



Determine[®] HIV-1/2 Ag/Ab Combo:
a revolutionary fourth generation rapid HIV test



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Abstract

Fourth-generation HIV-screening assays are the most advanced HIV serological tests. These assays have the capability to detect HIV antibody and HIV-1 p24 antigen simultaneously and were developed to detect acute as well as latent infections using a time and cost-saving strategy. Most fourth generation diagnostics, however, are laboratory based tests (e.g. ELISAs) and are not appropriate for point-of-care application or for use in challenging environments such as remote developing world settings. Inverness Medical has developed a rapid test, the Determine® HIV-1/2 Ag/Ab Combo, which enables simultaneous differential detection of HIV antibodies and p24 antigen enabling differentiation of acute infection from antibody only seropositives. Evaluation of the Determine® HIV-1/2 Ag/Ab Combo in-house and in 9 clinical sites in Africa, Asia, Europe and Latin America shows excellent performance with a clinical sensitivity of 100% (for patients at chronic stage of infection) compared to current standards of HIV detection as well as an improved detection of HIV infection of 2-20 days earlier (average of 5 days) than 3rd generation assays and, a specificity of 99.61% and 99.21% for the Antigen line and for the Antibody line respectively.

Introduction

Despite decades of aggressive prevention efforts and significant advances in effective treatment, HIV infection and AIDS remain a worldwide pandemic, with serious socio-economic consequences^(1, 2).

Primary (acute) or recent HIV infection can be difficult to diagnose since it is asymptomatic or presents as a common febrile illness with non specific symptoms⁽³⁾. Patients are typically highly infectious during this acute phase due to enormous viral burden in blood and genital secretions^(3, 4). In the United States, an estimated one-quarter of those infected patients remain unaware⁽⁵⁾ of their status, and are considered significant contributors to onward transmission of HIV. Therefore, identifying primary HIV infections is clearly a matter of global public health importance and it is essential to preventing further transmission and to identifying individuals which may benefit from early therapeutic intervention.

During acute HIV infection, the virus replicates extensively, reaching high levels of viremia, wide dissemination of virus and seeding of mucosal and lymphoid tissues^(3, 4). Over the following weeks, an early virus-specific immune response is generated, causing a subsequent viremia decline, before reaching a steady-state (viral set point) between viral and host factors^(4, 5, 6, 7, 8). Serologically, HIV specialists prefer to divide this period into three periods: pre-, per-, and seroconversion. The first one – the Pre – is the earliest, where only viral components can be detected. During the Per period, viral antigen can still be found, but it is declining, and HIV antibodies are starting to appear. During seroconversion, no antigen can be detected – mostly due the formation of antigen-antibody complexes as well as the virus integrating into the genome of infected cells – and only antibody can be detected (Figure 1).

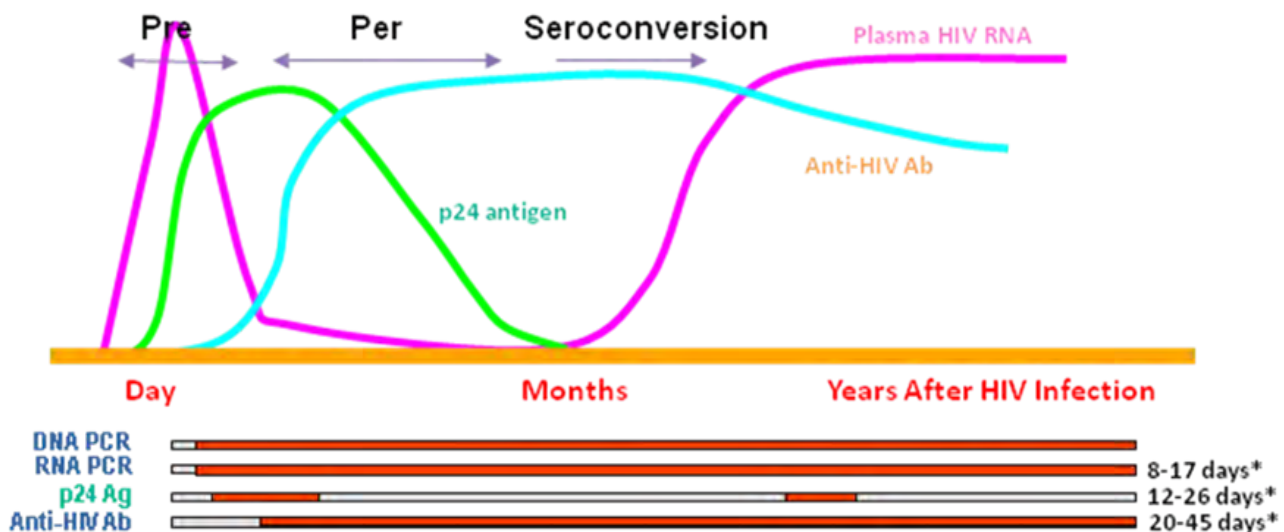


Figure 1: Kinetic of HIV markers during infection (Adapted from Pilcher et al, 2004 and ANAES report, 2000)

HIV antibody assays, including most sensitive third-generation tests, are limited in their ability to detect acute infections of HIV. As they do not target viral components, they do not identify infection during the pre-seroconversion phase and can only partially identify per-seroconversion cases. In order to address this period, fourth-generation screening assays were developed, shortening the duration of the “diagnostic window” by detecting HIV antibodies and HIV-1 p24 antigen simultaneously⁽⁹⁾. Most commercially available fourth generation HIV tests are in an ELISA format and are combined assays. They do not differentiate between the two markers and reveal only a single result, lacking the capability to specifically identify the status or phase of infection, whether it is an acute infection (incident case) or serological positive (prevalence case). Despite the increased use of laboratory-based fourth generation assays for routine diagnosis of HIV infection, in the developing world, their utility is limited by lack of laboratory infrastructure and the costs associated with these tests. Another inherent limitation to laboratory testing is the lengthy period to turn around test results and the significant loss to follow up when patients do not obtain their test results especially in remote areas⁽¹⁰⁾.

Inverness Medical has developed the Determine® HIV-1/2 Ag/Ab Combo, a rapid, point-of-care test on the globally recognized and simple to use Determine® platform. The test can simultaneously differentiate between HIV antibodies and p24 antigen allowing for:

- Shortening of the “diagnostic window” to detect pre- and per-seroconversion phases of HIV infection.
- Differential determination of the status of each marker during the window period, indicating the pre-, per- and seroconversion stages, and as a consequence more definite results as to the phase of infection.
- First screening in HIV rapid testing algorithms in areas with limited laboratory infrastructure.
- Testing when there may be malaise symptoms. As many people go to the doctor only when feeling sick, in the course of an HIV infection, the only time when a person may have symptoms is at the beginning of the infection.

Determine® HIV-1/2 Ag/Ab Combo System

Principle of the test

Determine® HIV-1/2 Ag/Ab Combo is an immunochromatographic test for the qualitative detection of p24 antigen and antibodies to HIV-1 and HIV-2 (Figure 2). Specimen is added to the sample pad. The specimen mixes with a biotinylated anti-p24 antibody and selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized avidin, recombinant antigens and synthetic peptides at the patient window sites. If antibodies to HIV-1 and/or HIV-2 are present in the specimen, the antibodies bind to the antigen-selenium colloid and to the immobilized recombinant antigens and synthetic peptides, forming one red bar at the patient HIV Antibody window site. If antibodies to HIV-1 and/or HIV-2 are absent the antigen-selenium colloid flows past the patient window, and no red bar is formed at the HIV Antibody window.

If free p24 antigen is present in the specimen, the antigen binds to the biotinylated anti-p24 from the sample pad and the selenium colloid anti-p24 antibody and it binds to an immobilized avidin forming a red bar at the patient HIV Antigen window site. If p24 antigen is not present both the biotinylated anti-p24 and selenium colloid anti-p24 antibody flow past the patient window, and no red bar is formed at the patient HIV Antigen window site.

To assure the user of assay validity, a procedural control bar is incorporated in the assay device.

Results are obtained in 20 minutes and can be read up to 30 minutes.

Test procedure and interpretation

Serum/Plasma

1) Remove tests



Note: Removal of the test units Should start from the right side of the test card to preserve the lot number which appears on the left side of the card

2) Remove cover



3) Add sample

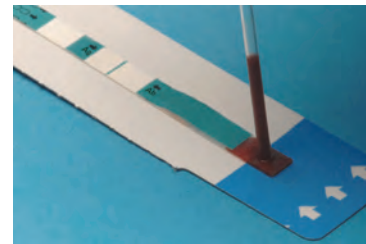


Add sample (50µl) to sample pad

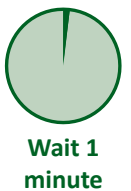


Figure 2: Determine® HIV-1/2 Ag/Ab Combo

3) Add sample



Add sample (50µl) to sample pad (finger stick or venipuncture)



Whole Blood

1) Remove tests



Note: Removal of the test units Should start from the right side of the test card to preserve the lot number which appears on the left side of the card

2) Remove cover



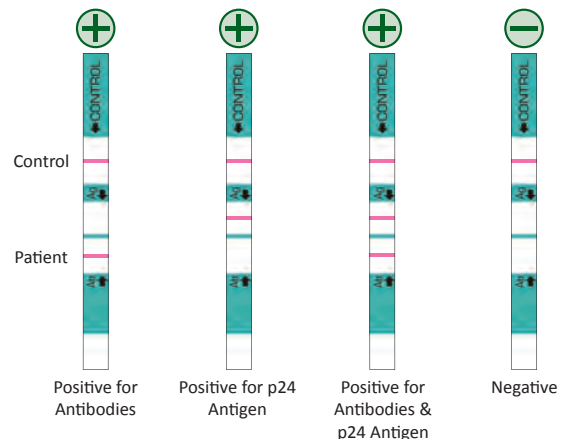
4) Add Chase Buffer (for whole blood only)



Add one drop of chase buffer



5) Read result



Performance Characteristics

The performance of Determine® HIV-1/2 Ag/Ab Combo was evaluated in-house and at nine (9) clinical sites in Africa, Asia, Europe and Latin America. It included 1179 positive and 2343 negative specimens from random blood donors, patients with HIV infection, patients considered high risk for HIV infection or in other clinical categories, 33 commercial seroconversion panels and an EFS Antigen panel for analytical sensitivity determination of the assay p24 antigen cut-off. The studies were designed for two types of specimens: retrospective (serum/plasma) frozen and fresh prospective (whole blood and serum/plasma) samples.

Sensitivity

The sensitivity has been evaluated by testing confirmed HIV antibody positive samples, commercial seroconversion panels and specimens from primary (acute) HIV infected patients. A total of 1179 confirmed HIV antibody positive specimens were tested (Tables 1,2,3) including 4 types of positive samples: HIV-1 (885), HIV-1 Group O (7), HIV-1 Non-B subtypes (154) together with circulating recombinant forms (CRFs) and HIV-2 (133).

Sensitivity for HIV antibodies

Table 1: Sensitivity by HIV type

Types	Number of Specimens Tested	Positive by Determine® HIV-1/2 Ag/Ab Combo	Sensitivity (%)
HIV-1	885	885	100.00%
HIV-1 group O	7	7	100.00%
HIV-1 non B subtypes*	154	154	100.00%
HIV-2	133	133	100.00%
Total	1179	1179	100.00%

*Subtype A, C, D, F, G, H, J, K, and CRF01_AE, CRF02_AG, CRF06_cpx, CRF09_cpx, CRF11_cpx, CRF35_AD, and BD

The global diagnostic sensitivity of the Determine® HIV-1/2 Ag/Ab Combo on the positive sample panel is calculated to be 100% (95% confidence interval: 98.04% - 100%).

Sensitivity by site

Table 2: HIV-1 Antibody*

Site	Number of Specimens Tested	Positive by Determine® HIV-1/2 Ag/Ab Combo	Sensitivity (%)
Belgium	140	140	100.00%
Uganda	139	139	100.00%
Senegal	128	128	100.00%
UK	115	115	100.00%
France	100	100	100.00%
Thailand	41	41	100.00%
Ethiopia	160	160	100.00%
South Africa	20	20	100.00%
Colombia	51	51	100.00%
In-house	139	139	100.00%
Total	1033	1033	100.00%

*includes the O Group and non-B subtypes detailed above

Table 3: HIV-2 Antibody

Site	Number of Specimens Tested	Positive by Determine® HIV-1/2 Ag/Ab Combo	Sensitivity (%)
Belgium	25	25	100.00%
Senegal	23	23	100.00%
UK	2	2	100.00%
France	25	25	100.00%
In-house	58	58	100.00%
Total	133	133	100.00%

Table 4: Comparison of Determine® HIV-1/2 Ag/Ab Combo sensitivity in whole blood and paired serum and plasma specimens*

No. of individuals	Paired specimens type				Correlation between matrices
	Serum	Plasma	WB venipuncture	WB fingerstick	
91	91	91	91	-	100.00%
20	-	-	20	20	100.00%
9	-	9	9	-	100.00%
22	22	22	-	22	100.00%
142	113	122	120	42	100.00%

*Were included in the sensitivity table above (Table 1)

Multiple (matched) specimens:

Seropositive specimens from a total of 142 individuals from Africa and Europe were tested. Multiple (matched) specimens were obtained from several of these donors. From these 142 individuals, 113 serum specimens, 122 plasma specimens, 120 whole blood (venipuncture) specimens and 42 whole blood (fingerstick) specimens were obtained in various combinations.

Whole Blood (venipuncture) specimens:

120 whole blood (venipuncture) specimens were tested. Of these, 91 were matched with serum and plasma, 9 were matched pairs with plasma and 20 were matched pairs with whole blood (fingerstick) specimens.

Whole Blood (fingerstick) specimens:

42 whole blood (fingerstick) specimens were tested. Of these, 22 were matched with serum and plasma and 20 were matched pairs with whole blood (venipuncture) specimens.

The results obtained from all specimen matrices showed 100% correlation, demonstrating that Determine® HIV-1/2 Ag/Ab Combo gives identical results for these types of specimen matrices.

HIV seroconversion panels

The capability of the Determine® HIV-1/2 Ag/Ab Combo to detect early infection was demonstrated on commercial seroconversion panels. As seen in Figure 3.a., when Panel BCP 6246 was tested on Determine® HIV-1/2 Ag/Ab Combo, p24 antigen was detected on bleed day 50, 7-days earlier than the appearance of HIV antibodies. Furthermore, when Panel BBI-AS was tested on Determine® HIV-1/2 Ag/Ab Combo, p24 antigen was detected on bleed day 12, 7-days earlier than the appearance of HIV antibodies (Figure 3.b).

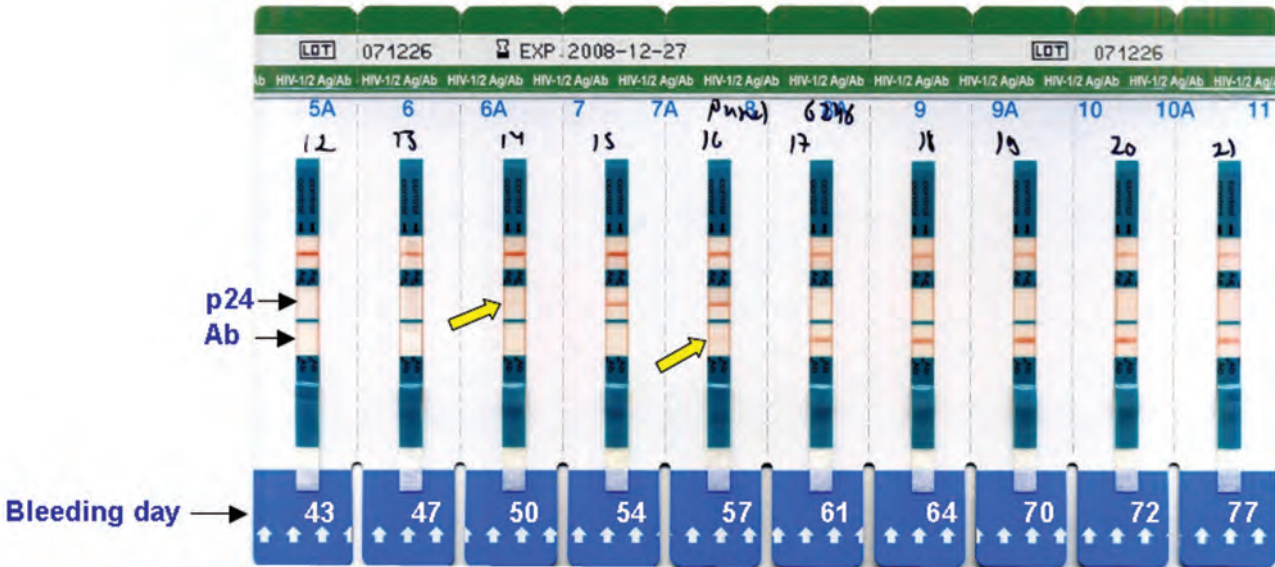


Figure 3.a: Seroconversion Panel BCP 6246

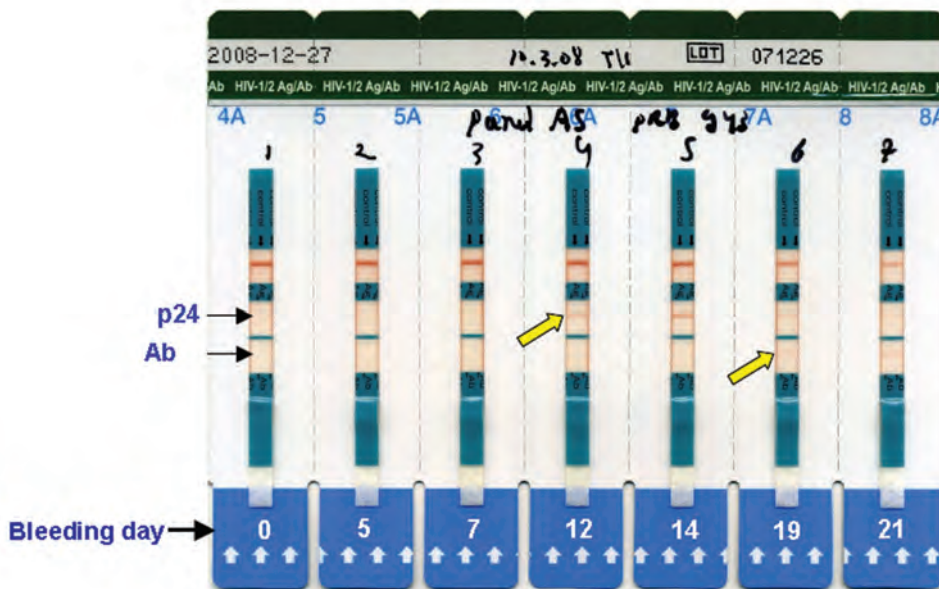


Figure 3.b: Seroconversion Panel BBI-AS

A summary of the results on 33 commercial panels is presented in Table 5. In 23 of 33 panels tested, Determine® HIV-1/2 Ag/Ab Combo detects infection earlier by at least one and up to five bleedings, than a 3rd-generation HIV antibody assay (Determine® HIV 1/2). 10 Panels showed equivalent detection.

Table 5: Sensitivity of Determine® HIV-1/2 Ag/Ab Combo in seroconversion panels

Panel	Bleeding day with first positive result					Earlier detection (days) than Determine® HIV-1/2
	Panels' reference test		Determine® HIV-1/2	Determine® HIV-1/2 Ag/Ab Combo		
	p24 Ag	3rd Gen EIA**		p24 Ag	Ab	
BBI-L	0	0	0	0	9	0
BBI-AY	18	20	18	18	20	0
BBI-AN-E	16	103	103	21	103	82
BBI-AM	0	9	9	0	9	9
BBI-Y	44	44	44	44	44	0
BBO-AI	0	7	7	0	7	7
BBI-AQ	9	18	18	18	18	0
BBI-BC	7	7	10	7	10	3
BBI-Z	7	27	27	7	27	20
BBI-AF	15	28	28	28	28	0
BBI-AP	7	11	11	7	11	4
BBI-AU	13	13	15	13	15	2
BBI-BA	8	19	19	11	19	8
BBI-BB	10	14	17	10	17	7
BBI-BD	17	-	-	-	-	0
BBI-BG	23	28	28	23	28	5
BBI-J	14	26	26	14	26	12
BBI-BE	3	12	12	7	12	5
BBI-AT	2	14	14	7	14	7
BBI-AE	3	7	7	3	7	4
BBI-Q	53	60	60	53	65	7
BBI-AG	27	34	27	27	3	0
BBI-AB	28	28	28	28	33	0
BBI-BF	47	-	-	47	47	47
BBI-BI	0	9	9	7	12	2
BBI-AS	12	14	19	12	19	7
BBI-AL	14	21	-	14	-	8
BBI-BH	9	15	15	9	15	7
BCPI-6240	23	28	28	23	30	5
BCPI-6244	28	33	33	33	35	0
BCPI-6246	50	57	57	50	57	7
BCPI-9020	95	98	95	95	95	0
BCPI-9021	50	57	57	50	57	7
Range of days: 2-20						Average days: 4.61*

* Panels AN-E and BF were excluded from the average calculation due to extended bleeding day gap

** Third-generation antibody test

Determine® HIV-1/2 Ag/Ab Combo detected HIV infection an average of 5 days (range: 2-20 days) earlier than the Determine® HIV-1/2 (3rd generation) antibody test, depending on the panel tested.

Primary HIV infection specimens

A total of 117 specimens from primary HIV infected patients (pre- or per-seroconversion) were tested (Table 6). Determine® HIV-1/2 Ag/Ab Combo detected 108 (92.3%) of the samples when compared to commercial CE marked 4th generation EIA.

Table 6: Sensitivity of Determine® HIV-1/2 Ag/Ab Combo on Primary HIV Infection: Pre and per seroconversion samples

Site	Number of Specimens Tested	Positive by Determine® HIV-1/2 Ag/Ab Combo	Sensitivity (%)
Belgium	10	8	80.00%
UK	18	16	88.89%
Thailand	7	2	28.57%
France	81	81	100.00%
Colombia	1	1	100.00%
Total	117	108	92.31%

Analytical sensitivity of Determine® HIV-1/2 Ag/Ab Combo

The analytical sensitivity of the Determine® HIV-1/2 Ag/Ab Combo was evaluated by testing the EFS Ag HIV panel (HIV Ag panel from the French Blood Establishment). A detection limit of 25pg/mL was reached.

The analytical sensitivity was also tested with purified HIV-1 p24 native protein (ABI, Maryland, USA) showing limit of detection of 12.5pg/mL.

Specificity

Diagnostic specificity was assessed by using a total of 2363 confirmed negative serum, plasma or whole blood specimens that were tested by Determine® HIV-1/2 Ag/Ab Combo (Table 7).

Table 7: Specificity of Determine® HIV-1/2 Ag/Ab Combo

Population	Number of Specimens Tested	Negative by Determine® HIV-1/2 Ag/Ab Combo Ab line	Specificity (%) of Ab line	Negative by Determine® HIV-1/2 Ag/Ab Combo Ag line	Specificity (%) of Ag line
Seronegative plasma or serum*	1783	1769	99.21%	1776	99.61%
Pregnant women	200	200	100.00%	199	99.50%
Disease States Other than HIV**	210	206	98.09%	210	100.00%
Potentially Interfering Substances***	150	150	100.00%	150	100.00%
Total	2343	2325	99.23%	2335	99.66%

* Conducted at in clinical trial sites detailed in Table 8

** Other viral or bacterial infections (HBV, HCV, HTLV, CMV, Toxo IgG, Syphilis, HSV 1/2, EBV, Flu vaccinated patients and Chlamydia IgG/IgM).
Cancer patients

*** IV drug users, multiparas pregnancy, rheumatoid factor, alcoholic cirrhosis, autoimmune (ANA), high cholesterol, lipemic, high bilirubin, hemolyzed, anti mouse IgG

The overall diagnostic specificity for markers, HIV antibodies and p24 antigen was over 99%: 99.23% (95% confidence interval: 98.90% – 99.58%) for HIV antibodies line and 99.66% for p24 antigen line (95% confidence interval: 99.54% - 99.78%)

A total of 1783 negative specimens were tested in nine clinical sites (Table 8)

Table 8: Specificity of Determine® HIV-1/2 Ag/Ab Combo by site

Study Site	Number of Specimens Tested	Negative by Determine® HIV-1/2 Ag/Ab Combo Ab line	Specificity (%) of Ab line	Negative by Determine® HIV-1/2 Ag/Ab Combo Ag line	Specificity (%) of Ag line
Belgium	100	100	100.00%	100	100.00%
Uganda	753	743	98.67%	753	100.00%
Senegal	221	221	100.00%	221	100.00%
UK	37	36	97.30%	37	100.00%
Thailand	82	82	100.00%	82	100.00%
France	100	100	100.00%	100	100.00%
Ethiopia	389	386	99.22%	382	98.2%
South Africa	20	20	100.00%	20	100.00%
Colombia	81	81	100.00%	81	100.00%
Total	1783	1769	99.21%	1776	99.61%

Effect of specimen matrix on specificity of Determine® HIV-1/2 Ag/Ab Combo

The effect of specimen matrices on the specificity of the Determine® HIV-1/2 Ag/Ab Combo was studied by comparing paired specimens. A total of 84 paired whole blood (venipuncture), serum and plasma, or whole blood and plasma only were tested (Table 9). All samples were found negative with 100% of concordance with the three matrices.

Table 9: A comparison of Determine® HIV-1/2 Ag/Ab Combo Specificity in whole blood and paired serum and plasma specimens

No. of individuals	Paired specimens type			Correlation between matrices
	Serum	Plasma	Whole Blood (venipuncture)	
64	64	64	64	100.00%
20	-	20	20	100.00%
84	64	84	84	100.00%

Summary

The Determine® HIV-1/2 Ag/Ab Combo is the first rapid diagnostic test that enables simultaneous and differential detection of HIV p24 antigen and antibodies for HIV-1 and HIV-2 in human serum, plasma and whole blood. The clinical trials conducted in-house and at nine(9) external sites clearly present the advantages of Determine® HIV-1/2 Ag/Ab Combo in improving early detection of HIV infection by identifying the presence of viral antigen before appearance of antibodies to the virus while also enabling the user to differentiate acute infection (incident case) and seropositive (prevalence case), due to the separate lines for HIV antigen and anti-HIV antibodies. Determine® HIV-1/2 Ag/Ab Combo is a rapid, point-of-care lateral flow test that provides clear visual results in 20-minutes. It permits HIV testing to be conducted in a broad range of clinical settings, from the physician's office, hospital or clinic in the developed world to the most remote and challenging environments of the developing world. The test makes a significant contribution to the effort to identify infection and, in turn, to stem the tide of further HIV transmission.

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