HIV-1 Viral Load Testing and the use of Dried Blood Spot (DBS) in Resource-Limited Settings

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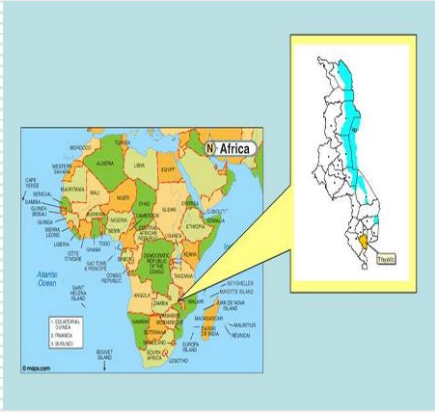
Key Facts for Providers and Patients

- VL is the best measure for the level of progression of HIV infection
- VL = number of HIV copies in a milliliter (copies/mL) of plasma
- More virus ⇨ Faster destruction of CD4 cells ⇨ More severe immunosuppression
- VL is done using an advanced lab method (RNA-PCR) on a blood sample.
- VL are costly
- VL can be done from:
  1. **Blood (plasma):** Transport in cooler box to lab within 24 hours.
  2. **Dried blood spot (DBS):** Transport in plastic bag with desiccant at ambient temperature, sample viable for 3 months or more.

MALAWI RECOMENDATIONS (July 2011)



ROUTINE VL

- Patients harbouring drug-resistant HIV when starting ART will be found with a VL after 6 months on ART > Important early sign for **poor adherence**.
- After that, patients who are adherent and clinically well have a low risk of ART failure. Therefore, routine follow-up VLs are done at 2 years, 4 years, 6 years, etc. after ART initiation.

TARGET VL

- Do additional VLs outside of this schedule for patients with suspected ART failure

VL>5000 copies x ml switch to 2nd line treatment

THYOLO - Malawi

- MSF in Thyolo since 1997
- Thyolo HIV prevalence = **14%** (N= 600,000).
- **34,730** people had ever intiated on ARVs by March, 2012.
- **27,446** people are alive and on ART
- <1% 2nd line ART

CAN WE USE VL IN A RURAL DISTRICT?

- VL laboratory in Thyolo as a pilot to validate the use of DBS finger prick compared to plasma and DBS from EDTA tubes

Choosing the Platform
NucliSENS EasyQ HIV-1 v2.0 Assay

- Use of DBS from EDTA venous blood validated and good correlation with plasma
- Cost =27.7 euros
- Technical support from bioMerieux South Africa
- Small size of the equipment
- Technically simple to operate
- Fast, results available in a few hours
- Low chances of cross-contamination
- Maintenance: daily / weekly/monthly → simple

Infrastructure

- 2 separate rooms (extraction/amplification)
- AC to maintain temperature 18-25°C
- Sink with running water

DETAILS:

- Sealed windows, protective film
- Separate benches for easyMAG and EasyQ analyzer from other "vibrating" equipment (centrifuge, vortex, etc)
- Common area: Refrigerator and freezer for reagents (2 if samples to be stored) – good quality!!!


Amplification
Extraction
2
1

Extraction Room

Amplification/ Detection Room

Equipment capacity
Number of tests

- easyMAG = 24 samples
- EasyQ = 48 samples
- Feasible → **72-96** tests/day = **1440** **(DBS)**-**1920** **(plasma)** tests/month
- Considerations:
  - Controls = 1 Positive and 1 Negative control / run → 22 samples / extraction
  - 2 easyMAG + 1 EasyQ (?)

Human Resources

- HR needs:
  - 2 lab techs full time → 1 in each room
  - 1 lab manager

(72/96 tests/day = **1440/1920** tests/mth)

- bioMerieux training = 3 days → need a molecular biologist to ensure transfer of knowledge to untrained lab staff
- bioMerieux technical support

HIV VL in Resource-Limited Settings
Challenges > Lessons Learned

MEDECINS SANS FRONTIERES

IMPLEMENTATION OF HIV VIRAL LOAD IN THYOLO- MALAWI

Lessons Learned

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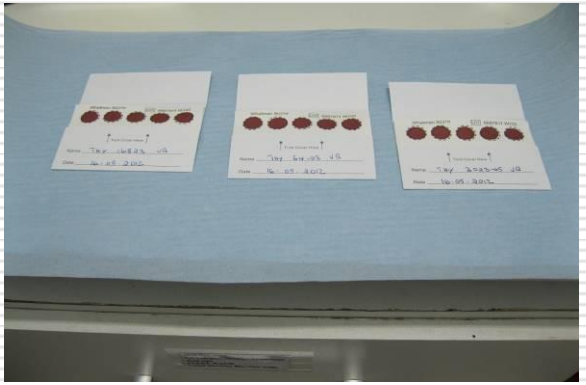
[October 2011]

MSF Operational Research

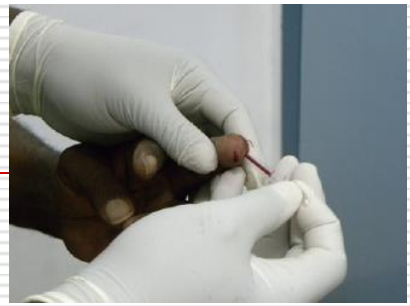
Validation of VL Testing Using DBS Samples Collected from Finger Prick



- **Time frame:**

MSF study started end of April 2011- April 2012

Study design:



265 HIV-1 infected patients

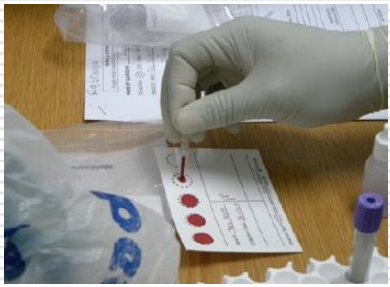
EDTA whole blood

Finger-prick

50 µL spotted on 5 Whatman circles with pipette

Centrifugation within 3 hours

50 µL spotted on 5 Whatman circles with Microsafe



EDTA venous blood DBS

Plasma

Finger-prick DBS

Stored at room temperature

Stored at -20° C

Stored at room temperature

EDTA DBS viral load

Vs.

Plasma viral load

Vs.

Finger-prick DBS viral load

All patients were asked to provide written informed consent

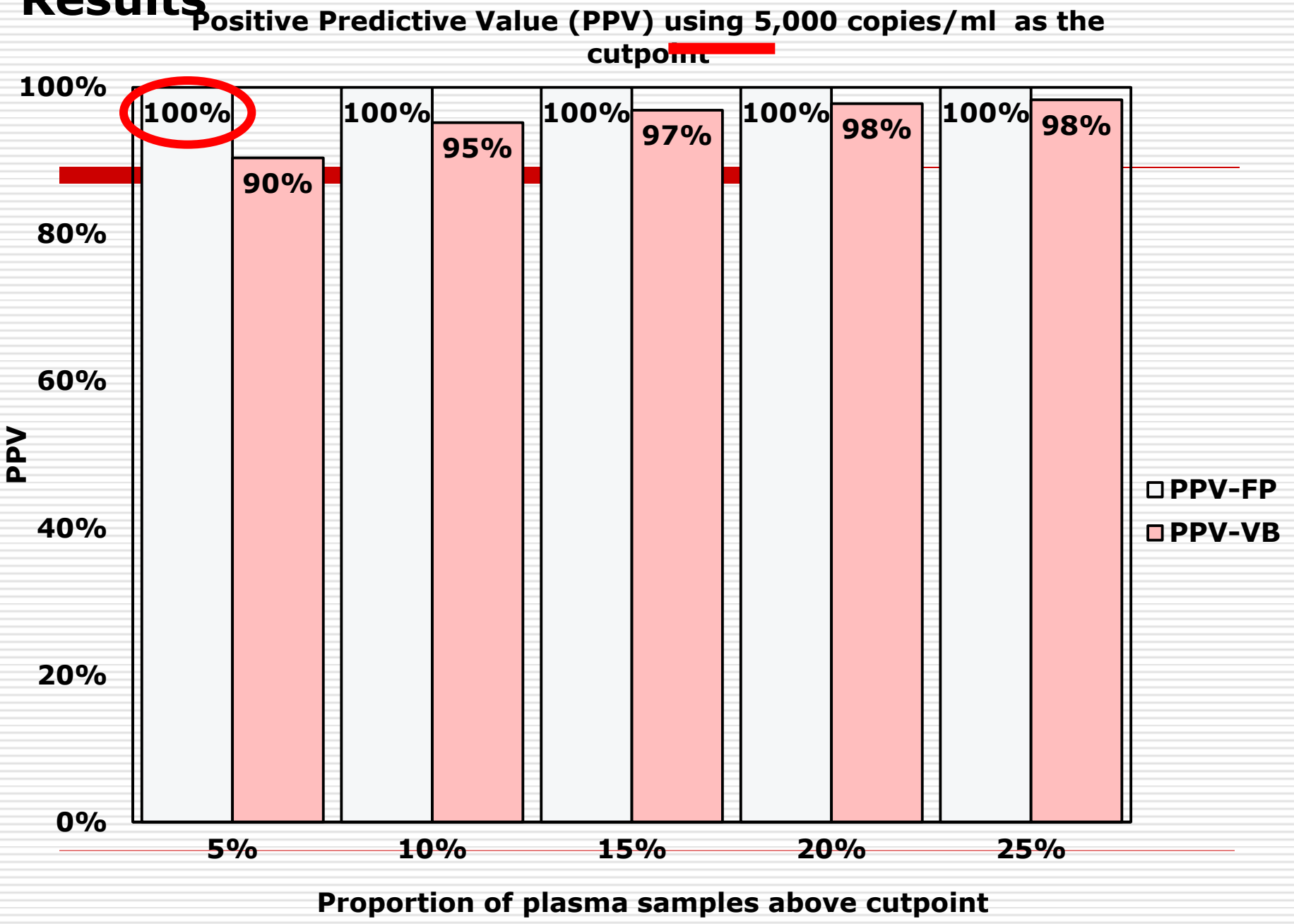
Population

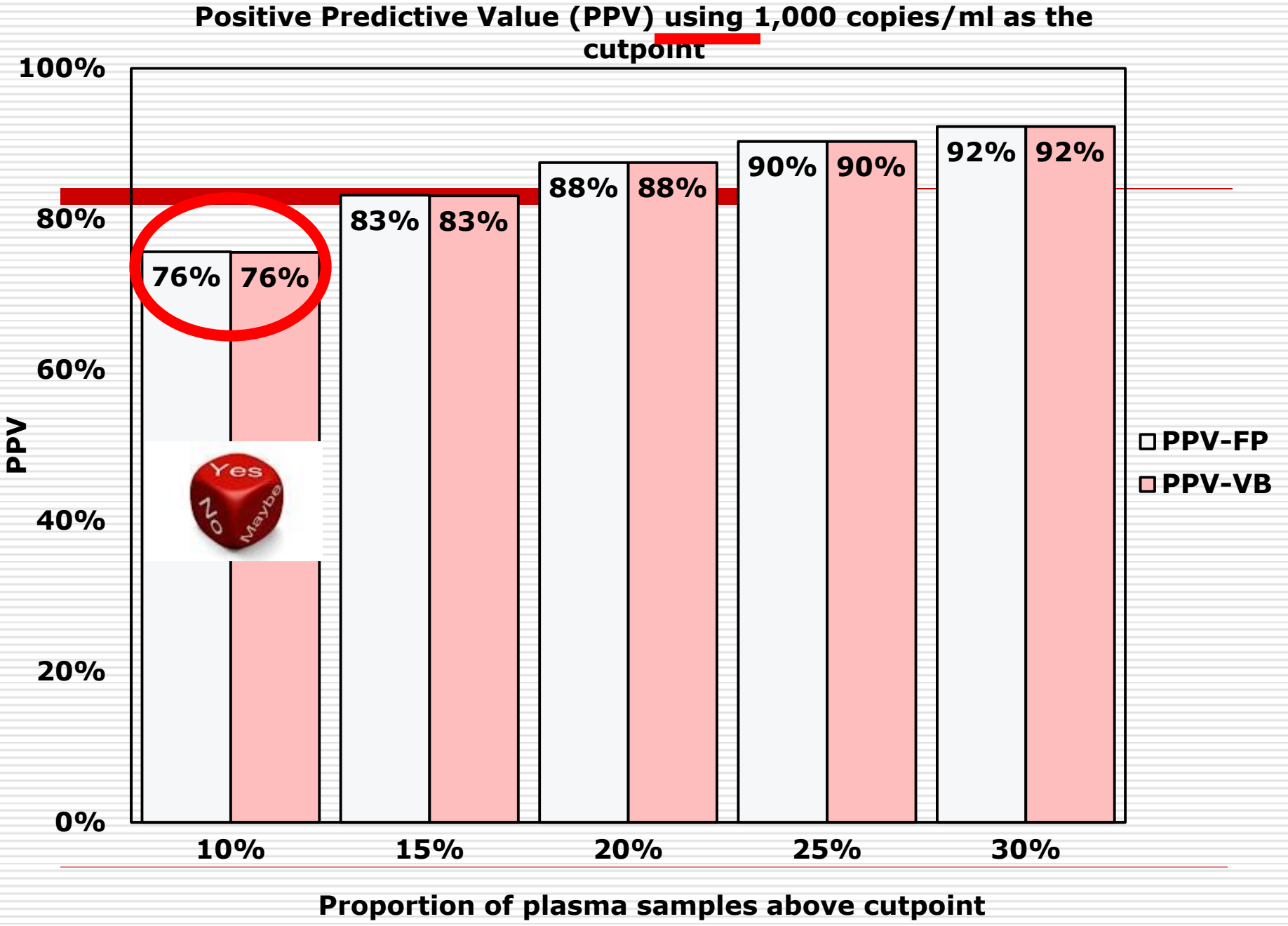
n = 265 with both FP + Plasma	
Gender (n = 265)	Male (46%) Female (54%)
Mean Age (n = 265)	38 years (15 – 70)
Months on ART	> 6 months – 9 years (3 years)
Routine monitoring vs. Treatment failure	59.2% vs 40.8%
Viral Load range	< 20 to 8’600.000 copies/mL

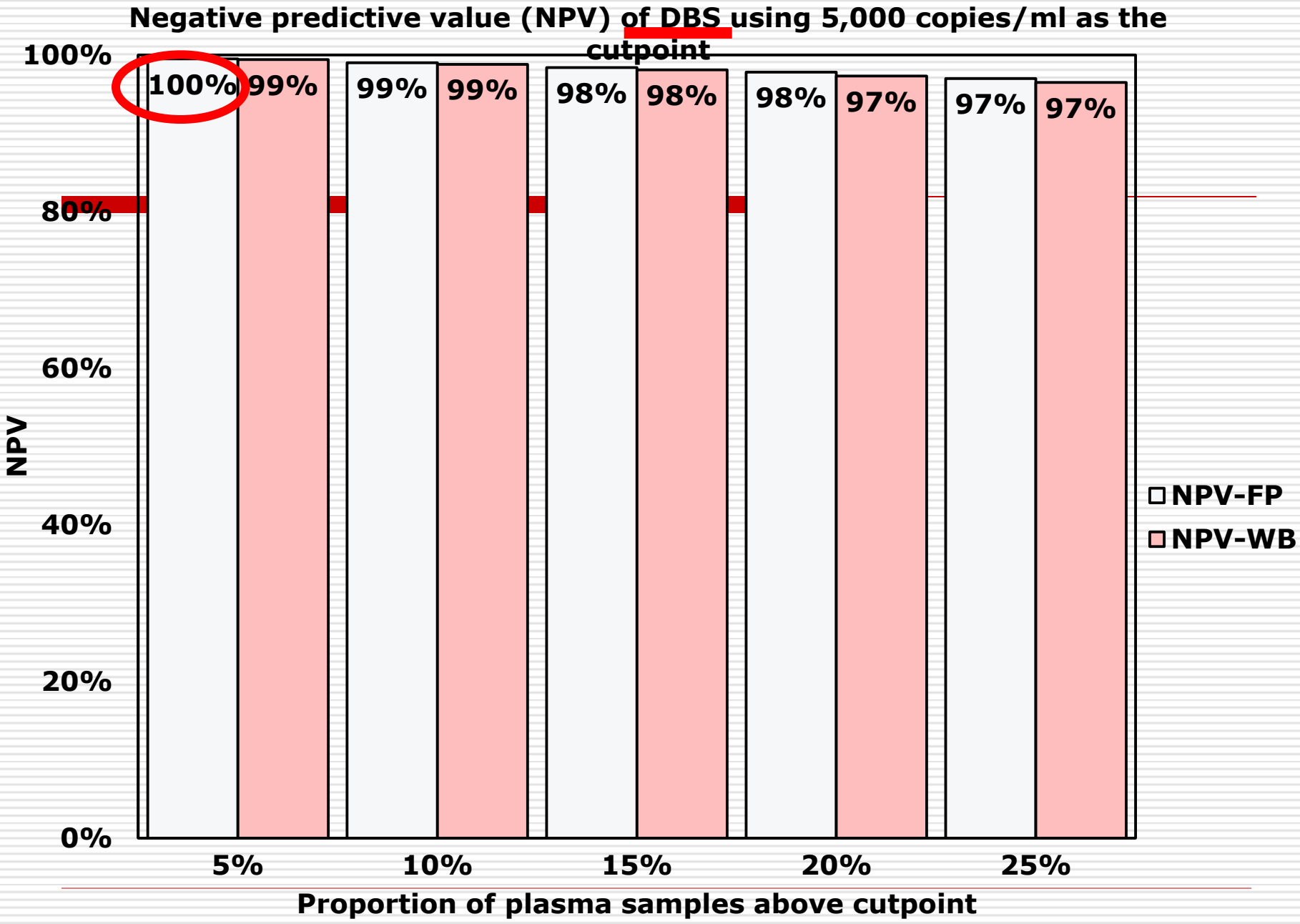
Sample collection Using Finger Prick **Our fears**

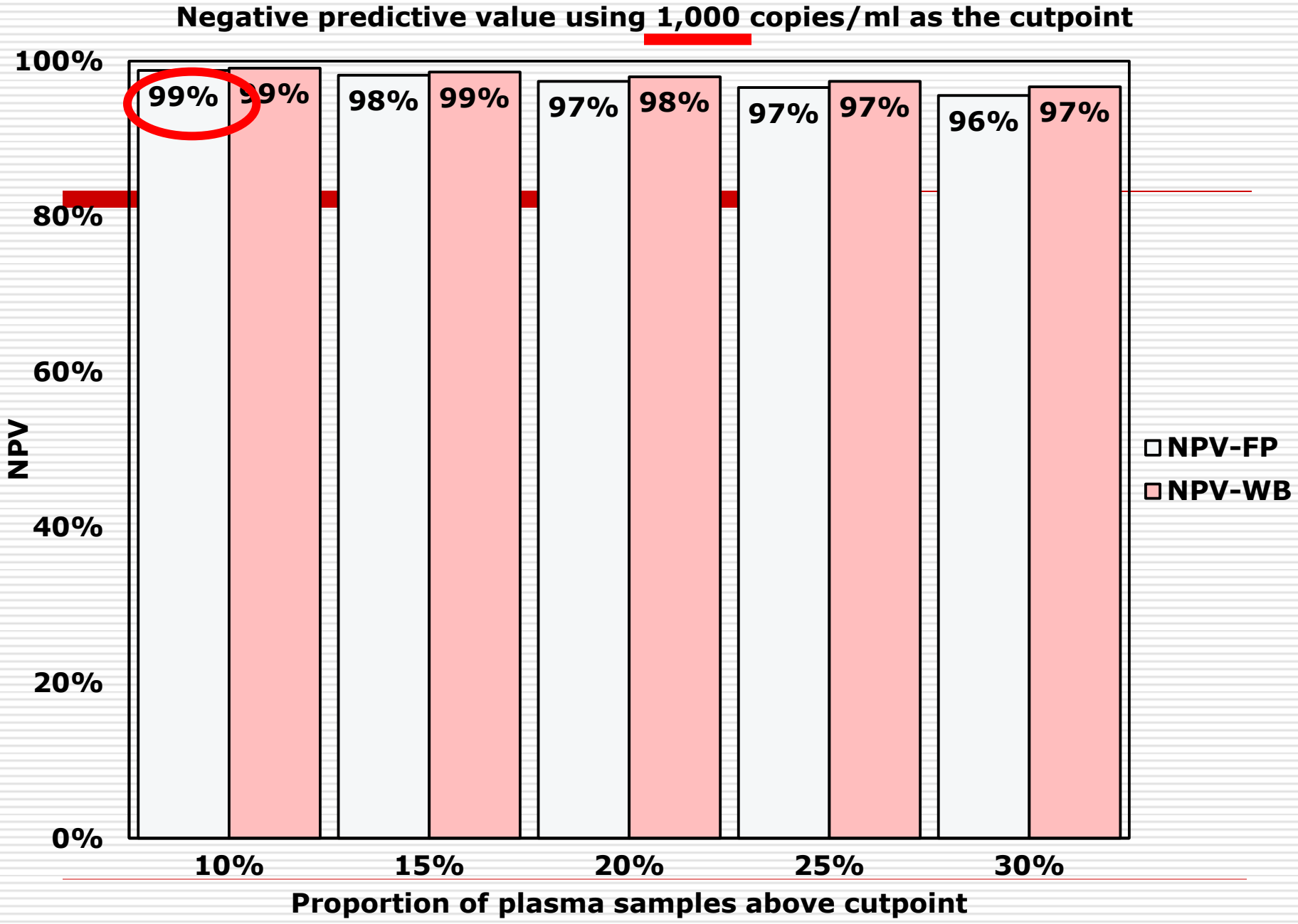
- RNAse contamination of samples (fingers, etc) → powder-free gloves, avoid touching circles with patient's finger
- Quantitative test: use of exact sample volume → 50µl/circle (Microsafe pipette) **?**
- Squeezing/milking the finger → anti-inflammatory cytokines, ratio plasma/cells
- Amount of virus in capillary X venous blood
- EDTA X non-anti-coagulated blood

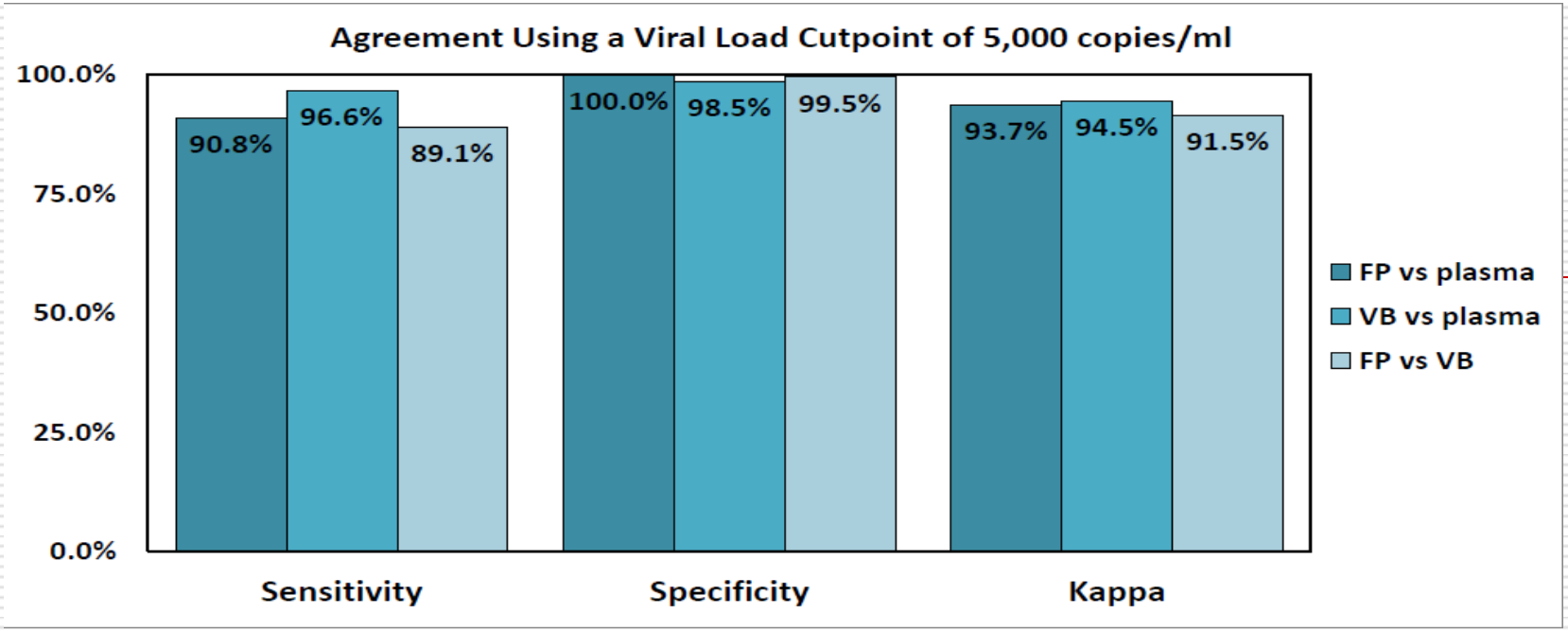
Results

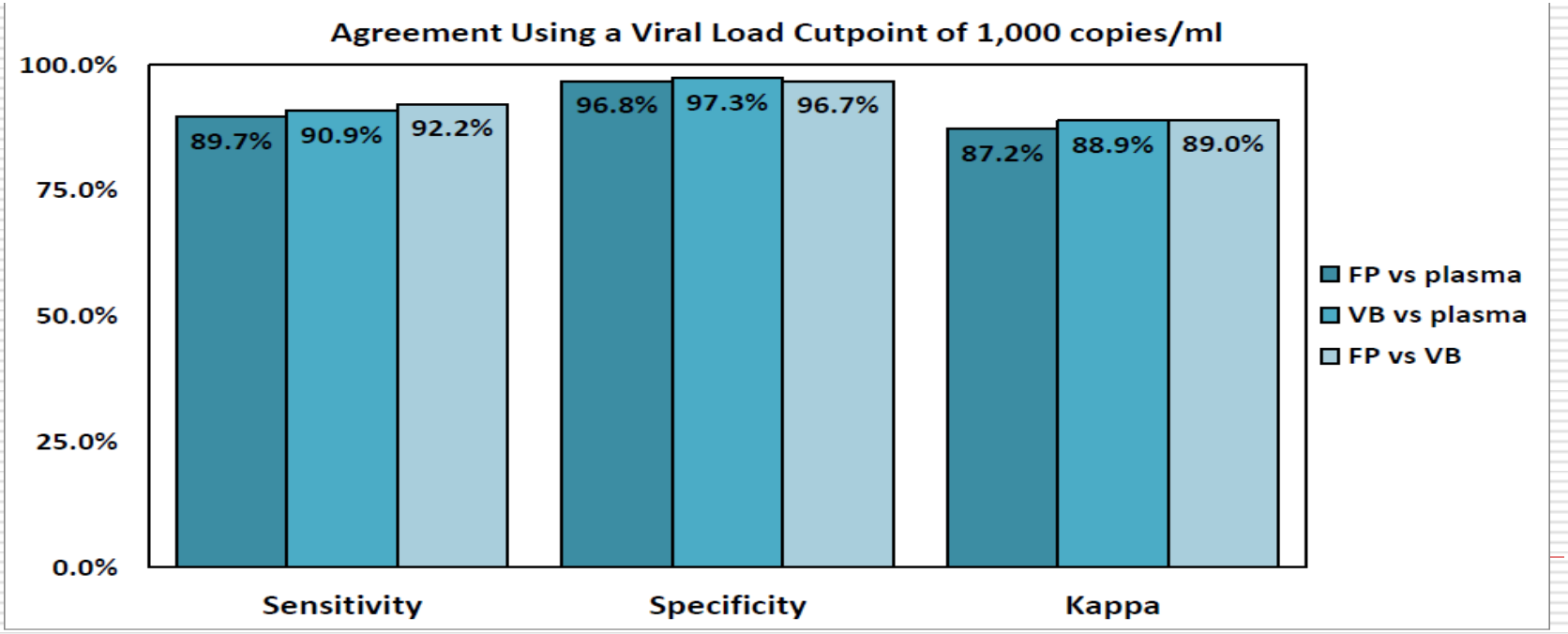

Positive Predictive Value (PPV) using 5,000 copies/ml as the cutpoint
100%
80%
60%
40%
20%
0%
PPV
100%
90%
100%
95%
100%
97%
100%
98%
100%
98%
5%
10%
15%
20%
25%
PPV-FP
PPV-VB
Proportion of plasma samples above cutpoint


Positive Predictive Value (PPV) using 1,000 copies/ml as the cutpoint
100%
80%
60%
40%
20%
0%
PPV
76%
76%
83%
83%
88%
88%
90%
90%
92%
92%
PPV-FP
PPV-VB
10%
15%
20%
25%
30%
Proportion of plasma samples above cutpoint

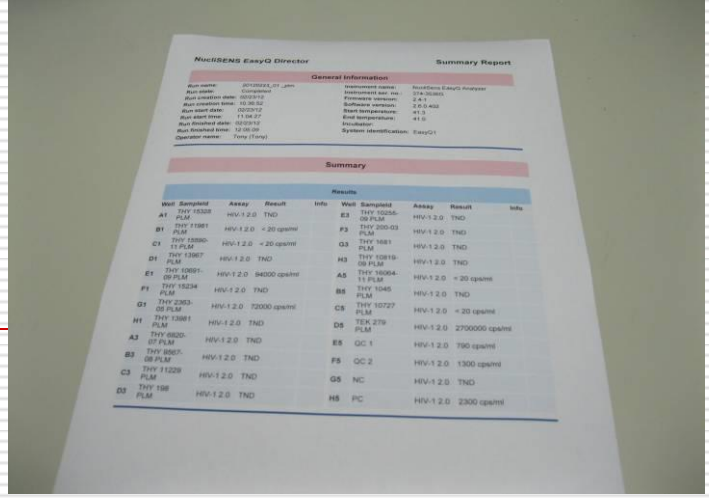

Negative predictive value (NPV) of DBS using 5,000 copies/ml as the cutpoint
100%
80%
60%
40%
20%
0%
NPV
100%
99%
99%
99%
98%
98%
98%
97%
97%
97%
5%
10%
15%
20%
25%
Proportion of plasma samples above cutpoint
NPV-FP
NPV-WB


Negative predictive value using 1,000 copies/ml as the cutpoint
100%
80%
60%
40%
20%
0%
NPV
99%
99%
98%
99%
97%
98%
97%
97%
96%
97%
10%
15%
20%
25%
30%
Proportion of plasma samples above cutpoint
NPV-FP
NPV-WB


Agreement Using a Viral Load Cutpoint of 5,000 copies/ml
100.0%
75.0%
50.0%
25.0%
0.0%
90.8%
96.6%
89.1%
100.0%
98.5%
99.5%
93.7%
94.5%
91.5%
Sensitivity
Specificity
Kappa
FP vs plasma
VB vs plasma
FP vs VB


Agreement Using a Viral Load Cutpoint of 1,000 copies/ml
100.0%
75.0%
50.0%
25.0%
0.0%
89.7%
90.9%
92.2%
96.8%
97.3%
96.7%
87.2%
88.9%
89.0%
Sensitivity
Specificity
Kappa
FP vs plasma
VB vs plasma
FP vs VB

Conclusion:



- Viral load measured using fingerprick DBS and viral load measured using EDTA DBS had comparable levels of agreement with plasma viral load results, demonstrating that, for measuring viral load, fingerprick DBS performs **as well as** EDTA DBS as an alternative to plasma.

Recommendations:

- The choice of sample type should be based on **practical considerations** and the prevalence of an elevated viral load specific to the setting

- Task shifting for FP needs to be validated before general roll-out (Phase 2)

Research Q:

"Can FP DBS and EDTA DBS be task shifted to other cadre at Health Center level and result's correlation be equally optimal?"

- Use of 5000 copies x ml threshold for **switching** vs. 1000 copies x ml threshold for **enhance adherence** sessions for all types of collection sample system

Algorithm: To be used for whole blood, EDTA DBS or fingerprick DBS

Viral load to be taken:

1 **Routine VL:** At Mth 6,24,48 then 2 yearly

2 **Target VL**: For confirmation on clinical or immunological failure at any time point

3 **For patients more than 6 months on D4T switching to TDF**

VL <1000 copies x ml

Groups 1 and 2 Continue first line regimen

Group 3 Switch to TDF

VL >1000 copies x ml

All groups start enhanced adherence

Repeat viral load with same method **3 months** after first viral load

VL <1000 copies x ml

Groups 1 and 2 continue first line

Group 3 switch to TDF

VL >1000 ≤ 5000 copies x ml

Groups 1 and 2 continue first line

Group 3 switch to AZT and repeat

For all repeat VL after **6 months**

VL> 5000 copies x ml

All groups

Consider switch to second line

If > 0.5 log drop repeat VL after 3 months

THANKS A LOT

Malawi MSF Team