Conflict of Interest

The author has declared no conflict of interest.

Diagnostic Performance of Lateral Flow Point-of-Care HIV-Combo Testing for Detection of Acute HIV Infection in Eswatini

Bernhard Kerschberger[1], Nombuso Ntshalintshali[1], Gugu Maphalala[2], Aung Aung[1], Charlie Mamba[1], Qhubekani Mpala[1], Dumile Sibandze[2], Lenhle Dube[2], R Rufaro Kashangura[3], Alex Telnov[4], Roberto de la Tour[4], Barbara Rusch[4], Alan Gonzalez[4], Alexandra Calmy[5], Iza Ciglenecki[4]

[1]Médecins Sans Frontières (MSF), Mbabane, Eswatini; [2]Ministry of Health, Mbabane, Eswatini; [3]Nhlangano Health Centre, Nhlangano, Eswatini; [4]MSF, Geneva, Switzerland; [5]University Hospital, Geneva, Switzerland

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Background

- Eswatini has one of the highest HIV incidence (1.36% per year) and prevalence (27.0%) globally (*SHIMS-2 2016*)
- MSF is supporting the Ministry of Health in HIV/TB care since 2007
- Despite a decline in HIV incidence by 44% since 2011, it remains high when compared to other countries
- **Sustained high HIV incidence possibly due to Acute HIV Infection (AHI)**

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

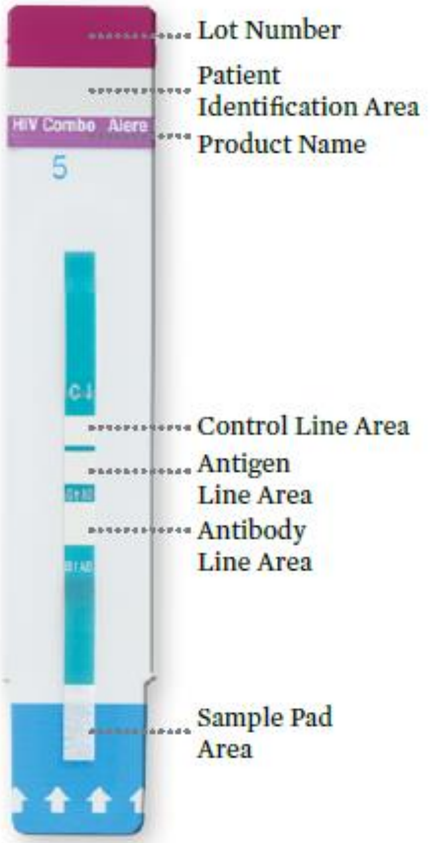
SCIENTIFIC DAYS

Acute HIV Infection (AHI)

- AHI is the time period between HIV infection and HIV sero-conversion (known as “window period”)
- **Window period** for antibody tests is between 3 weeks and 3 months
- AHI contributes up to 20% of all HIV infections in Sub-Saharan Africa, and up to 50% in men who have sex with men
- AHI is rarely suspected, diagnosed and treated in resource-limited settings but often standard care in Western countries
- **Diagnostic barriers**
  - High costs of viral load based diagnostic testing algorithms
  - The widely used antibody-based 3rd generation lateral flow HIV tests miss AHI
  - Lack of reliable antibody/p24 antigen based 4th generation point-of-care tests

The new Alere™ HIV Combo test

- **4th generation lateral flow point-of-care test**, detecting HIV-1/HIV-2 antibodies and p24 antigen
- The detection of p24 antigen suggests AHI, the detection of antibody only suggests recent/chronic infection
- Specimen: Serum/plasma**, fingerstick whole blood**, venipuncture whole blood
- Time to result: **20 minutes**
- **Storage 2-30°C**
- ~1.2 USD per test performed **(low cost)**
- **WHO prequalified since 2016**



Source of photo: https://www.globalpointofcare.abbott/en/product-details/alere-hiv-combo.html

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Objective

- **We aimed to assess the diagnostic performance of the new point-of-care rapid diagnostic (Alere™ HIV-Combo) test for the detection of AHI in Eswatini**

- Ethics approval was obtained from the MSF Ethics Review Board and the Eswatini Health and Human Research Review Board

- No conflict of interests

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Methods – Enrollment

- Study design: **Nested laboratory study** assessing the performance of a new point-of-care Alere™ HIV-Combo
- Location: Nhlangano Health Centre, Eswatini
- Duration: March 2019 to March 2020
- Enrollment: **HIV sero-negative/ sero-discordant adults (18-49 years) at risk of HIV infection attending the outpatient department**
    - **HIV sero-negative**: Alere™ Determine-negative (by finger-prick testing)
    - **HIV sero-discordant**: Alere™ Determine-positive and Uni-Gold™-negative (by finger-prick testing)

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Methods – Procedures

1. **Gold standard:** Participants were tested with the quantitative Xpert HIV-1 viral load assay for AHI.

    - AHI+ was defined as a VL result ≥40 copies/mL in HIV sero-negative/ sero-discordant specimens

2. **Comparator:** Leftover paired venepuncture whole blood and plasma specimens were tested with Alere™ HIV-Combo

    - Reactivity on the p24 antigen and/or antibody bars was considered as AHI+

    - A laboratory technician performed all testing in the lab

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Results – AHI+ cases (Gold standard)

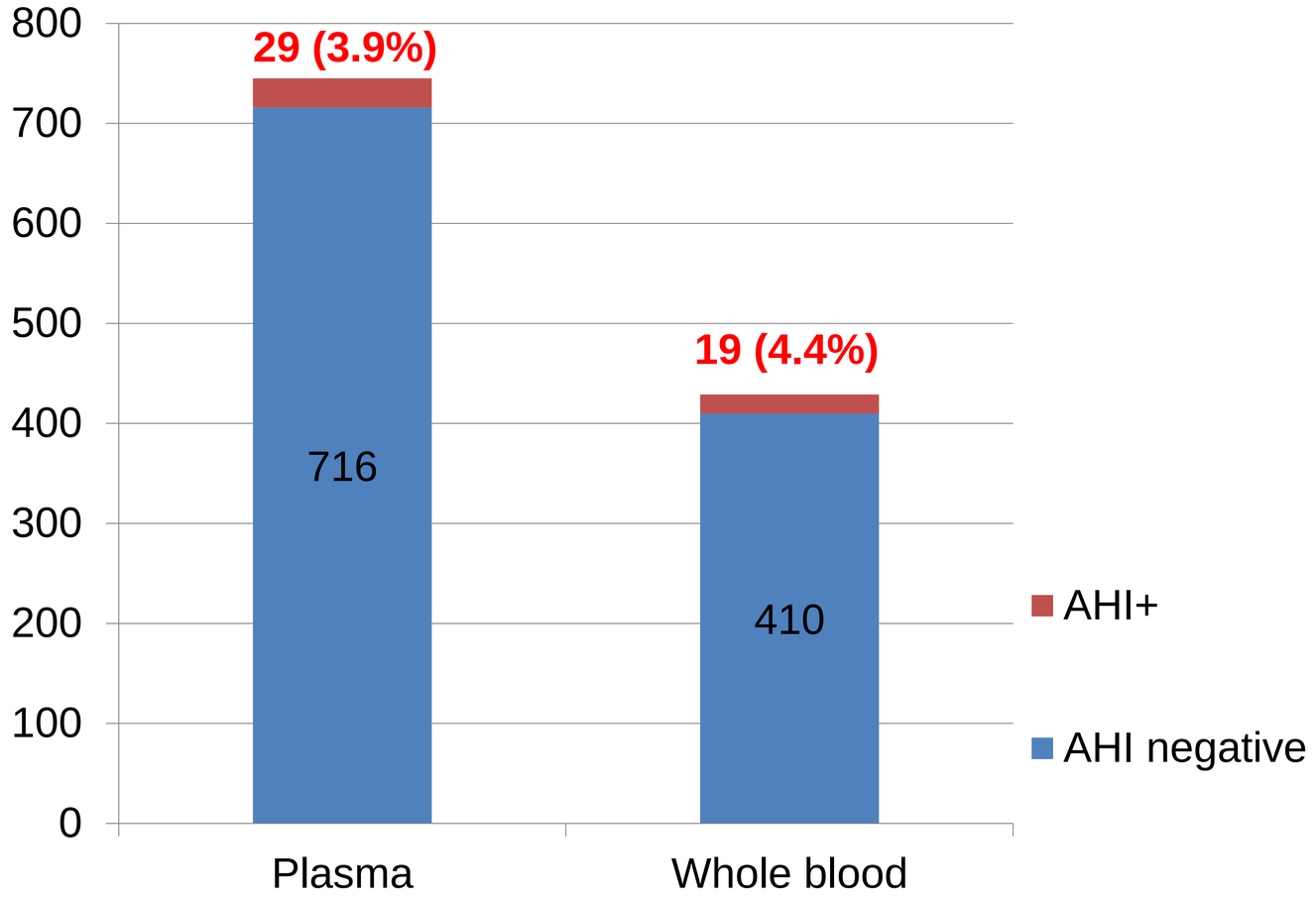
Gold standard

- **Plasma specimens**

  745 test results for HIV-Combo-plasma/Xpert: 3.9% (n=29) were true AHI+

- **Whole blood specimens**

  429 test results for HIV-Combo-whole blood/Xpert: 4.4% (n=19) were true AHI+



MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

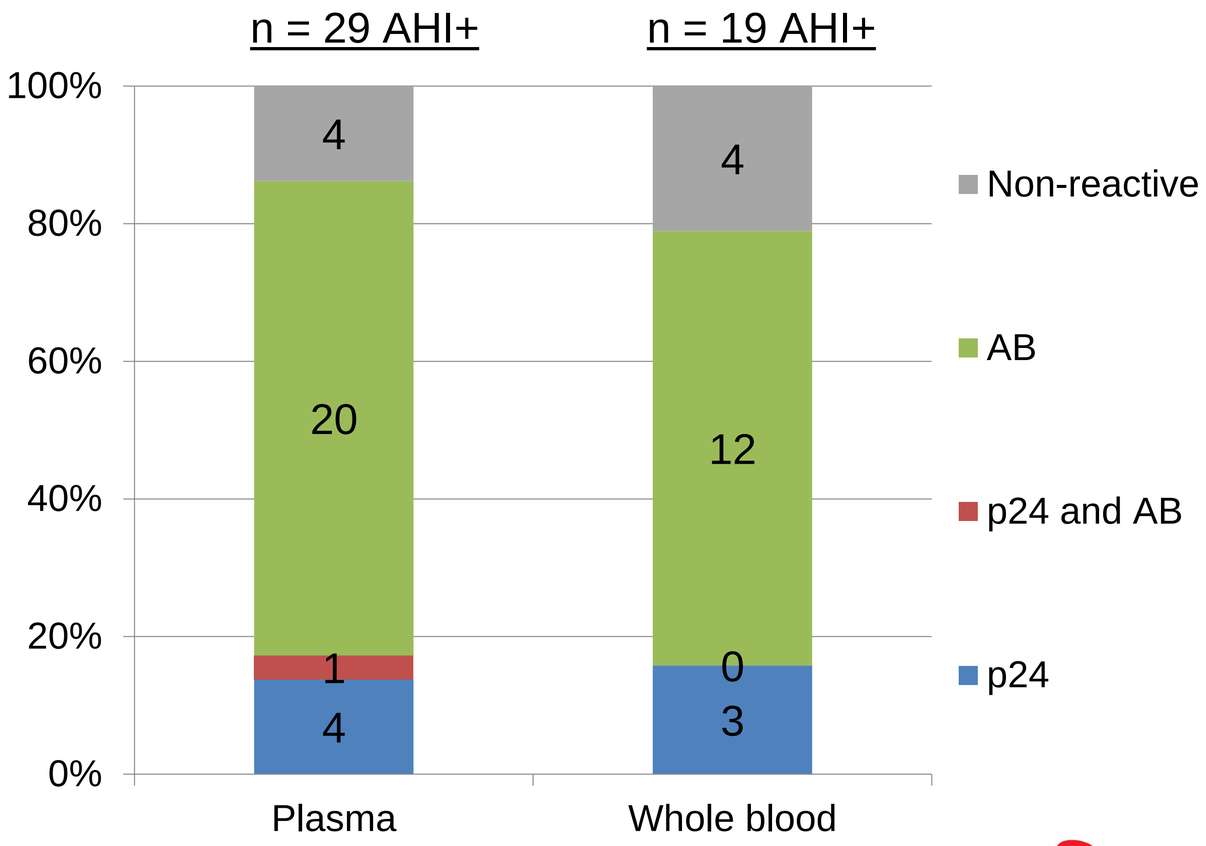
SCIENTIFIC DAYS

Results – HIV-Combo reactivity

HIV-Combo test results:

- **One false positive case** of AHI in both plasma and whole blood specimens
- **Among AHI+ confirmed specimens**
  - HIV-Combo missed ~20% of AHI+ cases
  - <20% were reactive to p24 or p24/antibodies
  - ~60% were reactive to antibodies only

Test results of HIV-Combo in AHI+ confirmed cases



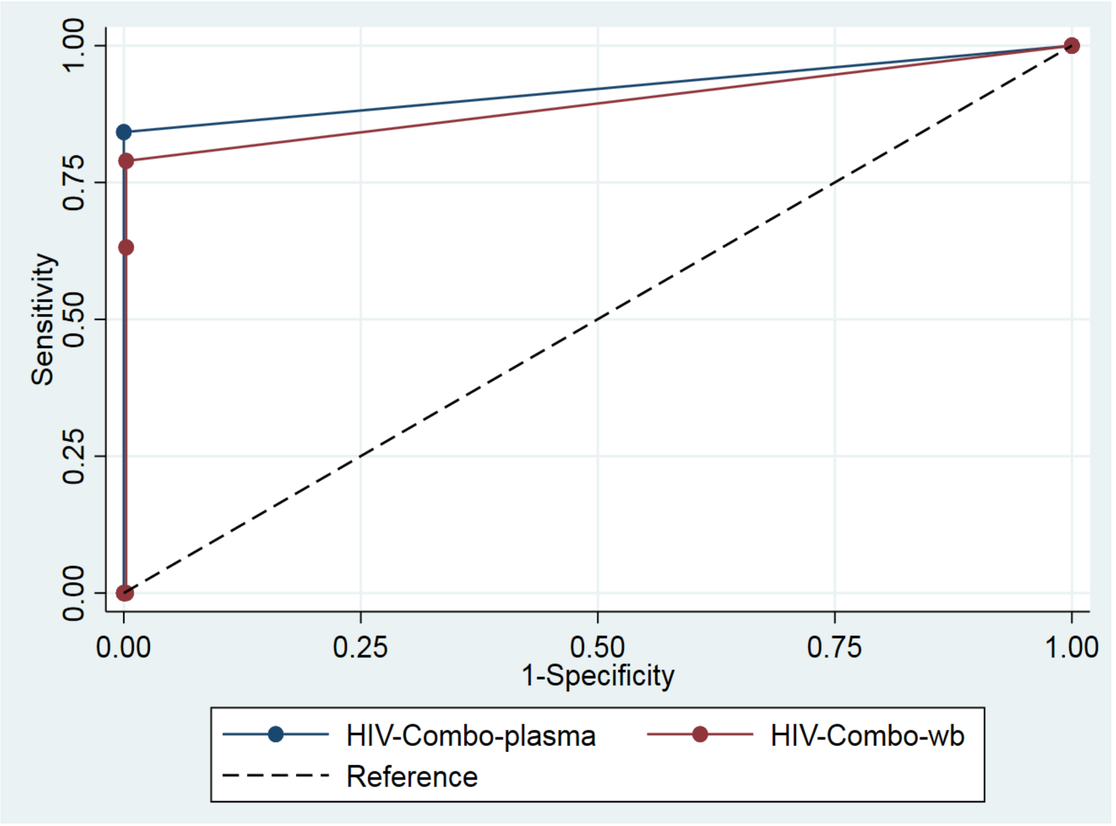
MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Results – ROC curves

The area under ROC were high for both specimen types:

- 0.93 (95% CI 0.87–0.99) for plasma specimens
- 0.89 (95% CI 0.80–0.99) for whole blood specimens



MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Results – Test performance

- **Sensitivity** was slightly higher for plasma (86.2%) vs whole blood (78.9%)
- **Specificity** was high for both specimen types (≥99.8%)
- **Negative predictive value** was above 99.0%
- **Positive predictive values** were 93.8% for whole blood vs 96.2% for plasma

	HIV-Combo Plasma (n=745)	HIV-Combo Whole blood (n=429)
Sensitivity	86.2% (68.3–96.1%)	78.9% (54.4–93.9%)
Specificity	99.9% (99.2–100%)	99.8% (98.6–100%)
Positive predictive value (PPV)	96.2% (80.4–99.9%)	93.8% (69.8–99.8%)
Negative predictive value (NPV)	99.4% (99.6–99.8%)	99.0% (97.5–99.7%)

Limitations

- HIV-Combo testing was performed **under laboratory conditions by a lab-technician** using plasma/ whole blood specimens
    - **Different specimen types** may perform differently
    - In many routine settings, **HIV counselors use finger-prick testing** → Test performance may differ
- **Positive and negative predictive values may change for populations with less AHI transmission**
- **The HIV-Combo test does not always allow to differentiate between established and acute/recent HIV infection** → p24 antigen can be immunocomplexed with antibodies and thus be undetectable, therefore falsely suggesting established HIV infection

Conclusions

- **The 4th generation Alere HIV-Combo test diagnosed most cases of AHI**
- **This test has potential for use in routine settings and for specific populations experiencing high HIV transmission**
- A follow-up study will evaluate its performance when used in routine outpatient care settings by lay counsellors on finger-prick samples
- HIV programmes should start considering more sensitive tests for the detection of AHI in order to achieve HIV epidemic control

MEDECINS SANS FRONTIERES
DOCTORS WITHOUT BORDERS

SCIENTIFIC DAYS

Acknowledgements

- Patients and health workers of Nhlangano Health Centre
- Ministry of Health of Eswatini
  - National Reference Laboratory (NRL)
  - Eswatini National AIDS Programme (ENAP)
- Current and former MSF staff involved in the study