

# Use of the **VioOne**<sup>TM</sup> HIV Profile<sup>TM</sup> for Detection of Recent HIV-1 Infection

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# VioOne™ HIV Profile™

## HIV Supplemental Assay (For Research Use Only):

A serologic assay for confirmation and differentiation of individual antibodies directed to different gene products of HIV-1 and HIV-2 in human serum or plasma specimens. It is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2.

## Study Objective:

To determine if the supplemental assay can correctly identify samples from patients with recent and longstanding HIV-1 infections.



# VioOne™ HIV Profile™

Each 8-well strip (column) of an ELISA plate consists of the following solid phase antigens:

- Well A – Non-Viral Antigen
- Well B – recombinant HIV-1 p65 (*pol*)
- Well C – recombinant HIV-1 gp160 (reduced) (*env*)
- Well D – recombinant HIV-1 gp160 (*env*)
- Well E – recombinant HIV-1 gp41 (*env*)
- Well F – recombinant HIV-1 p24 (*gag*)
- Well G – HIV-2 specific peptide (reduced)
- Well H – HIV-2 specific peptide

# VioOne™ HIV Profile™

- Negative Control is added to ELISA Plate Column 1
- Positive Control (HIV-1/HIV-2) is added to ELISA Plate Column 2
- Samples are added to ELISA Plate Columns 3-12
- Assay requires 160 µL Control or Sample per Column
- The HIV Profile Assay utilizes the following two Conjugates:
  - Conjugate 1 – Biotinylated HIV-1 p24
  - Conjugate 2 – Streptavidin-HRP
    - HIV-1 recombinant gp160-HRP
    - HIV-1 recombinant p65-HRP
    - HIV-2 peptide-HRP

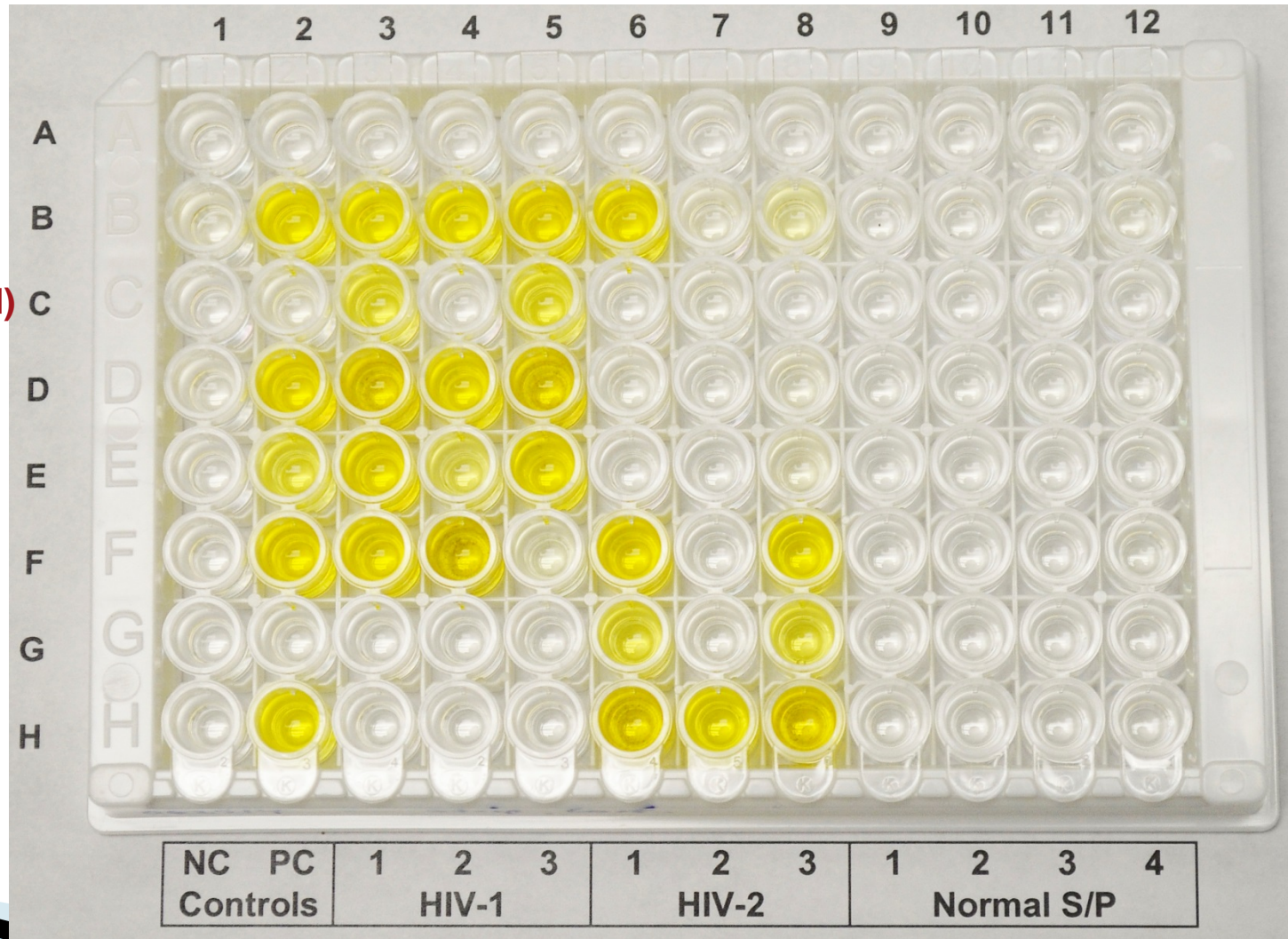
# VioOne™ HIV Profile™

- Add 80 µL Sample Diluent to all wells
- Add 20 µL Controls/Samples to respective plate strips/columns
  - 37°C for 90 minutes/Wash
- Add 100 µL Conjugate 1 (p24-biotin) to each well
  - 37°C for 30 minutes/Wash
- Add 100 µL Conjugate 2 (SA-HRP, gp160-HRP, p65-HRP, HIV-2-HRP) to each well
  - 37°C for 30 minutes/Wash
- Add 100 µL Substrate to each well
  - RT for 30 minutes
- Add 100 µL 2N Sulfuric Acid STOP solution/read at 450 nm



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Example Results



Non-Viral Ag

HIV-1 p65

HIV-1 gp160 (reduced)

HIV-1 gp160

HIV-1 gp41

HIV-1 p24

HIV-2 (reduced)

HIV-2



# Results of SeraCare HIV-1 Incidence/Prevalence Performance Panel PRB-601

**Recency Index =  
Reduced gp160 S/CO (Row C) x Standard gp160 S/CO (Row D)**

## Incidence Panel Members (S/CO Values)

		Sample 1	Sample 2	Sample 5	Sample 7	Sample 9	Sample 12	Sample 14
A	No Antigen	0.401	0.426	0.418	0.426	0.459	0.398	0.406
B	HIV-1 p65	4.392	2.280	1.879	1.077	6.873	17.905	22.385
C	HIV-1 gp160 (Reduced)	0.434	0.418	0.401	0.434	0.468	0.571	0.398
D	HIV-1 gp160	4.885	4.092	3.958	5.787	9.269	15.569	3.255
E	HIV-1 gp41	3.883	3.023	6.280	3.691	8.234	11.313	2.692
F	HIV-1 p24	29.954	30.213	12.952	14.555	20.585	32.041	10.559
G	HIV-2 Peptide (Reduced)	0.409	0.401	0.443	0.401	0.409	0.422	0.422
H	HIV-2 Peptide	0.409	0.401	0.418	0.392	0.426	0.414	0.398
	<b>Recency Index</b>	<b>2.12</b>	<b>1.71</b>	<b>1.59</b>	<b>2.51</b>	<b>4.34</b>	<b>8.89</b>	<b>1.30</b>

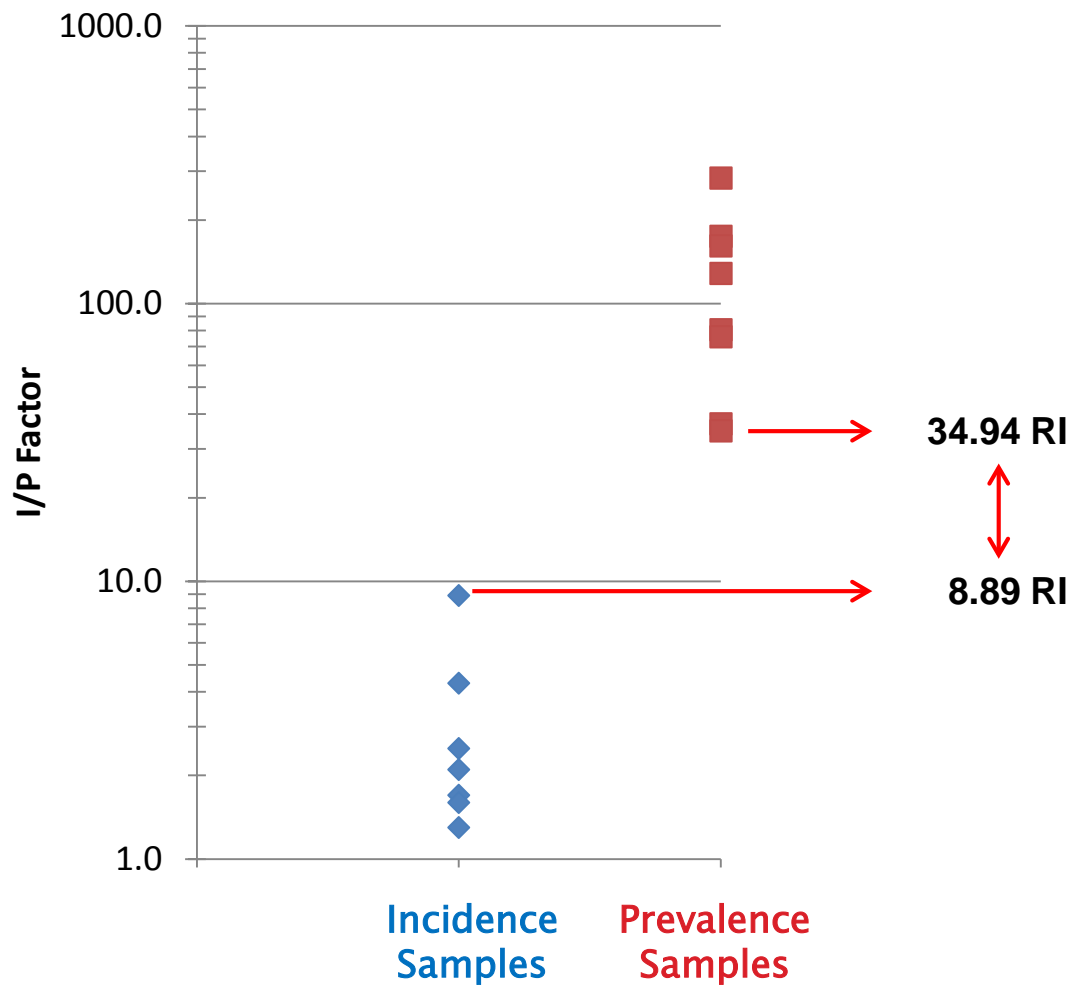
# Results of SeraCare HIV-1 Incidence/Prevalence Performance Panel PRB-601

## Prevalence Panel Members (S/CO Values)

		Sample 3	Sample 4	Sample 6	Sample 8	Sample 10	Sample 11	Sample 13	Sample 15
A	No Antigen	0.418	0.418	0.434	0.493	0.409	0.389	0.406	0.406
B	HIV-1 p65	19.324	29.177	8.384	27.916	29.344	28.389	16.820	25.300
C	HIV-1 gp160 (Reduced)	2.614	4.058	1.370	8.860	5.628	5.226	2.534	1.342
D	HIV-1 gp160	30.873	31.691	27.006	32.058	31.140	30.915	30.079	26.046
E	HIV-1 gp41	24.117	24.326	22.514	32.401	30.455	29.193	29.913	16.472
F	HIV-1 p24	31.775	32.209	27.641	32.084	6.205	28.447	32.141	32.240
G	HIV-2 Peptide (Reduced)	0.451	0.434	0.468	0.618	0.409	0.422	0.431	0.464
H	HIV-2 Peptide	0.426	0.418	0.409	0.501	0.409	0.414	0.455	0.431
	Recency Index	80.69	128.62	36.99	284.04	175.27	161.55	76.22	34.94



# Recency Index for Incidence/Prevalence Panel Members



# HIV Recency Biomarker Screening Panel (HRBS)

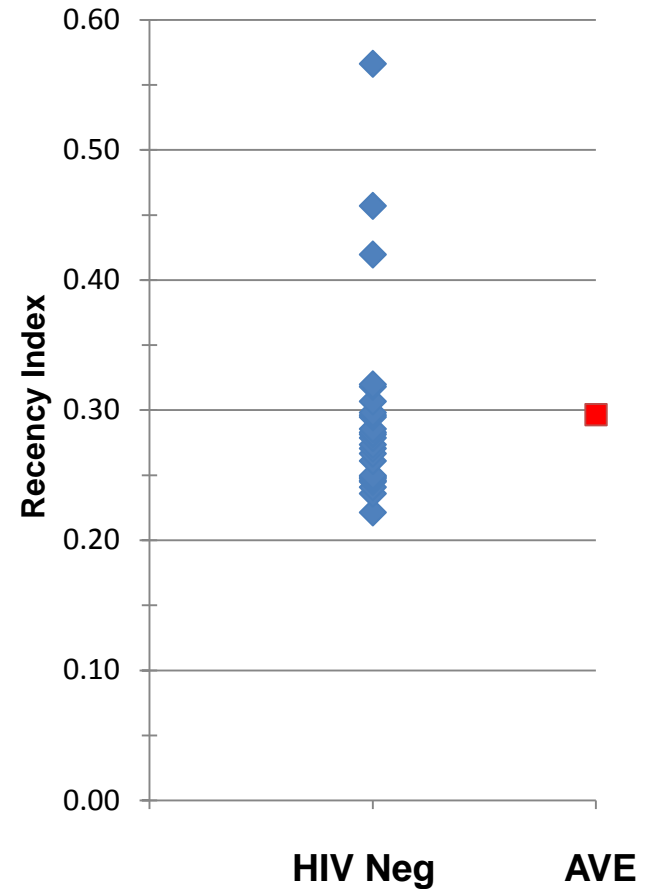


Panel includes 124 well characterized plasma samples:

- HIV Negative (n=25)
- Early HIV Infection <12 mo (n=24)
- Longstanding HIV Infection >12 mo (n=42)
- ART Initiated Early (n=10)
- ART Initiated Late (n=11)
- Elite Controllers (n=11)

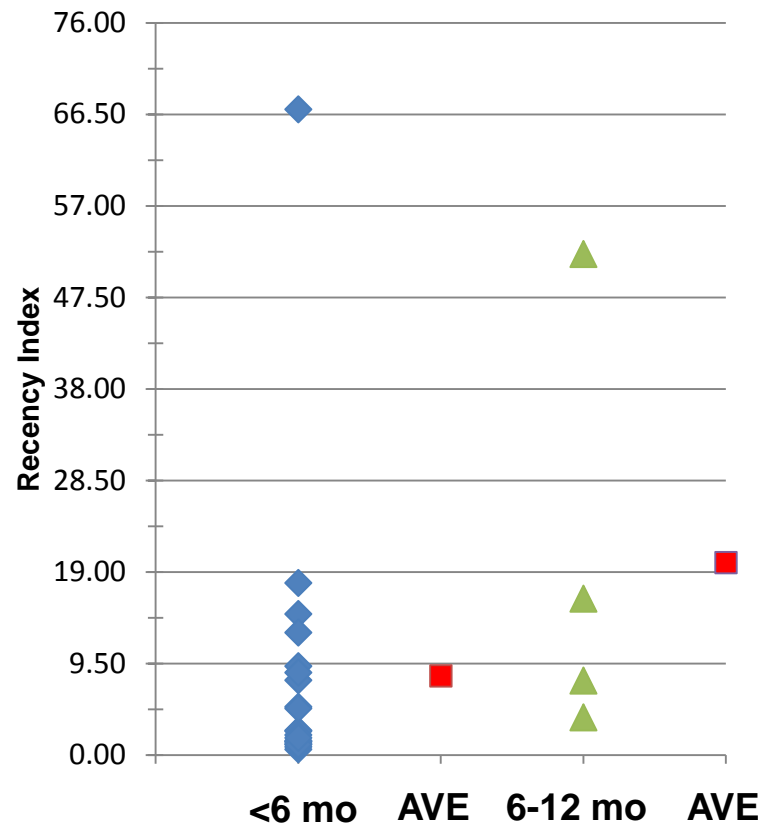
# Profile Assay Results of HRBS HIV Negative Samples

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)
0912-02	0.62	0.92	0.57
1027-02	0.43	0.55	0.24
1695-02	0.44	0.67	0.29
1844-02	0.50	0.92	0.46
2245-02	0.45	0.68	0.31
2509-02	0.39	0.61	0.24
3869-02	0.54	0.59	0.32
3966-02	0.41	0.67	0.27
4509-02	0.42	0.71	0.30
4713-02	0.42	0.67	0.28
4908-02	0.41	0.68	0.28
5022-02	0.44	0.67	0.30
5478-02	0.41	0.66	0.27
5605-02	0.41	0.61	0.25
5755-02	0.43	0.97	0.42
5987-02	0.42	0.59	0.24
5998-02	0.40	0.79	0.32
6395-02	0.41	0.69	0.28
6591-02	0.39	0.64	0.25
7269-02	0.39	0.68	0.27
7357-02	0.39	0.63	0.25
7850-03	0.42	0.68	0.29
8773-02	0.40	0.61	0.25
8995-02	0.38	0.59	0.22
9333-02	0.41	0.64	0.26



# Results of HRBS Samples <12 months from EDI

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)	Months from EDI
0950-02	0.44	5.79	2.56	<6
1316-02	0.56	2.40	1.36	<6
1476-02	0.47	10.73	5.02	<6
1885-03	0.60	15.33	9.26	<6
2427-02	0.52	4.82	2.49	<6
3152-03	0.43	4.94	2.11	<6
3365-02	0.68	11.52	7.79	<6
3458-04	0.72	20.43	14.66	<6
3834-02	0.39	1.52	0.59	<6
5299-04	0.43	2.72	1.18	<6
5683-02	0.64	1.38	0.88	<6
6094-02	0.69	18.37	12.72	<6
6831-02	0.57	15.15	8.58	<6
6953-03	0.81	22.08	17.88	<6
7115-02	0.68	7.07	4.84	<6
7268-02	2.28	29.39	67.01	<6
8119-03	0.47	3.05	1.44	<6
8580-03	0.45	3.33	1.50	<6
8800-02	0.43	2.53	1.10	<6
9888-04	0.39	4.58	1.80	<6
2424-03	0.50	7.84	3.91	6-12
3083-02	0.79	20.64	16.23	6-12
4190-02	1.81	28.73	52.00	6-12
8423-02	0.73	10.63	7.73	6-12

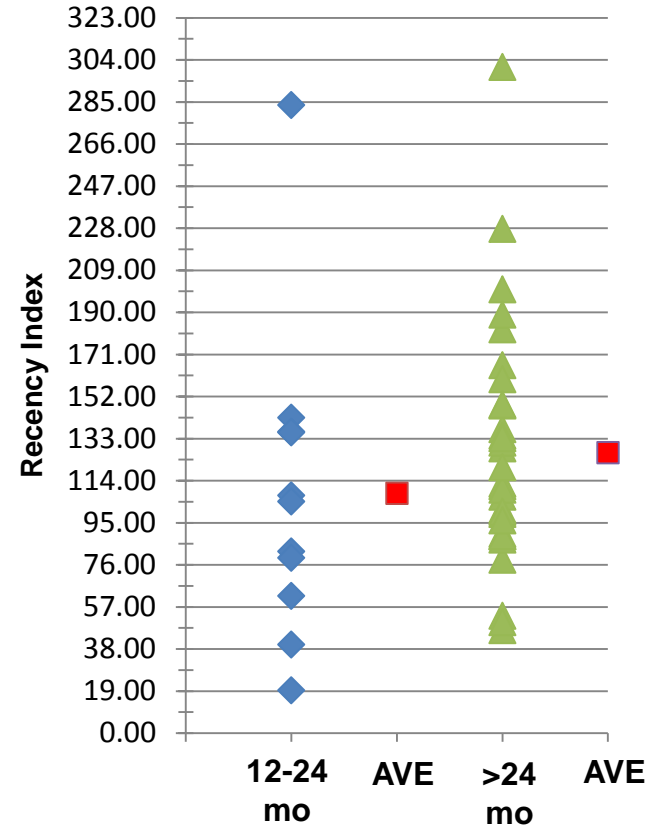


**Using a RI cutoff of 19.00,  
22/24 Samples Classified Correctly -  
92% Accuracy**

# Results of HRBS Samples >12 months from EDI

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)	Months from EDI
0515-02	5.69	25.04	142.55	12-24
0738-02	3.23	25.36	82.02	12-24
2342-03	3.62	29.62	107.33	12-24
2522-03	0.82	23.59	19.34	12-24
3059-03	1.35	29.56	39.99	12-24
4155-03	4.38	31.10	136.10	12-24
7846-02	2.56	30.92	79.16	12-24
7918-03	3.32	31.54	104.57	12-24
7962-03	4.17	32.60	135.84	12-24
8624-02	11.00	25.79	283.60	12-24
9218-02	2.41	25.74	61.96	12-24

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)	Months from EDI
0097-02	2.11	25.15	53.09	>24
0847-03	3.55	31.44	111.58	>24
0906-02	3.53	31.97	112.76	>24
0929-03	2.95	30.54	90.09	>24
1503-03	6.63	30.24	200.37	>24
1701-03	4.61	32.08	147.94	>24
1735-02	5.43	25.26	137.23	>24
2367-03	4.71	27.32	128.64	>24
2399-03	2.88	30.89	88.86	>24
2696-03	3.10	32.55	100.86	>24
2910-03	5.70	32.03	182.44	>24
2971-03	4.43	30.31	134.37	>24
3093-02	6.49	25.61	166.18	>24
3185-02	3.15	24.85	78.33	>24
3396-02	3.52	24.86	87.41	>24
3717-03	3.95	33.24	131.23	>24
4240-02	9.34	32.23	300.87	>24
4471-03	1.80	25.84	46.50	>24
4678-02	3.52	25.30	89.03	>24
6385-02	5.90	32.03	188.96	>24
7423-03	4.35	30.64	133.30	>24
7570-02	7.06	32.25	227.79	>24
7805-03	3.50	30.52	106.85	>24
7833-02	3.18	31.10	98.97	>24
7839-03	3.24	33.95	109.95	>24
8362-03	3.89	30.92	120.14	>24
8553-02	6.27	25.49	159.86	>24
9402-03	4.70	31.55	148.41	>24
9528-02	3.99	25.04	99.97	>24
9749-02	2.04	24.56	50.20	>24
9895-02	3.89	24.75	96.16	>24



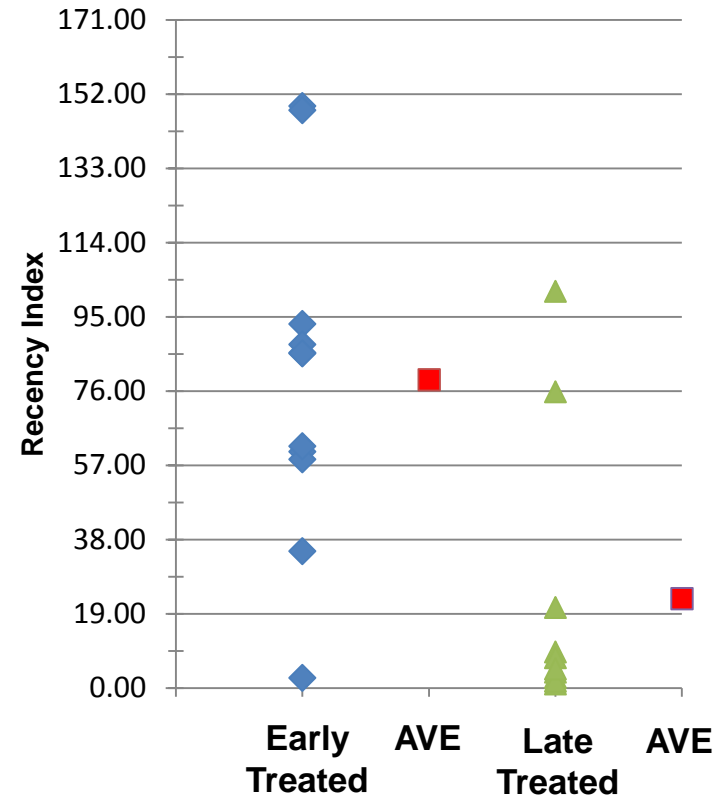
**Using a RI cutoff of 19.00, 42/42 Samples Classified Correctly - 100% Accuracy**



# Results of HRBS Samples from Patients on Early vs Late Treatment

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)	Early Treatment
1300-03	2.35	25.79	60.53	No
2733-03	2.71	34.35	93.19	No
3440-03	0.45	5.85	2.63	No
6588-03	2.11	27.74	58.55	No
6620-03	2.41	25.67	61.94	No
7237-03	3.19	26.92	85.81	No
7667-03	2.75	32.01	87.97	No
7875-03	4.85	30.71	148.95	No
1532-02	6.05	24.46	147.93	No
2159-02	1.67	21.06	35.08	No
4790-02	2.75	31.18	85.69	No

	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)	Early Treatment
5571-03	0.44	2.43	1.07	Yes
0059-02	0.41	4.36	1.79	Yes
8157-03	0.43	6.30	2.71	Yes
4344-02	0.71	5.81	4.13	Yes
9690-02	0.58	8.47	4.91	Yes
7419-03	0.65	12.05	7.83	Yes
7265-03	0.64	14.49	9.27	Yes
9386-02	1.02	20.28	20.69	Yes
7371-02	3.06	24.74	75.81	Yes
8998-03	3.74	27.14	101.54	Yes

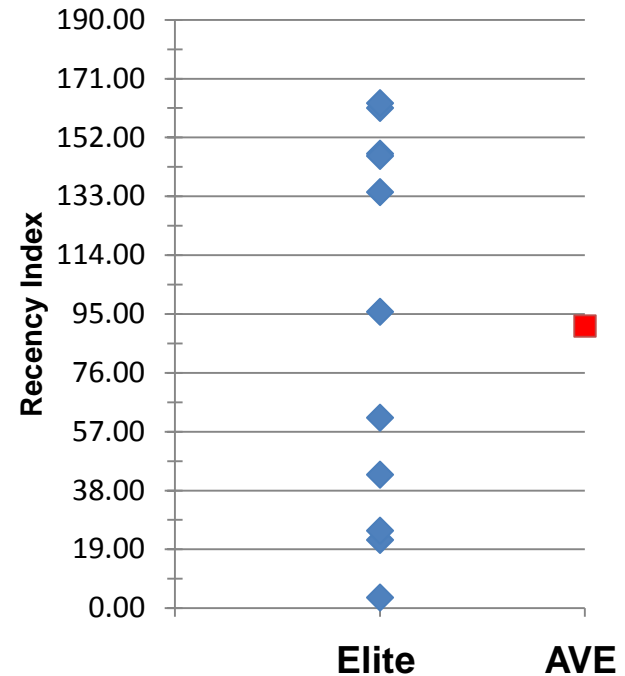


**Using a RI cutoff of 19.00,  
10/11 Samples from Late Treatment &  
3/10 Samples from Early Treatment  
Classified Correctly**



# Results of HRBS Samples from Elite Controllers

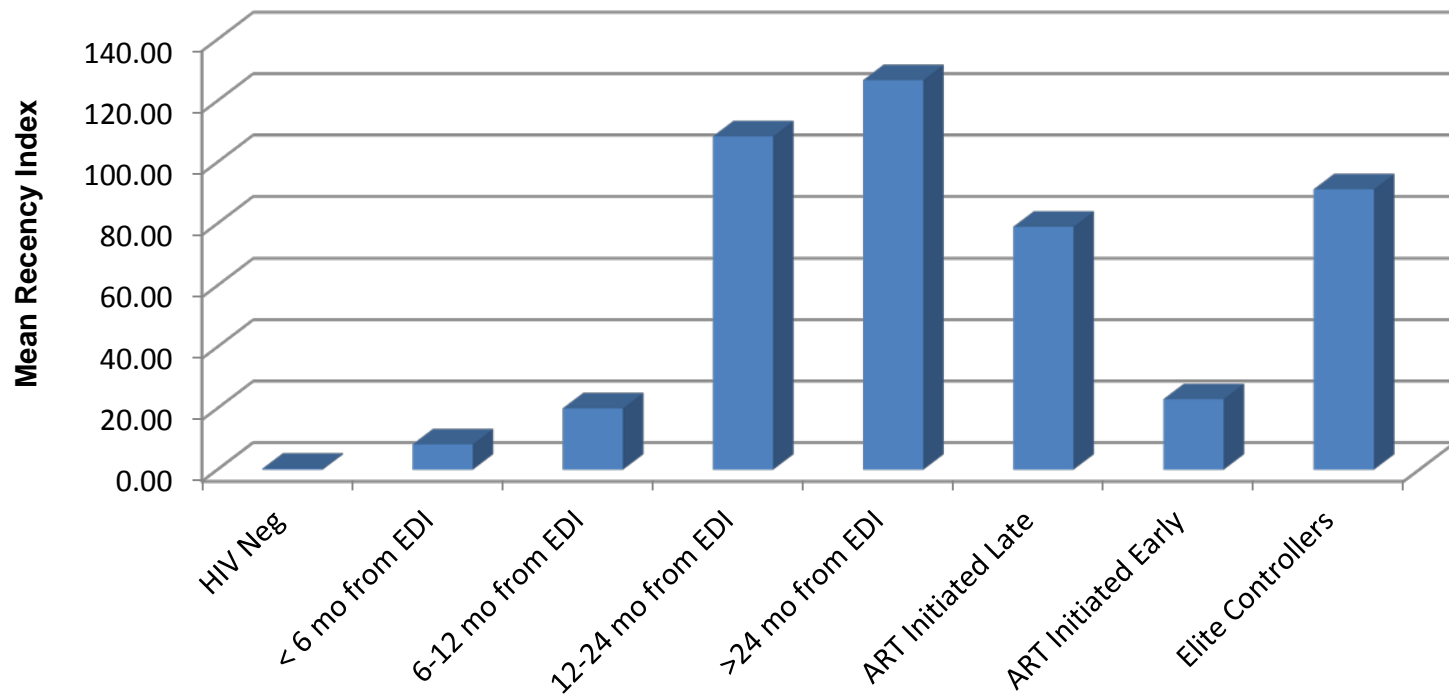
	HIV-1 gp160 Reduced	HIV-1 gp160 Optimal	Recency Index (RI)
1278-03	3.03	31.57	95.69
1665-03	5.18	31.17	161.53
1848-03	4.70	31.26	146.81
3526-03	0.68	5.08	3.45
5002-03	1.02	21.59	22.02
6812-03	2.06	29.83	61.53
7106-03	4.56	32.02	146.06
7623-03	1.24	20.19	25.06
7786-03	1.56	27.58	43.09
7920-02	4.28	31.40	134.34
1284-02	5.21	31.32	163.15



**Using a RI cutoff of 19.00,  
10/11 Samples from Elite Controllers  
Classified Correctly**

# Profile Assay Mean Recency Index Results from Different Sample Populations Included in the CEPHIA HRBS Panel

	HIV Neg	< 6 mo from EDI	6-12 mo from EDI	12-24 mo from EDI	>24 mo from EDI	ART Initiated Late	ART Initiated Early	Elite Controllers
Mean RI	0.30	8.24	19.97	108.41	126.72	78.94	22.97	91.16



## Comparison of Avioq Profile Assay Results vs LAg Assay Results on the CEPHIA HRBS Panel

<b>Specimen Populations</b>	<b>n</b>	<b>HIV Profile Correct Classification</b>	<b>LAg Assay Correct Classification</b>
<b>HIV Negative</b>	<b>25</b>	<b>25</b>	<b>25</b>
<b>&lt;12 months from EDI</b>	<b>24</b>	<b>22</b>	<b>20</b>
<b>&gt;12 months from EDI</b>	<b>42</b>	<b>42</b>	<b>40</b>
<b>Challenge: Elite Controllers</b>	<b>11</b>	<b>10</b>	<b>7</b>
<b>Challenge: ART initiated early</b>	<b>10</b>	<b>3</b>	<b>2</b>
<b>Challenge: ART initiated late</b>	<b>11</b>	<b>10</b>	<b>10</b>

# VioOne™ HIV Profile™

## ► Conclusions:

- Avioq's VioOne HIV Profile differentiated Incidence from Prevalence samples in a commercially available SeraCare panel.
- Using a Recency Index cutoff of 19.00, the Profile Assay differentiated samples from patients with HIV infection of less than 12 months vs greater than 12 months, similar to an established LAg assay.
- Next step is to test a Proof of Concept panel "Pitt Panel 2" from CEPHIA for tuning and optimization of Recency threshold.