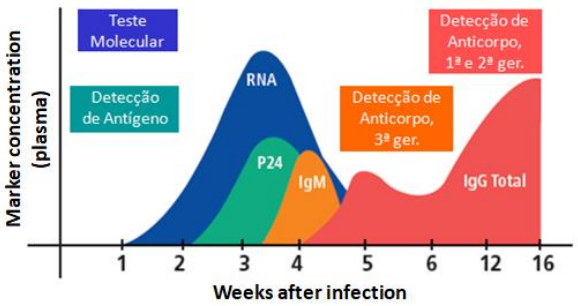
Fiebig classification system

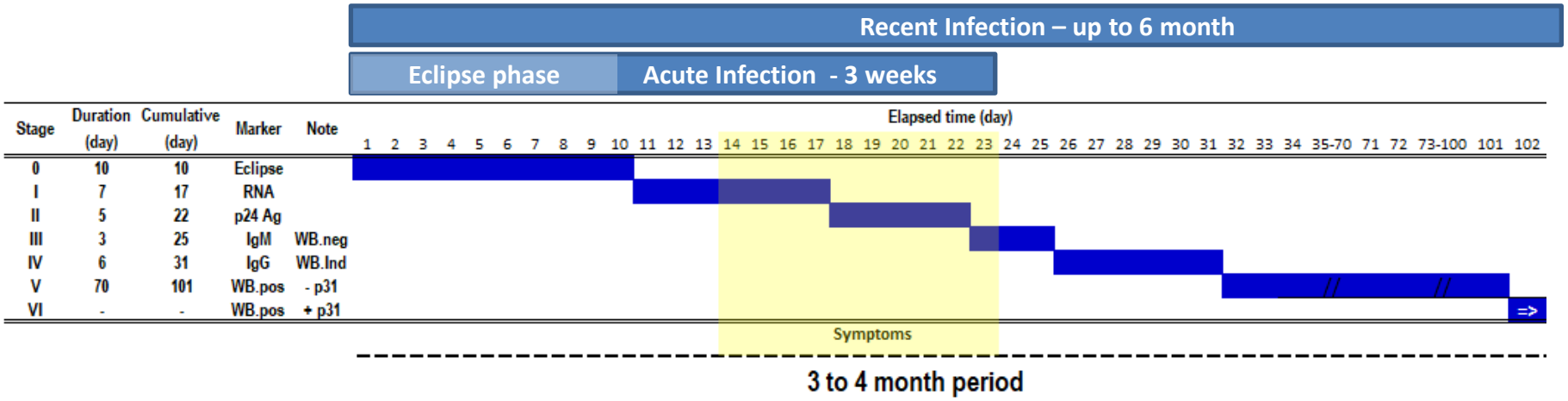
Stage	Marker					Duration in days	
	RNA	p24 Ag	EIA (3rdG)	WB	LAg[b]	Individual	Cumulative
0[a]	-	-	-	-	-	10 (7-21)	10
I	+	-	-	-	-	7 (5-10)	17
II	+	+	-	-	-	5 (4-8)	22
III	+	+	+	-	-	3 (2-5)	25
IV	+	+/-	+	Ind	-	6 (4-8)	31
V	+	+/-	+	+ (-p31[c])	-	70 (40-122)	101
VIa	+	+/-	+	+ (+p31)	-	~ 80	~ 180
VIb	+	+/-	+	+ (+p31)	+	Open-ended	Open-ended

[a] Eclipse; [b] Limiting Antigen Avidity Assay; [c] p31 protein (integrase)

Fiebig et al. AIDS 2003, 17:1871–1879

Four biological markers of HIV infection.





Three types of "Determine-like" rapid test

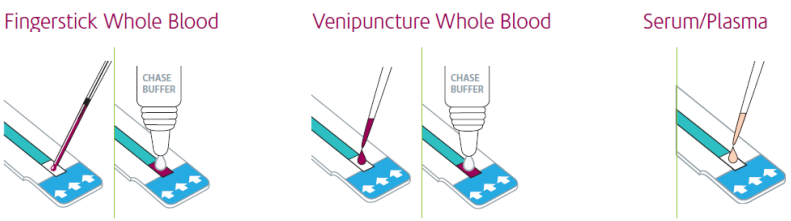
Alere HIV Combo

IS NOT

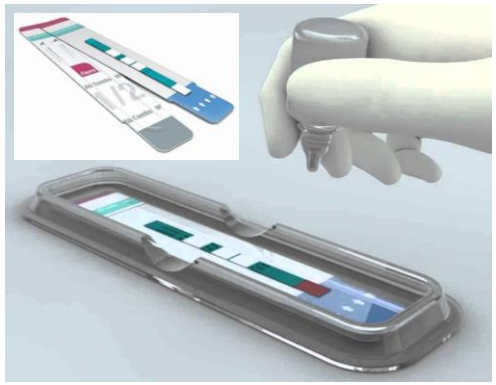
Alere Determine HIV-1/2 Ag/Ab Combo

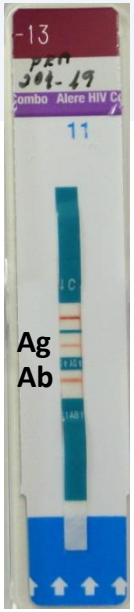
Determine Ac	Alere Determine HIV-1/2 Ag/Ab Combo	**Alere HIV Combo**
c2000	**c2010**	**2015**
Very good RT for Ab detection	**Did not perform well in Africa and LA**	**Superior performance**

Test Procedure



20 minutes to results





Alere HIV Combo - Antibody component

1. Specificity of the Alere HIV Combo RT

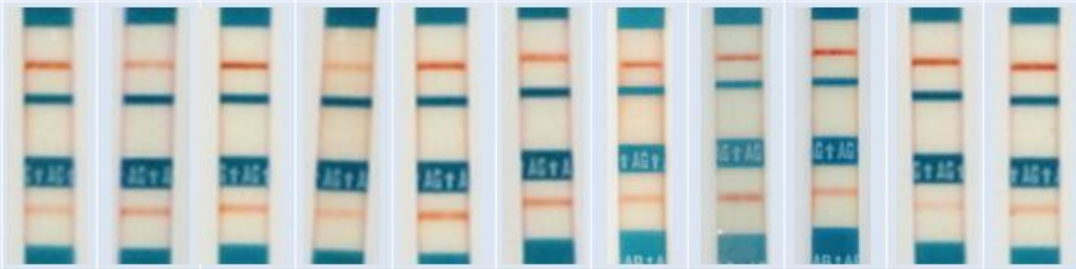
Rapid Test	Sample matrix	Total number assayed	Number of True-Negative	Specificity		
				Spe (%)[a]	CI 95% L[b]	CI 95% U[c]
Alere HIV Combo	Whole blood	611	608	**99.51**	98.56	100
	Plasma	1,686	1,677	**99.47**	98.99	99.76

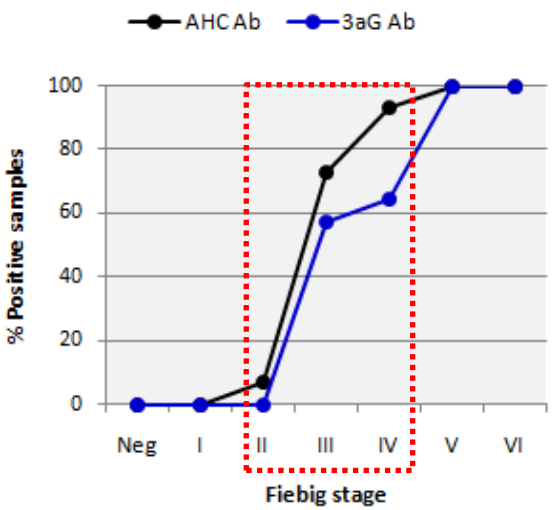
2. Sensitivity of the Alere HIV Combo RT

Rapid Test	Sample matrix	Total number assayed	Number of True-Positive	Sensitivity		
				Sen (%)[a]	CI 95% L[b]	CI 95% U[c]
Alere HIV Combo	Whole blood	203	203	**100**	98.20	100
	Plasma	424	424	**100**	99.13	100

Alere HIV Combo - Antibody component

Broad range of reactivity against different HIV-1 subtypes, including HIV-1 group O and HIV-2 (n=82)

Sample #	WW RB-1	WWR B-15	WW RB-3	WW RB-6	WWR B-10	WW RB-8	1101 /01	HUB 1207	PS 2257	WW RB-9	WWR B-11
Type/ Subtype	HIV-1 A	HIV-1 B	HIV-1 C	HIV-1 D	HIV-1 F	HIV-1 G	HIV-1 BC	HIV-1 BF	HIV-1 CRF02	HIV-1 gO	HIV-2
Ab --->											
N	3	30	7	2	13	2	3	2	1	1	18



RT	Fiebig Stage				
	II	III	IV	V	VI
Alere HIV Combo	6,7	72,7	92,9	100,0	100,0
MedTeste HIV	0,0	57,1	64,3	100,0	100,0

Ag | Ag IgM | Ag IgG

← 14 dias →

Alere HIV Combo - Antigen component

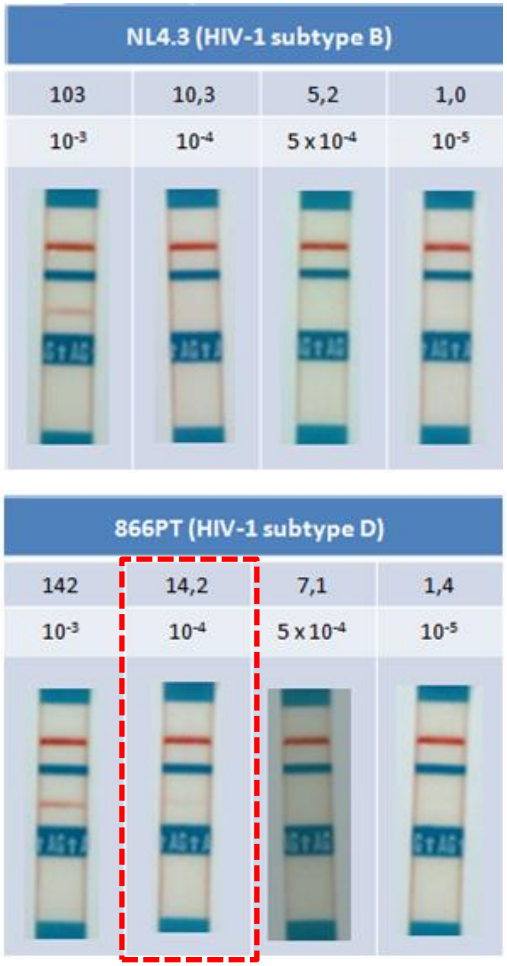
1. Specificity of the Alere HIV Combo RT

Rapid Test	Sample matrix	Total number assayed	Number of True-Negative	Specificity		
				Spe (%)[a]	CI 95% L [b]	CI 95% U [c]
Alere HIV Combo	Whole blood	611	0	**100**	99.40	100
	Plasma	1,686	1,677	**99.47**	98.99	99.76

- **How to evaluate sensitivity?**
  - By determining the Limit of Detection of
    - Antigen (Ag) and;
    - Viral Load (VL)
    - .... and looking whether these parameters would be found at early stages of HIV infection (Fiebig System)
  - By using panels of early seroconversion samples

Limit of Detection of HIV p24 Ag

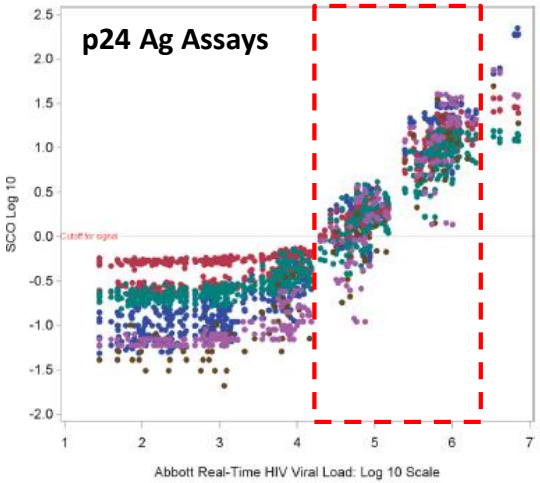
- Make *in vitro* culture of HIV-1 subtype D and subtype B to generate HIV p24 Ag;
- Collect culture supernatant (full of p24 Ag);
- Quantify p24 Ag in culture supernatant (immunoassay);
- Spike culture supernatant in HIV negative human plasma;
- Make serial dilution of known quantity of p24 Ag;
- Challenge Alere HIV Combo!



Detection level: 14 pg/mL of HIV p24 Ag

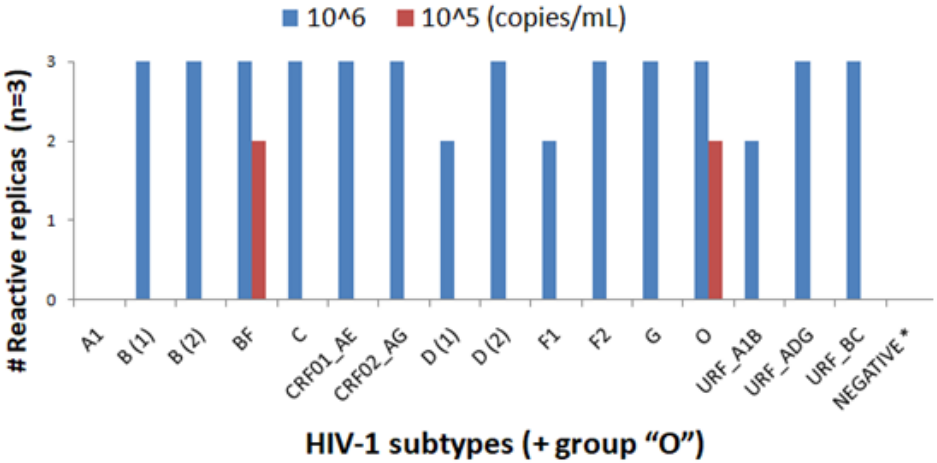
Limit of Detection of HIV Viral Load

- Make *in vitro* culture of several HIV-1 group M subtypes and group O to generate HIV viruses;
- Collect culture supernatant (full of HIV viruses);
- Quantify HIV VL of culture supernatant;
- Spike culture supernatant in HIV negative human plasma;
- Make serial dilution of known quantity of HIV VL;
- Challenge Alere HIV Combo!



Blind panel of HIV subtypes, from cultured viruses: The EQAPOL proficiency testing program

Stone, M et al. J Clin Microbiol, 2018

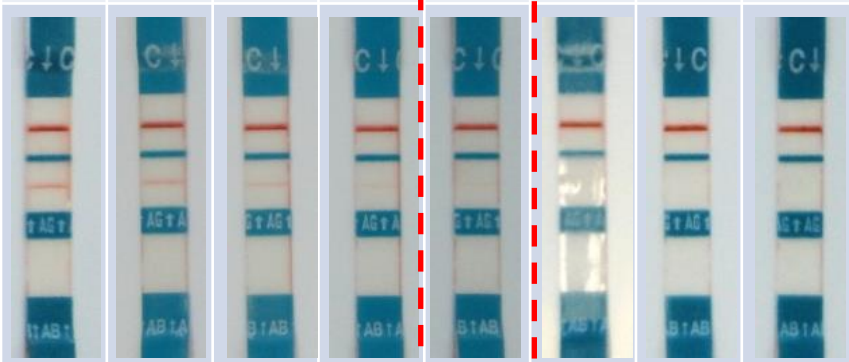
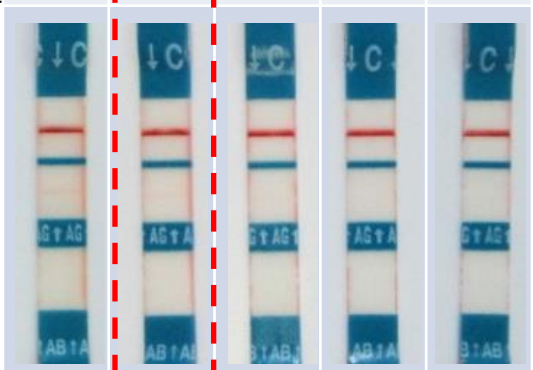


Detection of VL between 100K and 1,000K cp/mL and of different HIV subtypes (including group O)

However, EQAPOL panel did not precisely defined de Limit of Detection of Alere HIV Combo

Limit of Detection of HIV Viral Load

HIV-1 subtype B virus spiked in HIV negative human plasma:
Viral Load validated by 74 laboratories

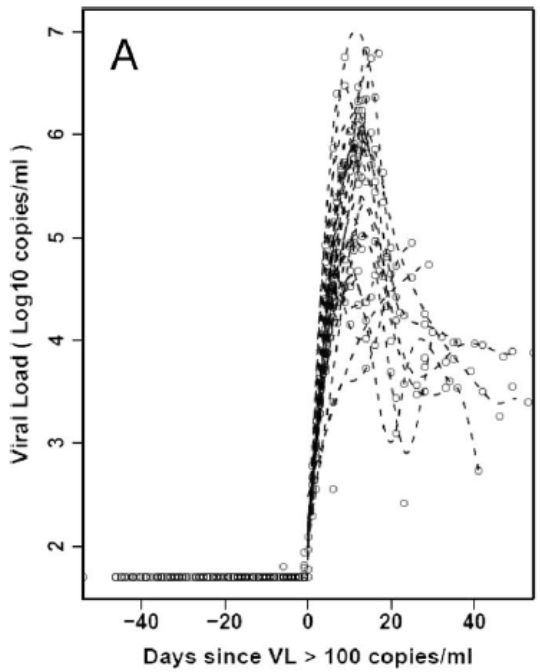
Sample 1 – dilution series									Sample 2 – dilution series				
Pure	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Dilution titer	Pure	1/2	1/4	1/8	1/16
3,020 K	1,510 K	755 K	375 K	188 K	94,400	47,200	23,500	Viral Load (cp/mL)	468 K	234 K	117 K	58,500	29,200
													
+	+	+	+	+	-	-	-		+	+	-	-	-

HIV p24 Ag detected between
188,000 to 234,000 VL (copies/ml)

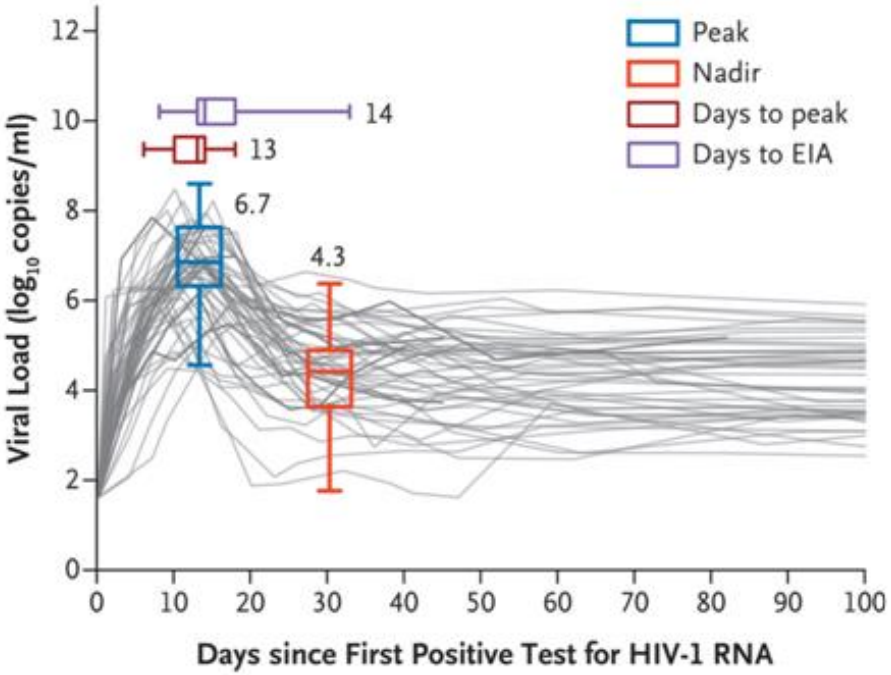
The question now is …

- When during the early stages of HIV infection (Fiebig System) we first find 200,000 cp/mL (LoD of Alere HIV Combo for HIV VL)?
  - Two characteristics of Viral Dynamics of Acute HIV-1 Infection :

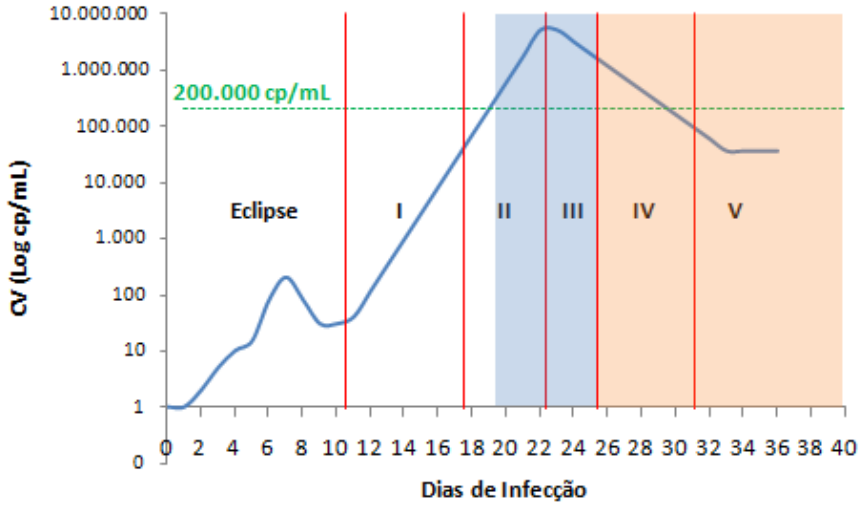
Sharp VL increase

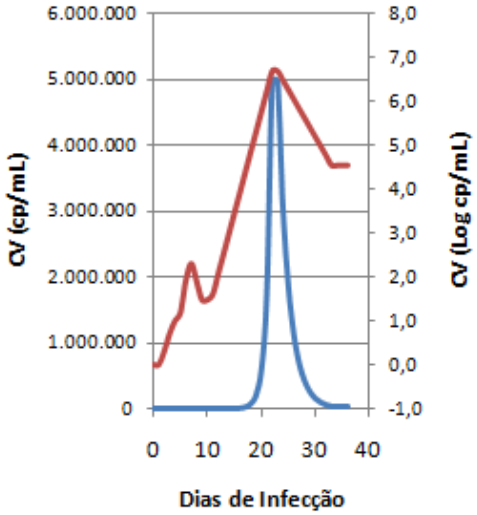


10 to 15 days to reach peak VL once first detected



200,000 cp/mL crosses Fiebig stages II to IV





Ribeiro RM, J. Virol., 2010
Hill AL, Immunol. Rev., 2018

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[a] Eclipse; [b] Limiting Antigen Avidity Assay; [c] p31 protein (integrase)

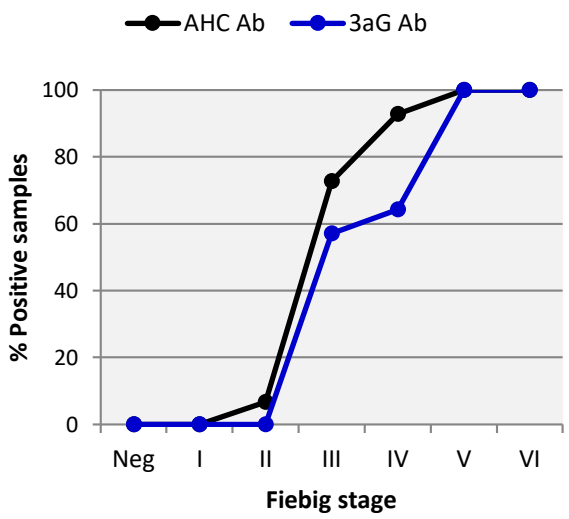
Fiebig et al. AIDS 2003, 17:1871–1879

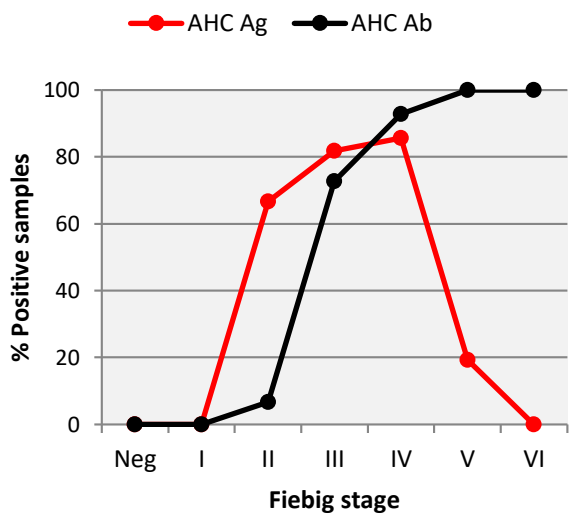
Alere HIV Combo - Antigen component

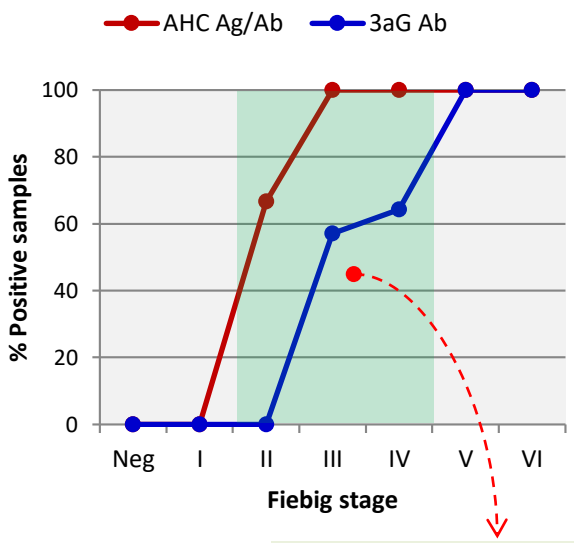
Using HIV early seroconversion panels

Performance of Alere HIV Combo x 3rd generation RT

Neg	I	II	III	IV	V	VI
14	9	15	22	14	26	15





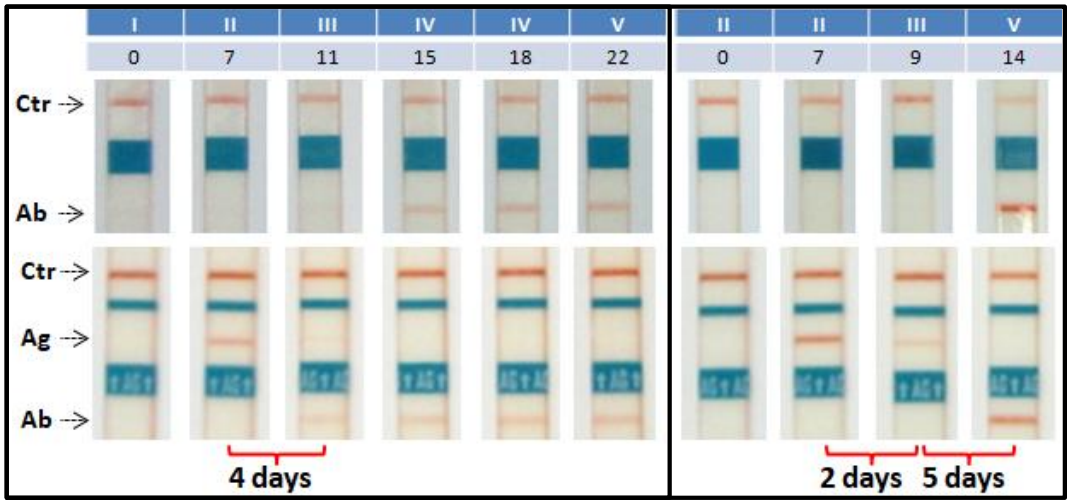


Opportunity for early HIV diagnosis

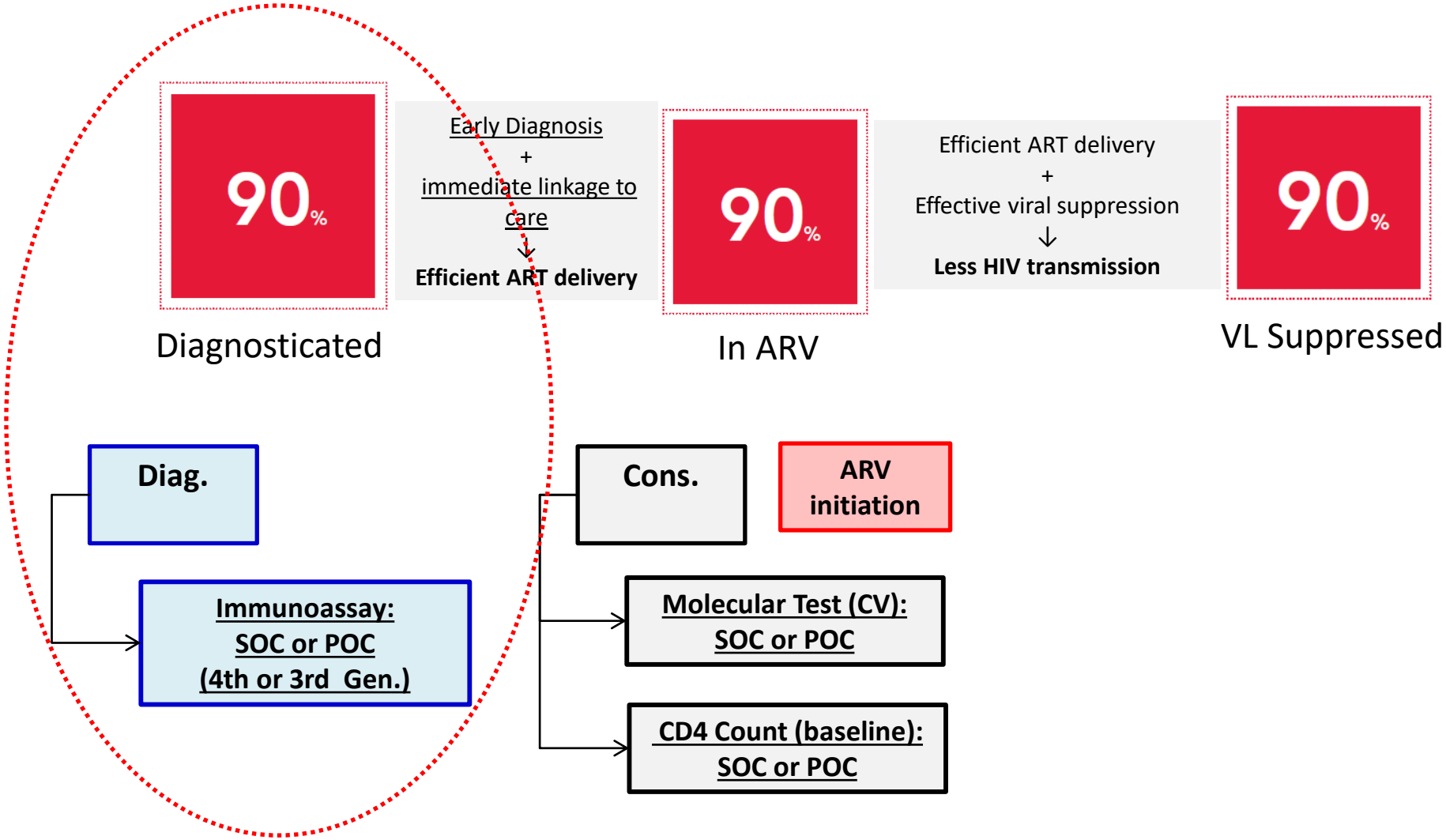
Alere HIV Combo - Antigen component

Using HIV early seroconversion panels

Determine Ab x Alere HIV Combo

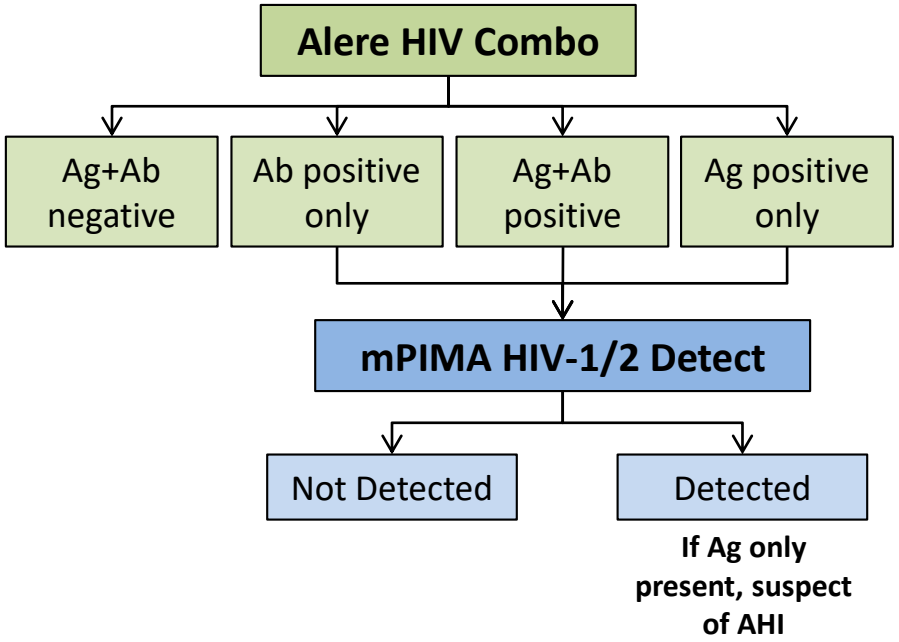


Steps and Challenges of 90/90/90



Is there a "rapid" molecular assay for HIV confirmation?

- A qualitative and rapid molecular assays (RMA) is available
  - **mPIMA HIV-1/2 Detect (POC):**
    - Widely used for Early Infant Diagnosis
- Two POC assays: Alere HIV Combo + mPIMA HIV-1/2 Detect



Diagnosis confirmed within 90 minutes (RT + RMA);

Only 75µL (50µL RT + 25µL RMA) of blood collected by fingerstick;

Can be performed at any site;

Possible onsite identification and confirmation of acute cases of HIV infection.

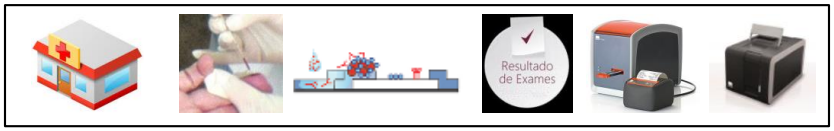
Advantages of a "*rapid*" 4thG algorithm

Laboratory assays
(SOC)

<u>Public Health Cost</u>:
X days => ~20% loss of diagnosis
More HIV transmission ….

<u>Cost to patient</u>: multiples visits =
transportation cost +
day lost from job +
Health deterioration

"Rapid" 4thG Algorithm
(POC)



Linkage to care in one single visit!

How can we wisely apply the Combo assay?

- Choose appropriate population
  - high incidence group
- Repeat testing frequently
  - Most effective
- PreP?
  - Yes: to select individuals for entry

Key populations and 4thG POC – Example

Estimates of HIV incidence from 2004 to 2013 (15 years old and over)

- Population: 29.4 per 100,000 (95% CI 27.0-31.5)

General population 0,6%

Men 15-49 0,7%

Women 15-49 0,4%

Exposure category	Incidence rate of HIV (100.000 inhabitants) - 2013	
	Recife	Curitiba
MSM	1.469	923,7
Men (Others)	37,2	28,1

40 times higher than general male population

FSW

HIV **Sífilis**

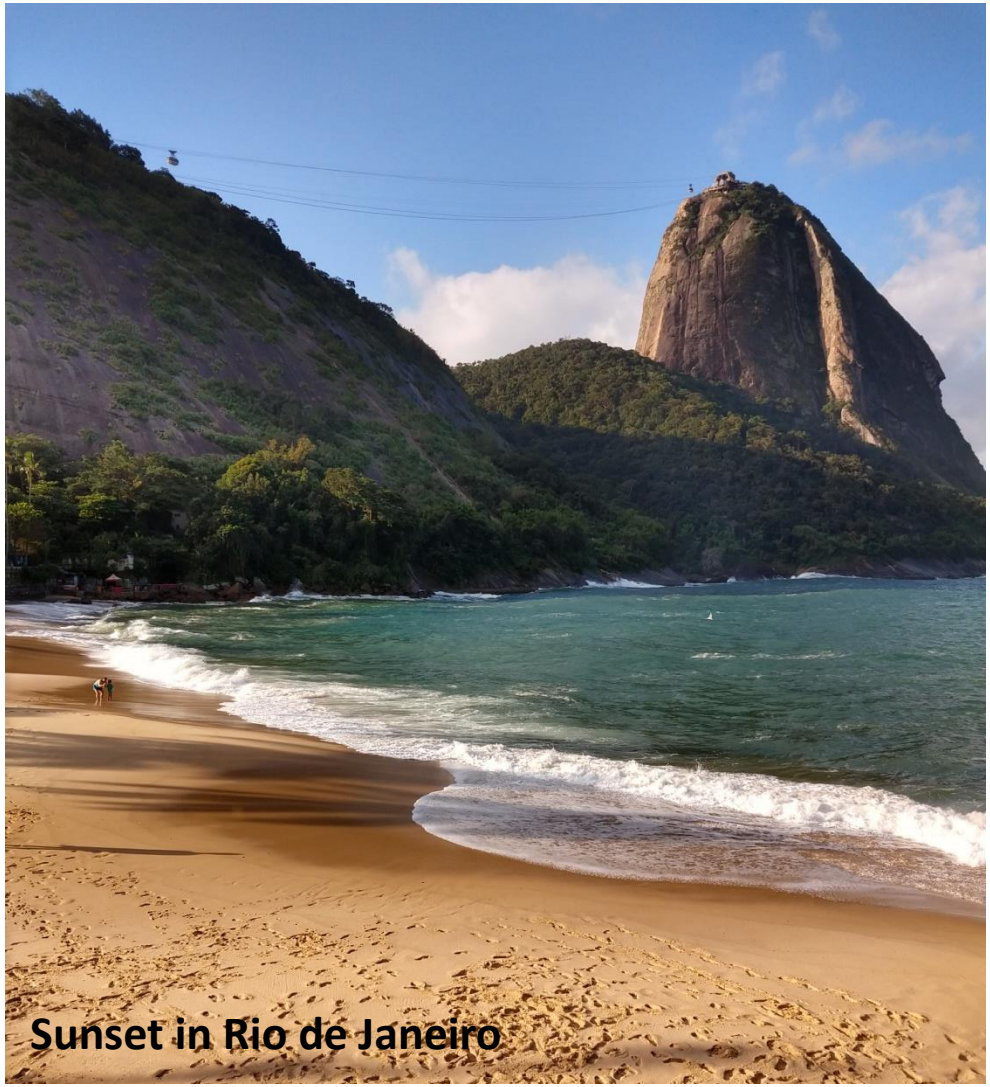
4,9% - 2009 – 2,4%

5,3% - 2016 – 8,5%

MSM 10,5%

PWID 5,9%

Incidencia = 388 per 100.000
(IC95%: 376-400)
24 times higher than general female population.



Thank you!