VioOne™ HIV Profile™ Supplemental Assay for Confirmation and Differentiation of HIV-1 and HIV-2 Antibodies

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Disclaimer: Product FDA and CE Mark Approval Pending

HIV Profile Intended Use

- The VioOne™ HIV Profile™ Supplemental Assay (Profile) is an enzyme-linked immunosorbent assay (ELISA) for confirmation and differentiation of individual antibodies directed to various gene products of HIV-1 (Group M & Group O) and HIV-2 in human serum or plasma.
- Profile is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2.
- It is intended as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens repeatedly reactive in diagnosis or screening procedures, including pediatric patients (ages 2-20).
- Results of Profile can also be used to distinguish recent from long standing HIV-1 infection and thus be used for HIV-1 incidence estimation.

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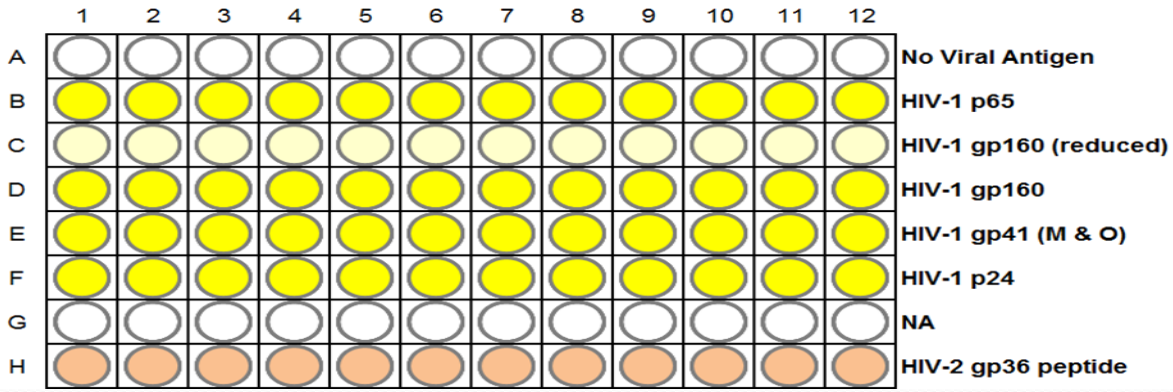
HIV Profile Assay Features

The VioOne™ HIV Profile™ Supplemental Assay contains 3 capabilities

- Detection and confirmation of antibody to HIV-1 antigens
- Detection and confirmation of antibody to HIV-2 antigen



	HIV-1 Antibody Confirmation	HIV-2 Antibody Confirmation	Recency Determination
No Viral Antigen			
HIV-1 p65	X		
HIV-1 gp160 (reduced)			X
HIV-1 gp160	X		X
HIV-1 gp41 (M & O)	X		
HIV-1 p24	X		
NA			
HIV-2 gp36 peptide		X	

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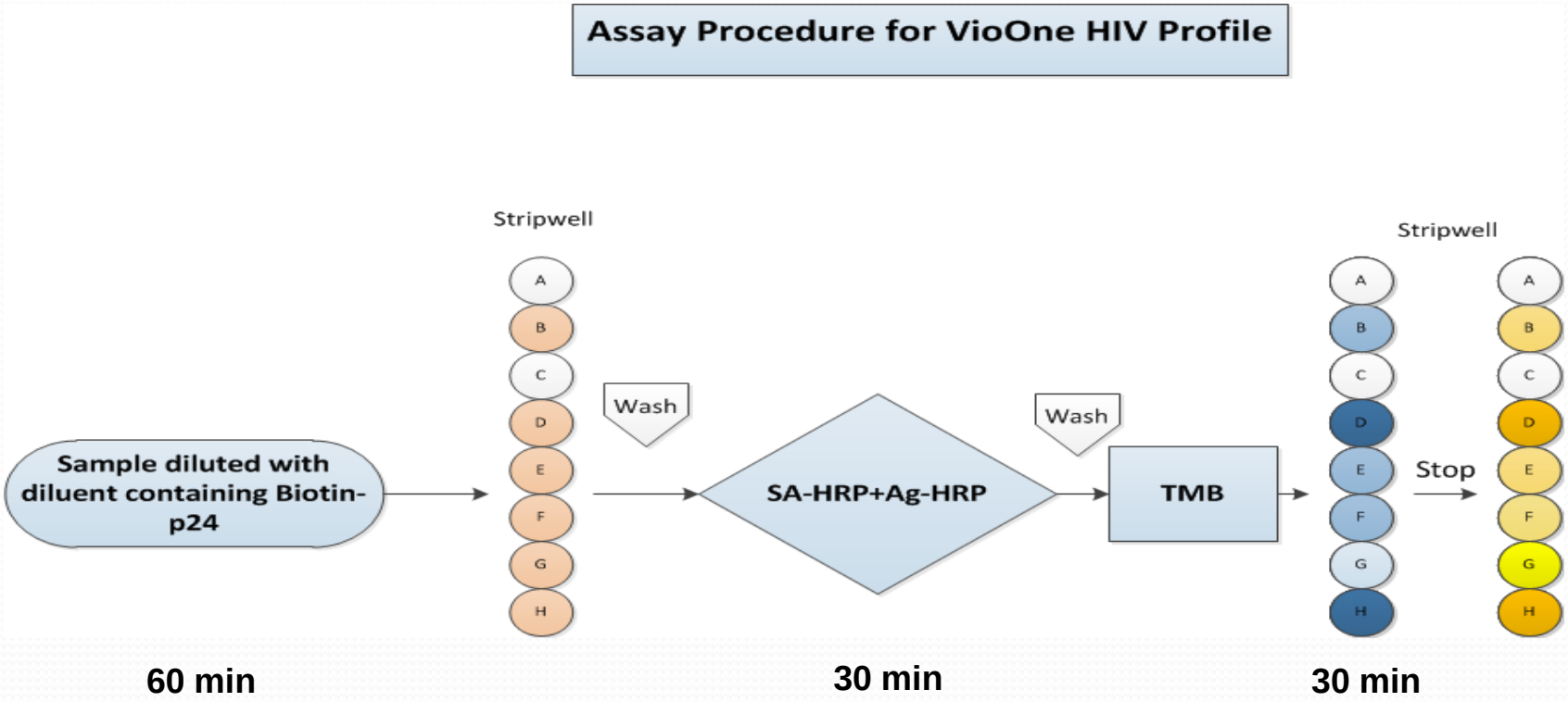
HIV Profile Assay Procedure



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HIV Profile Analytical Testing
(Across 3 Validation Lots)

- Preclinical Anticoagulant Study
  - Serum, EDTA, Citrate, Heparin Plasma from 20 Matched Donors
  - Tested Unspiked and Spiked at 3 Levels with HIV-1/HIV-2 Ab
- Repeat Reactive Samples Confirmed Negative (n=100)
- Repeat Reactive Samples Confirmed Indeterminate (n=107)
- Limit of Detection Panels (4 HIV-1 & 4 HIV-2)
- Seroconversion Panels (n=20) & Performance Panels (n=2)
- HIV-1 Group M Clades (n=83) and CRFs (n=13)
- Interfering Conditions (Unspiked & Spiked with HIV-1, HIV-2 and HIV-1/HIV-2, n=14)
- Cross Reactive Conditions / Sample Types (Unspiked Only, n=13)

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HIV Profile Clinical Testing

- Reproducibility
  - Seven member panel; Negative (n=2), HIV-1 (n=2), HIV-2 (n=2), HIV-1/HIV-2 (n=1)
  - Tested in 9 plates/day; 3 HIV Profile Validation Lots; 1 Analyst
  - Duration 5 days at 3 External Sites
- Specificity
  - 300 Plasma, 300 Serum (Negative in Screening Assays)
  - Tested across 3 HIV Profile Validation Lots; 3 External Sites
- Sensitivity
  - 400 HIV-1, 202 HIV-2 (52 at CDC), 50 AIDS, 40 HIV Peds, 10 HIV Coinfection, 10 HIV-1 Group O, 27 HIV Pregnant Females
  - Tested across 4 HIV Profile Validation Lots; 4 External Sites

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Limit of Detection

- Analytical sensitivity of Profile was compared to FDA approved predicate Supplemental Assay
- Study panel consisted of 4 HIV-1 and 4 HIV-2 positive samples terminally diluted to undetectable antibody levels

			VioOne™ HIV Profile™ Assay					
			HIV-1 (n=24)			HIV-2 (n=29)		
			POS	IND	NEG	POS	IND	NEG
Predicate Assay	HIV-1	POS	0	0	0			
		IND	2	0	0			
		NEG	10	2	10			
	HIV-2	POS				12	0	0
		IND				5	0	1
		NEG				1	0	10

- Profile detected 12/24 HIV-1 samples as positive & 2 as indeterminate
- Predicate detected 0/24 HIV-1 samples as positive & 2 as indeterminate
- Profile detected 18/29 HIV-2 samples as positive & 0 as indeterminate
- Predicate detected 12/29 HIV-2 samples as positive & 6 as indeterminate

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Seroconversion Panels

- A total of 40 samples from 10 additional commercially available seroconversion panels were tested with Profile and compared to predicate results

Panel			First Confirmed Positive Bleed (Days)	
Panel ID	Number of Panel Members	Collection Days	VioOne HIV Profile Supplemental Assay	PredicateSupplemental Assay
1	4	17, 38, 49, 51	49	None
2	3	59, 62, 67	67	67
3	3	3, 10, 49	49	49
4	5	4, 9, 18, 21, 25	21	21
5	4	7, 10, 14	None[a]	None[a]
6	5	5, 8, 12, 16, 19	12	12
7	5	10, 14, 18, 21, 25	21	18
8	3	18, 25, 30	25	30
9	4	33, 35, 40, 42	40	40
10	5	0, 4, 7, 25, 31	0	4

[a]Both assays detected panel member 14 as HIV-1 Indeterminate.

- Profile detected HIV-1 positive samples within one bleed or better in 10/10 panels for a total of 50.0% (20/40) as reactive compared to Predicate that detected 42.5% (17/40) samples as reactive.

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Profile Analytical Performance

HIV-1 Incidence / Prevalence Panel

- All 15 members of the SeraCare PRB601 Incidence / Prevalence Panel, consisting of 7 known HIV-1 positive incidence (new infections) members and 8 known HIV-1 positive prevalence (long standing infections) members, were found to be HIV-1 antibody positive & HIV-2 antibody negative with Profile

HIV-1 / HIV-2 Performance Panel

- All 15 members of the SeraCare HIV-1 / HIV-2 Performance Panel 0800-0331, containing 7 HIV-1 positive, 7 HIV-2 positive, and 1 negative panel member were correctly identified and differentiated by Profile matching Multispot test results

Elevated Level of Biotin

- An HIV negative human serum sample was spiked with 3600 ng/mL of exogenous biotin, which is three times the therapeutic level.
- The human serum sample containing 3600 ng/mL of biotin performed similarly to the same sample without added biotin, regardless whether the samples were spiked or unspiked with HIV antibody (HIV-1 antibody, HIV-2 antibody, or a blend of HIV-1 & HIV-2 antibodies).

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HIV-1 Group M Clades

- Nine (9) major HIV-1 Group M clades and 13 CRFs consisting of a total of 96 samples found in the US and Europe were tested positive for HIV-1 with Profile
- 100% positivity rate (95% confidence interval: 96.2% - 100%).

HIV-1 Group M Clade	Number of Samples	VioOne™ HIV Profile™		
		NEG	IND	POS
A	10	0	0	10
B	10	0	0	10
C	10	0	0	10*
D	10	0	0	10
F	11	0	0	11
G	10	0	0	10
H	10	0	0	10
J	3	0	0	3
K	9	0	0	9
CRF_01_AE	4	0	0	4
CRF02_AG	2	0	0	2
CRF06_cpx	2	0	0	2
CRF11_cpx	1	0	0	1
CRF14_BG	2	0	0	2
CRF18_cpx	1	0	0	1
CRF25_cpx	1	0	0	1
TOTAL	**96**	**0**	**0**	**96**

*1 Clade C sample tested HIV-1 positive with reactivity to HIV-2 antigen

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Samples Repeatedly Reactive with an FDA Diagnostic or Screening HIV Assay but Confirmed Negative with a Supplemental Assay

- Of 100 Repeatedly Reactive samples confirmed Negative by the Geenius, MultiSpot or IFA, 99 samples were negative and 1 sample was indeterminate with Profile. None of these samples were confirmed positive with Profile.
- All 22 samples confirmed negative by Geenius were confirmed negative with Profile with 100% concordance between the two assays.
- All of 18 samples confirmed negative by MultiSpot were confirmed negative with Profile with 100% concordance between the two assays.
- Of the 60 IFA negative samples, 59 were negative and 1 indeterminate with Profile. Thus, there was 98.33% concordance between the test results of Profile and the IFA assay.

	Supplemental Assay Results	Number	VioOne™ HIV Profile™ Supplemental Assay		
			Negative	Indeterminate	Positive
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	Bio-Rad Geenius[a] NEG	22	22	0	0
	MultiSpot[b] NEG	18	18	0	0
	IFA[c] NEG	60	59	1	0
	Total	**100**	**99 (99.0%)**	**1 (1.0%)**	**0**

[a] Geenius™ HIV 1/2 Supplemental System
[b] Multispot HIV-1/HIV-2 Rapid Test
[c] Fluorognost HIV-1 IFA

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Samples Repeatedly Reactive with an Approved Diagnostic or Screening HIV Assay but Indeterminate with a Supplemental Assay

- Of 8 samples confirmed indeterminate by Geenius, 1 was indeterminate, 3 were negative, and 4 were HIV-1 antibody positive by Profile
- Of the 99 samples confirmed indeterminate by IFA, 92 were negative, 3 were indeterminate, 1 was HIV-1 antibody positive and 3 were HIV-2 antibody positive by Profile.
- Also tested were 19 samples which were repeatedly reactive with a diagnostic or screening HIV assay and confirmed positive with an IFA assay, but tested indeterminate with a Western Blot test. All of these 19 samples were confirmed to be positive by Profile.

	Supplemental Assay Result	Number	VioOne™ HIV Profile™ Supplemental Assay		
			Negative	Indeterminate	Positive
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	Bio-Rad Geenius[a] IND	8	3	1	4
	IFA[b] IND	99	92	3	4
	Total	**107**	**95 (88.8%)**	**4 (3.7%)**	**8 (7.5%)**
	IFA[b] POS, Western Blot[c] IND	19	0	0	19

[a] Geenius™ HIV ½ Supplemental System
[b] Fluorognost HIV-1 IFA
[c] Cambridge Biotech HIV-1 Western Blot Kit

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Potential Interfering Factors or Medical Conditions

- A panel of 130 retrospective samples from patients not known to be infected with HIV representing 13 categories of potentially interfering medical conditions unrelated to HIV infection were tested with Profile.
- Samples were tested unspiked (negative), spiked with an HIV-1 positive antibody, spiked with HIV-2 positive antibody, or spiked with both HIV-1 and HIV-2 positive antibodies.
- Profile results of testing 130 potentially interfering factor samples tested **unspiked** are as follows:

Potentially Interfering Factor	Number Tested	VioOne™ HIV Profile™		
		NEG	IND	POS
AutoImmune disease	10	10	0	0
Dialysis patients	10	10	0	0
EBV infection	10	10	0	0
HBsAg infection	10	10	0	0
HCV infection	10	10	0	0
High rheumatoid factor	10	10	0	0
Multiparous (pregnant) females	10	9	0	1[a]
Post influenza vaccine	10	9	1[b]	0
Yeast (Candida) reactive	10	7	3[c]	0
Vaccinia vaccine samples	10	10	0	0
HTLV-I/II antibody positive	10	10	0	0
Multiple transfusions	10	10	0	0
Hemophilia	10	0	0	0
TOTAL	**130**	**125 (96.15%)**	**4 (3.08%)**	**1 (0.77%)**

[a]HIV-2 positive that was negative on duplicate repeat testing.
[b]HIV-1 indeterminate that was negative on repeat testing in singlicate due to volume limitation.
[c]Of these three HIV-1 indeterminate samples, one was indeterminate upon repeat testing in duplicate, one was negative upon repeat testing in duplicate and, one was invalid for repeat testing.

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Potential Interfering Factors or Medical Conditions

- Of the 130 potentially interfering factor samples **spiked with a blend of HIV-1 and HIV-2 antibodies**, all were tested positive for both HIV-1 and HIV-2 antibody with Profile.

Potential Interfering Factor	Number Tested	VioOne™ HIV Profile™		
		NEG	HIV-1 POS	HIV-2 POS
AutoImmune disease	10	0	10	10
Dialysis patients	10	0	10	10
EBV infection	10	0	10	10
HBsAg infection	10	0	10	10
HCV infection	10	0	10	10
High rheumatoid factor	10	0	10	10
Multiparous (pregnant) females	10	0	10	10
Post influenza vaccine	10	0	10	10
Yeast (Candida) infection	10	0	10	10
Vaccinia vaccine samples	10	0	10	10
HTLV-I/II antibody positive	10	0	10	10
Multiple transfusions	10	0	10	10
Hemophilia	10	0	10	10
TOTAL	**130**	**0**	**130 (100%)**	**130 (100%)**

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Cross Reactivity Study

- In a separate cross-reactivity study, a panel of 47 potentially cross-reactive samples representing nine different disease states was tested with Profile.
- All 47 samples were tested negative with Profile, indicating that the samples from these disease states did not result in cross reactivity in the Profile assay.

Disease State Samples	Number Tested	VioOne™ HIV Profile™		
		NEG	IND	POS
Cirrhosis	5	5	0	0
Hepatitis A	7	7	0	0
Cancer	5	5	0	0
HSV IgG	5	5	0	0
Malaria: P. falciparum	5	5	0	0
Rubella IgG	5	5	0	0
Syphilis	5	5	0	0
Toxoplasmosis IgG	5	5	0	0
CMV IgG	5	5	0	0
TOTAL	**47**	**47 (100%)**	**0**	**0**

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Cross Reactivity Study

- Commercially available samples containing various levels of bilirubin, lipid (triglycerides), hemoglobin, or HAMA (human anti-mouse antibody) were tested with Profile.
- As the table shows, these substances did not cause cross reactivity in the Profile assay.

Panel Member Samples		Number Tested	VioOne™ HIV Profile™		
			NEG	IND	POS
Total Bilirubin	0.20 mg/dL	1	1	0	0
	2.00 mg/dL	1	1	0	0
	4.00 mg/dL	1	1	0	0
	6.70 mg/dL	1	1	0	0
	11.43 mg/dL	1	1	0	0
Lipemia - Triglycerides	150 mg/dL	1	1	0	0
	272 mg/dL	1	1	0	0
	379 mg/dL	1	1	0	0
	1013 mg/dL	1	1	0	0
	2375 mg/dL	1	1	0	0
Hemoglobin	Normal	1	1	0	0
	140 mg/dL	1	1	0	0
	275 mg/dL	1	1	0	0
	550 mg/dL	1	1	0	0
	1100 mg/dL	1	1	0	0
HAMA (Human anti mouse antibody)	Negative	1	1	0	0
	Negative	1	1	0	0
	4.0 ng/mL	1	1	0	0
	4.7 ng/mL	1	1	0	0
	7.2 ng/mL	1	1	0	0
	9.6 ng/mL	1	1	0	0
	13.0 ng/mL	1	1	0	0
	27.1 ng/mL	1	0	1*	0
	30.0 ng/mL	1	1	0	0
	38.8 ng/mL	1	1	0	0
	52.7 ng/mL	1	1	0	0
	74.0 ng/mL	1	1	0	0
TOTAL		**27**	**(26/27) 96.30%**	**(1/27) 3.70%**	**(0/27) 0.00%**

*HIV-1 Indeterminate that was negative upon repeat testing in duplicate.

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Profile Reproducibility Results

- Of the 1215 replicates tested throughout the precision/reproducibility study (kit controls included), there were 7 replicates with test results resulting in incorrect interpretation, i.e., interpretations different from expected interpretations.
- Therefore, for Profile, the total percent (%) agreement of observed vs expected results was 99.42% with a 95% CI of 98.82 – 99.77%.
- Predicate showed an overall % agreement of 97.23% with a 95% CI of 96.28 – 98.00%.

Sample			Test Results			
Code Name	**Description**	**Expected Results**	**# of Replicates**	**# of Correct Test Results**	**% Agreement**	**95% CI**
Kit PC	HIV-1/HIV-2 Antibody Positive	HIV-1 / HIV-2 POS	135	135	100%	97.30% - 100%
Kit NC	HIV-1/HIV-2 Antibody Negative	NEG	135	135	100%	97.30% - 100%
R1	HIV-1/HIV-2 Antibody Negative	NEG	135	134	99.26%	95.94% - 99.98%
R2	HIV-1/HIV-2 Antibody Negative (high background)	NEG	135	132	97.78%	93.64% - 99.54%
R3	HIV-1 Low Antibody Positive	HIV-1 POS	135	133	98.52%	94.75% - 99.82%
R4	HIV-1 Moderate/High Antibody Positive	HIV-1 POS	135	134	99.26%	95.94% - 99.98%
R5	HIV-2 Moderate Antibody Positive	HIV-2 POS	135	135	100%	97.30% - 100%
R6	HIV-2 High Antibody Positive	HIV-2 POS	135	135	100%	97.30% - 100%
R7	HIV-1/HIV-2 Low Antibody Positive	HIV-1 / HIV-2 POS	135	135	100%	97.30% - 100%
Overall Agreement (including Kit Controls)			**1215**	**1208**	**99.42%**	**98.82%-99.77%**

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Profile Clinical Specificity

- A total of 599 serum and plasma, including 20 serum pediatric samples, were collected from individuals at low risk for HIV infection and tested at 3 external sites across 3 Profile validation lots.
- The overall clinical specificity for the VioOne™ HIV Profile™ in the low risk population was 98.16% (95% CI: 96.74% - 99.08%).
- Predicate tested a total of 416 serum and plasma samples and showed a clinical specificity of 95.67% and 95% CI of 93.26% - 97.24%.

SAMPLE TYPE	NUMBER	NEGATIVE	INDETERMINATE	POSITIVE
SERUM	279[A]	272	5[B]	2[D]
PEDIATRIC SERUM	20	20	0	0
PLASMA	300	296	3[C]	1[E]
TOTAL	**599**	**588 (98.16%)**	**8 (1.36%)**	**3 (0.50%)**

[a]One sample was repeatedly invalid and excluded from analysis.
[b]All 5 samples were HIV-1 indeterminate and negative upon repeat testing in duplicate.
[c]All 3 samples were HIV-1 indeterminate and negative upon repeat testing in duplicate.
[d]Of the 2 samples, 1 was HIV-1 positive and 1 was HIV-2 positive and both were negative upon repeat testing in duplicate.
[e]Sample was HIV-2 positive and negative upon repeat testing in duplicate.

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Profile Clinical Sensitivity Results

SAMPLE TYPE	NUMBER	POSITIVE	INDETERMINATE	NEGATIVE
HIV-1 POSITIVE	400	398[A]	1[B]	1
HIV-2 POSITIVE	202	201[C]	0	0
HIV-1/HIV-2 COINFECTION	10	10[D]	0	0
HIV-1 GROUP O	15	15	0	0
AIDS	50	50[E]	0	0
HIV-1 POSITIVE PEDIATRICS	40	38[F]	2	0
HIV-1 POSITIVE PREGNANT FEMALES	27	27	0	0
TOTAL	**744**	**739 (99.33%)**	**3 (0.40%)**	**1 (0.13%)**

[a]One sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen.
[b]HIV-1 indeterminate and HIV-1 upon repeat testing in duplicate.
[E]Eighty-four of 202 samples were interpreted as HIV-2 positive with reactivity to HIV-1 antigens. One (1) HIV-2 sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen. All other samples were HIV-2 positive.
[d]Ten samples were HIV-2 positive and 6 of 10 were interpreted as HIV-2 positive with reactivity to HIV-1 antigens.
[e]Four samples were interpreted as HIV-1 positive with reactivity to HIV-2 antigen.
[f]One sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen.

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Profile Clinical Sensitivity

- Serum or plasma (n= 744) repository samples from individuals infected with HIV were tested at 4 external sites and at Avioq with 4 Profile validation kit lots.
- Sensitivity samples consisted of 400 HIV-1 positives, 202 HIV-2 positives, 50 AIDS positive, 40 pediatric HIV positive, 15 HIV Group O positives, 10 HIV-1/HIV-2 coinfected positives and 27 samples from HIV positive pregnant females (7 each from the 1st and 2nd trimesters and 13 from the 3rd trimester).
- The overall clinical sensitivity of the Profile assay against these 744 samples is 99.33% with a 95% CI of 98.44% - 99.78%.
- As shown in the Geenius package insert, the overall clinical sensitivity of the Geenius assay against a population of 2024 samples taken from 769 patients is 99.26% with a 95% CI of 98.78% - 99.55%.

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