Performance evaluation of BIO-FLASH HIV 1+2 Ag/Ab on Biokit's BIO-FLASH® analyser

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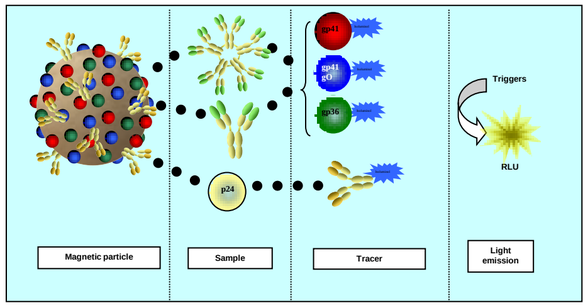
INTRODUCTION AND PURPOSE

Two related retrovirus, human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2) are the etiological agents of the acquired immunodeficiency syndrome (AIDS).

Biokit's **BIO-FLASH HIV 1+2 Ag/Ab** is a fourth generation chemiluminescent two-step immunoassay that uses recombinant antigens with sequences belonging to both HIV-1 (gp41 and gp41gO) and HIV-2 (gp36), as well as monoclonal antibodies against HIV p24. It is designed for the simultaneous detection of HIV-1/HIV-2 antibodies and HIV p24 antigen, allowing an early diagnosis of HIV infection. The aim of this study was to evaluate the performance of the assay on the **BIO-FLASH® analyser.**



When **BIO-FLASH HIV 1+2 Ag/Ab** paramagnetic microparticles are mixed and incubated with the sample and the assay buffer, the specific anti-HIV antibodies and/or HIV p24 antigen, if present in the sample, may bind to the HIV antigens and/or to the HIV p24 antibodies coated on the microparticles. A magnetic separation, followed by a wash step, is done to remove the residual sample. Immediately after, a tracer consisting of isoluminol-labeled HIV antigens and HIV p24 antibodies is added and may bind to the anti-HIV antibodies and/or HIV p24 antigens captured by the microparticles. After a second incubation, a magnetic separation, and a wash step, reagents that trigger the chemiluminescent reaction are added. The emitted light is measured as relative light units (RLU) by the BIO-FLASH luminometer. The RLUs are directly proportional to the anti-HIV antibodies and/or HIV p24 antigen concentration in the sample.

METHODS

Specificity was assessed by testing 6702 fresh unselected serum and plasma samples from three different blood banks (Banc de Sang i Teixits de Catalunya (Spain), EFS Pyrénées-Méditerranée site de Montpellier (France) and DRK Blutspendedienst Baden-Württemberg-Hessen (Germany)), plus 200 non-selected serum samples of hospitalized patients. Sensitivity was assessed by testing 695 pre-characterized positive frozen samples from Hospital routine and Viral Testing Screening Laboratories, including samples of the different anti-HIV-1 subtypes, anti-HIV-2, HIV-1 p24 antigen as well as fresh samples (less than 1 day after extraction). In addition 30 commercial seroconversion panels were analysed. Samples were evaluated in three different sites: Biokit, EFS Pyrénées-Méditerranée and Paul-Ehrlich-Institut. Other relevant characteristics like analytical sensitivity, sample matrix, precision, cross-reactivity, potential endogenous interferences, time to first result, throughput, repeatability and reagent on-board stability, were also assessed.

Relative Specificity and Sensitivity

- 4000 Negative samples Banc de Sang i Teixits de Catalunya (Spain)
- 2102 Negative samples EFS Pyrénées-Méditerranée (France)
- 600 Negative samples DRK Blutspendedienst Baden-Württemberg-Hessen (Germany)
- 200 Hospitalized patients
- 515 HIV-1 Positive samples
- 113 HIV-2 Positive samples
- 30 fresh HIV-1 Positive samples (≤ 1 day after sampling)

Interferents

- 2 samples at different levels *vs*. Hemoglobin, Bilirubin (complex and free) and Triglycerides

Potential Cross-reaction

- 119 samples in 21 different medical conditions: RF, ANA, Pregnant women (including multiparous), HSV-1 IgG, HSV-2 IgG, elevated IgG levels, elevated IgM levels, HBsAg, anti-HCV, anti-CMV IgG, anti-CMV IgM, EBV, Heterophile antibodies (EBV), Syphilis, anti-Rubella, anti-VZV IgG, anti-VZV IgM, HTLV, anti-Toxo IgG, anti-Toxo IgM and anti-*E.coli*

RESULTS

Samples were tested with **BIO-FLASH HIV 1+2 Ag/Ab** in comparison with reference methods (CE Mark).

Sample Group	n	% Sensitivity
anti-HIV-1 subtype A (Group M)	6	100%
anti-HIV-1 subtype C (Group M)	11	100%
anti-HIV-1 subtype D (Group M)	2	100%
anti-HIV-1 subtype CRF01_AE (Group M)	4	100%
anti-HIV-1 subtype F (Group M)	4	100%
anti-HIV-1 subtype G (Group M)	1	100%
anti-HIV-1 subtype CRF02 (Group M)	2	100%
anti-HIV-1 subtype CRF02-AG (Group M)	4	100%
anti-HIV-1 subtype CRF03-AB (Group M)	1	100%
anti-HIV-1 subtype CRF11-cpx (Group M)	1	100%
anti-HIV-1 subtype K (Group M)	1	100%
anti-HIV-1 Group O	8	100%
anti-HIV-2	113	100%
anti-HIV-1 unknown subtype	477	100%
HIV-1 p24 Antigen	30	100%
Same day (≤ 1 day after sampling)	30	100%

BIO-FLASH HIV 1+2 Ag/Ab	n	Value
Relative Specificity (Negative sera and plasma)	6702	99.8 %
Relative Specificity (Hospitalized patients)	200	100 %
Relative Sensitivity (HIV-1 and HIV-2 Positive samples, HIV-1 Ag p24 Positive samples and Fresh samples)	695	100%

Analytical sensitivity results are detailed in the following table:

Sample	Analytical sensitivity
British Working Standard HIV-1	8.17 S/CO
British Working Standard HIV-1, 1 in 5 dilution	1.66 S/CO
Monitor Sample HIV-2	4.58 S/CO
WHO Standard HIV-1 p24 (concentration at the cut-off level)	0.64 IU/mL
Bio-Rad HIV-1 Antigen Standard (concentration at the cut-off level)	15.87 pg/mL

BIO-FLASH HIV 1+2 Ag/Ab results are not affected by the potentially endogenous interferents (CLSI EP7-A Guidelines) and other potential interfering medical conditions, showing an overall agreement of 100%.

Within-Run (Repeatability) and total precision (Within-Lab) were calculated following CLSI EP05-A2. Results are summarized in the table below:

Precision											
Level	Negative Control	anti-HIV1 Positive Control	anti-HIV2 Positive Control	Ag p24 Positive Control	Negative Sample	anti-HIV1 cut-off Sample	anti-HIV2 cut-off Sample	Ag p24 cut-off Sample	anti-HIV1 Medium Positive Sample	anti-HIV2 Medium Positive Sample	Ag p24 Medium Positive Sample
Mean (S/CO)	0.15	2.88	2.98	2.83	0.28	0.90	0.76	1.05	5.61	4.42	5.36
Within-Run (Repeatability)	SD	% CV	% CV	% CV	SD	% CV	% CV	% CV	% CV	% CV	% CV
	0.023	2.4	3.5	2.1	0.022	3.4	3.6	3.5	2.2	3.2	1.8
Total (Within-Lab)	SD	% CV	% CV	% CV	SD	% CV	% CV	% CV	% CV	% CV	% CV
	0.026	4.2	5.5	3.1	0.034	4.9	5.7	5.3	3.8	4.4	3.1

Other functional characteristics of the **BIO-FLASH HIV 1+2 Ag/Ab** assay:

Throughput and Time to first result	• 61 tests/hour and 42 minutes, respectively
On-board stability	• Up to 13 weeks
Other characteristics	• Equivalent results with all sample matrixes assessed (serum, serum separator tube (SST), EDTA-plasma, lithium heparin plasma, lithium heparin plasma separator tube (PST), sodium heparin plasma, sodium citrate plasma, CPDA plasma, CPD plasma, ACD plasma and K-Oxalate plasma) • Demonstrated lot-to-lot interchangeability between Reagents, Calibrator and Controls • 100 tests / kit

CONCLUSIONS

BIO-FLASH HIV 1+2 Ag/Ab assay on the **BIO-FLASH® analyzer** shows an excellent performance in terms of specificity, sensitivity, precision, cross-reactivity, endogenous interferences and repeatability. Due to its throughput, ease of use, random-access sample load capability and long on-board stability, the robust **BIO-FLASH HIV 1+2 Ag/Ab** assay could be an excellent choice for routine use in a clinical or blood bank laboratory.

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