Excellent sensitivity of a rapid 4th generation HIV test (Determine HIV-1/2 Ag/Ab) - based on a blinded evaluation in chronically HIV infected women and men

LBPE12

C. Kahlert[1,2], S. Daneel[1], A. Witteck[1], A. Züst[1], P. Schmid[1], J. Thierbach[3], P. Vernazza[1] and the Swiss HIV Cohort Study

[1]Cantonal Hospital St. Gallen, Switzerland, [2]Children Hospital St. Gallen, Switzerland, [3]Cantonal Hospital, Transfusion, St. Gallen, Switzerland

christian.kahlert@kssg.ch

SWISS HIV COHORT STUDY

BACKGROUND

Rapid point-of-care HIV testing is an essential screening tool for widespread detection of HIV infection and thus an important contribution to HIV prevention. Recently, a 4th generation test with the ability to detect p24-antigen and HIV-antibodies has become available. Pavie et al. (1) challenged the use of this test by reporting an unacceptably low sensitivity at the 2009 IAS meeting. 198 HIV positive individuals with chronic HIV infection have been sequentially tested by several rapid tests using capillary blood. To the great surprise of most experts, the test yielded a low sensitivity for the Determine® 3rd and 4th generation assays (*Table 1*).

Table 1: Results by Pavie et al, 2009, IAS (1)

n=198	Oraquick blood	Oraquick oral fluid	Vikia blood	Determine 3rd blood	INSTI blood	Determine 4th blood
Negative	9	22	1	7	2	5
Indeterminate	4	13	1	1	3	5
Negative Ctr	0	0	0	3	2	31
Positive	185	163	196	187	191	157
% False neg	4.6	11.9	0.5	3.6	1	3.1

Possible explanations for the low sensitivity claimed by Pavie et al. (1) and others might include:

- use of capillary whole blood
- insufficient training of testing personnel
- combined antiretroviral treatment (cART)

STUDY AIMS and HYPOTHESIS

The present study was conducted to confirm or reject the low sensitivity presented by Pavie et al. (1).
Determine® HIV-1/2 Ag/Ab, the most widely used rapid test in Switzerland was prospectively evaluated

- using capillary blood
- in chronically HIV-infected cART treated individuals
- by adequately trained personnel

Hypothesis: sensitivity of Determine® 4th is > 99%

METHODS and STUDY DESIGN

Study design

Prospective blinded evaluation of Determine® HIV-1/2 Ag/Ab in a routine HIV testing site
Ethical approval and informed consent were obtained

Subjects

Patients, fully documented HIV positive under cART
Blood donors, documented HIV negative

Test set up and blinded interpretation

Capillary blood draw and HIV rapid test according to manufacturer's instruction
Results were interpreted by a blinded and trained reader on a prepared worksheet (*Fig 1*) within 20-30 minutes
HIV positive and negative tests were randomly mixed by an independent co-investigator to ascertain at least 10% (1-4 per day) of negative tests
All study personnel received standard training

Statistics

Fisher's exact test was used to compare relative frequencies of indeterminate tests
Sensitivity was calculated as test-positive / true positive
95% CI for fractions with a zero denominator were calculated according to the "*rule of three*" (2)

STUDY SPONSOR

This study was set up by the authors independent of the manufacturer of the test. Inverness Medical® provided all the testing material and supported the personnel conducting the study. The analysis was independently

RESULTS

Demographics

Between May and July 2010, **300** HIV positive patients and **69** HIV seronegative blood donors (controls) were tested in our institution. Both groups were comparable concerning sex and age. All patients were under cART. The demographic data are depicted in *Table 2*.

Table 2: Study population

n=369	HIV infected	controls
sex (male)	70%	68%
age (mean)	46 years	45 years
Antiretroviral treatment status	100%	N/A
duration HIV infection (median)	**11.5** (0.4–27.4) years	N/A

N/A = not applicable

Detailed test results

The detailed results of the blinded evaluation of the rapid tests in numbers is given in Fig. 1. One single sample reacted in all three bands (antigen, antibody, control).

Fig 1: Worksheet for documentation of test results and corresponding results in numbers

Test result	HIV +	HIV -
	0	0
	33	0
	0	0
	0	**69**
	1	0
	0	0
	266	0

Sensitivity of the Assay

- None of the chronically infected patients tested false negative
- None of the controls tested false positive.
- Crosstabulation (see table 3) of the results demonstrate a sensitivity of **100%** for the Determine® HIV-1/2 Ag/Ab (95% CI 99%-100%)

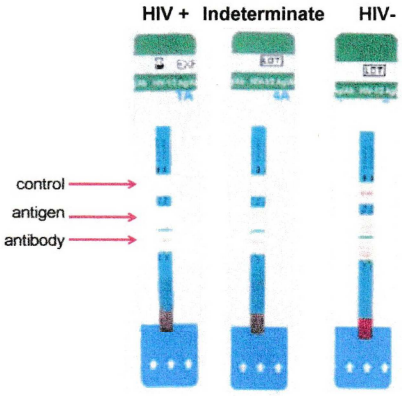
Table 3: Crosstabulation of the results

Test result	HIV +	HIV -
positive	267	0
negative	0	69
indeterminate	33	0

Indeterminate results

- As reported previously (1), a high rate of indeterminate test results (example, see *Fig 3*) was observed in samples from HIV-positive subjects. **(8.9%)**
- Indeterminate results were characterized by a missing control band but showing a strong HIV-positive antibody line (Fig 2)
- Indeterminate results appeared to be more likely in samples were the positive HIV-Antibody reaction band was very strong, suggesting a competitive effect
- None of the test from HIV-neg control individuals was indeterminate (0%, n=**69**)
- The frequency of indeterminate results differed significantly in HIV-pos. and HIV-neg. samples (33/300 vs. 0/69; p<0.01).

Fig 2: Weak or absent control reaction in 3 representative examples of antibody tests



The representative samples nicely demonstrate the weak or absent control band usually found in HIV-positive samples and the strong control reaction in HIV negative individuals.

DISCUSSION

Sensitivity of the assay

- This study confirms a **100%** (99-100%) sensitivity of Determine® HIV-1/2 Ag/Ab in chronically infected individuals tested by fingerpick
- Improper use of the test results, rather than whole blood methodology or antiretroviral treatment are more likely to explain previous findings (1).

Indeterminate results

- Indeterminate results (not reactive control band) were only found in strongly reactive HIV-positive samples, but in a relevant frequency of 9%
- Competition is the most likely explanation for this observation

CONCLUSION

Sensitivity:
Point-of care HIV testing with the "Determine rapid HIV Ag/Ab" test showed a high reliability and ability to identify HIV infection in our cohort.

Indeterminate Results:
HIV tests with undetectable control lines were only found in HIV-positive individuals. Since any reactive test would have to be re-evaluated with a second alternative test strategy, the negative control band in HIV-positive individuals has no impact on the routine use of the HIV-rapid test in testing clinics.

REFERENCES

(1) Pavie, J., et al, *High rates of false negative results with oral fluid and blood specimens using different kits of rapid testing for HIV diagnosis* – IAS 2009, Abstract MOPDB104
(2) Hanley et al, *If nothing goes wrong, is everything all right? Interpreting zero numerators* JAMA 1983;249:1733

ACKNOWLEDGMENT

The study was conducted in the framework of the Swiss HIV cohort study.

The members of the Swiss HIV Cohort Study are Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Bürgisser P, Burton-Jeangros C, Calmy A, Cavassini M, Dubs R, Egger M, Elzi L, Fehr J, Fischer M, Flepp M, Francioli P (President of the SHCS), Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (Chairman of the Scientific Board), Hasse B, Hirsch HH, Hirschel B, Hösli I, Kahlert C, Kaiser L, Keiser O, Kind C, Klimkait T, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Müller N, Nadal D, Pantaleo G, Rauch A, Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P,Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Taffé