

# Indeterminate HIV-1 Serological Results and the Cost of Resolution

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## Background

### HIV tests on the market

- Aptima NAT RNA assay detects HIV-1/2 positive specimens 6 days post infection (1).
- The 4th generation assay detects HIV-1/2 positive specimens 11 days post infection (2).
- 3<sup>rd</sup> generation Bio-Rad assay detects HIV-1/2 positive specimens 17 days post infection (3).
- Multi-spot Rapid test detects HIV-1/2 positive specimens 24 days post infection (4).
- HIV-1 Western blot detects positive specimens 32 days post infection (5).

### Pre-2011 CDC HIV testing algorithm

- A repeatedly reactive specimen on 3<sup>rd</sup> generation assay followed by a confirmation by Western blot.
- This allows for detection as early as 17 days post infection, but there is a 15 day gap between the confirmation test, which allows for a potential negative or indeterminate result by Western blot.

### Post-2011 CDC testing algorithm

- A repeatedly reactive specimen on 4<sup>th</sup> generation assay followed by Bio-Rad Multispot. Discrepant results go to Aptima/Aptima NAT RNA assay.
- This allows for detection as early as 11 days post infection.

### HIV testing in Tennessee Public Health Clinics

- Early detection of HIV is crucial to control the spread of HIV. Public Health Clinics offer HIV diagnostic services at free or reduced prices to customers who do not have the ability to pay.
- The current algorithm used by the Tennessee Department of Health Laboratory Services is 3<sup>rd</sup> generation testing EIA followed by Western blot testing for confirmation.
- If an indeterminate result is obtained, the recommendation is for the patient be re-tested; however, the patient population at the public health clinic is less likely to return for a re-test.
- The Aptima NAT RNA assay would be ideal for testing on our patient population, but it is cost prohibitive for low or moderate testing facilities due to its short shelf life and large package volume.

## Objectives

1. To examine the resolution of indeterminate serological status of the Public Health Clinic patient population to determine if switching from the pre-2011 HIV diagnostic algorithm to the post-2011 algorithm makes sound clinical and financial sense.
2. To look at the cost associated with switching algorithms and make suggestions on how the Public Health Laboratories can absorb this cost.

## Methods

### Study setting and design

Specimens submitted for HIV testing to TDOH Laboratory Service from July 1, 2009 to June 30, 2011 were analyzed for their status: negative, positive, or indeterminate. Next, indeterminate results were analyzed for whether or not their status was resolved.

### EVOLIS automated EIA

The Bio-Rad GS HIV-1/HIV-2 Plus O antigen EIA (3<sup>rd</sup> generation) was performed on the Bio-Rad EVOLIS automated EIA instrument following the protocol in the package insert (3).

### HIV-1 / HIV-2 Western blot

The Bio-Rad HIV-1 and Immunetics HIV-2 Western blots were performed manually following the protocols in the package inserts(5,6).

## Results

### Results of indeterminate HIV status reports submitted by the Tennessee Division of Lab Services

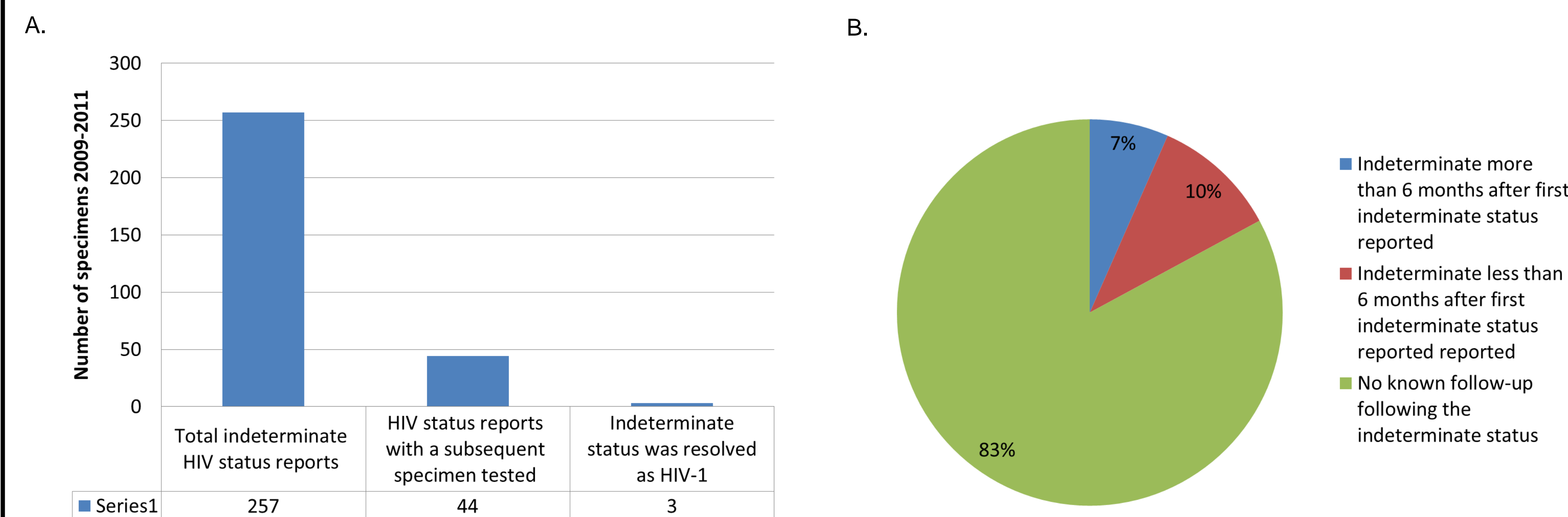


Figure 1. (A) Resolution of HIV-1 Indeterminate status reports . (B) Percentage of HIV-1 status reports resolved based on time.

### HIV diagnostic tests timeline and the immune response to HIV

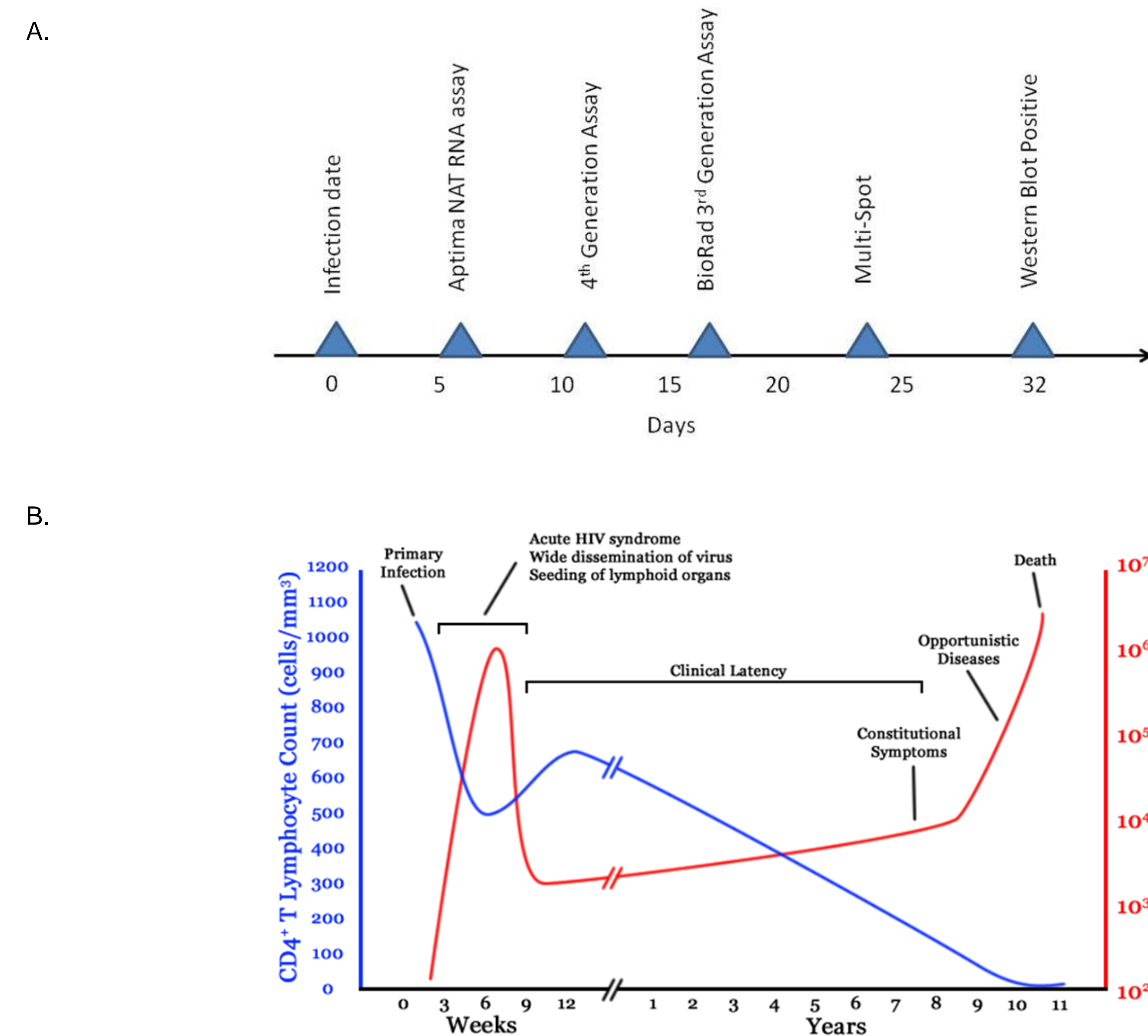


Figure 2. (A) Timeline in which the available assays can detect HIV status post infection.(B) Viremia and CD4 count for HIV infection over time (7).

## Results

### Discrepancies between 3<sup>rd</sup> and 4<sup>th</sup> generation EIA

Sample ID	Initial screen	First-gen or second-gen EIA		WB	Third-gen EIA					Viral load (copies/ml)*	Fourth-gen IA		
		Avg S/C	Result		Avg S/C	Result	RT	RT	RT		RT	Avg S/C	Result
A	First-gen EIA	0.351	NR	I	0.127	NR	N	N	N	N	5.770†	0.37	NR
B	First-gen EIA	0.602	NR	I	0.955	CNR	N	N	N	N	>500,000†	61.14†	R
C	First-gen EIA	0.440	NR	I	>14.658	R	N	N	N	N	15.183†	1.62	R
E	First-gen EIA	0.368	NR	I	0.233	NR	N	N	N	N	0.377†	0.65	NR
F	First-gen EIA	0.329	NR	I	13.433	R	N	N	R1		>500,000†	65.73	R
G	First-gen EIA	0.317	NR	N	0.084	NR	N	N	N	N	12.852†	0.74	NR
H	First-gen EIA	0.338	NR	I	0.109	NR	N	N	N	N	14.062†	0.68	NR
I	First-gen EIA	0.646	NR	I	>14.658	R	R	R	R	R1	>500,000†	67.70	R
K	First-gen EIA	0.358	NR	N	0.196	NR	N	N	N	N	5.921†	0.23	NR
L	First-gen EIA	0.346	NR	N	4.574	R	R	R	R	R1	>500,000†	43.92	R
M	First-gen EIA	0.373	NR	N	0.175	CNR	N	N	N	N	>500,000†	35.52	R
N	First-gen EIA	0.337	NR	N	1.527	R	N	N	N	N	>500,000†	368.21	R
O	First-gen EIA	0.301	NR	N	0.127	NR	N	N	N	N	1.177†	0.21	NR
P	First-gen EIA	0.755	NR	N	>14.658	R	N	N	R1		>500,000†	136.62	R
Q	First-gen EIA	0.311	NR	N	0.277	NR	N	N	N	N	0.411†	1.59	R
R	First-gen EIA	0.642	NR	I	0.117	CNR	N	N	N	N	30.341†	2.65	R
S	First-gen EIA	0.466	NR	N	13.276	R	N	N	R1		>500,000†	219.97	R
T	First-gen EIA	0.401	NR	N	4.929	R	N	N	N	N	>500,000†	268.30	R
U	First-gen EIA	0.325	NR	I	0.195	CNR	N	N	N	N	>500,000†	317.71	R
V	First-gen EIA	0.512	NR	N	0.188	NR	N	N	N	N	>500,000†	303.53	R
W	First-gen EIA	0.504	NR	I	>12.403	R	N	N	R1		>500,000†	121.68	R
Y	First-gen EIA	0.340	NR	N	>12.900	R	N	N	N	N	>500,000†	237.06	R
Z	First-gen EIA	0.378	NR	N	0.201	NR	N	N	N	N	102.288†	2.00	R
AA	First-gen EIA	0.343	NR	N	0.237	NR	N	N	N	N	29.433†	2.24	NR
AB	First-gen EIA	0.375	NR	N	0.189	NR	N	N	N	N	>500,000†	168.67	R
AC	First-gen EIA	0.396	NR	I	0.289	NR	N	N	N	N	0.390	0.53	R
AD	First-gen EIA	0.426	NR	N	0.371	NR	N	N	N	N	>500,000†	132.59	R
AE	First-gen EIA	0.369	NR	N	0.145	NR	N	N	N	N	382.359†	12.94	R
AF	First-gen EIA	0.761	NR	I	0.907	NR	N	N	R1		41.146†	13.39	R
AG	First-gen EIA	0.436	NR	I	0.165	NR	N	N	N	N	46.610†	12.23	R
AH	First-gen EIA	0.371	NR	N	0.195	NR	N	N	N	N	353.030†	10.66	R
AJ	First-gen EIA	0.416	NR	N	1.528	R	N	N	R1		>500,000†	24.25	R
AK	First-gen EIA	0.6	NR	N	0.147	NR	N	N	N	N	0.460†	0.25	R
AM	First-gen EIA	0.441	NR	N	0.187	NR	N	N	N	N	>500,000†	309.58	R
AN	First-gen EIA	0.333	NR	I	0.17	NR	N	N	N	N	>500,000†	115.50	R
AO	First-gen EIA	0.45	NR	N	9.634	R	N	N	R1		>500,000†	22.83	R
AP	First-gen EIA	0.34	NR	N	>12.854	R	N	N	R1		>500,000†	115.50	R
AR	First-gen EIA	0.45	NR	N	0.097	NR	N	N	N	N	>500,000†	10.57	R
AS	First-gen EIA	0.48	NR	I	>14.641	R	N	N	R1		>500,000†	46.610	R
AT	First-gen EIA	0.48	NR	N	0.174	NR	N	N	N	N	>500,000†	175.33	R
AU	First-gen EIA	1.48	R	P	13.69	R	N	N	R1		106.211	31.25	R
AV	First-gen EIA	0.5	NR	N	0.271	NR	N	N	N	N	333.066	5.68	R
AW	First-gen EIA	0.582	NR	P	12.466	R	N	N	R1		255	14.28	R
AX	First-gen EIA	0.36	NR	N	1.554	R	N	N	R1		>10,000,000	360.32	R
AY	First-gen EIA	0.45	NR	N	0.215	NR	N	N	N	N	9.509	1.46	R
AZ	First-gen EIA	0.59	NR	P	9.454	R	N	N	R1		2,975,306	80.82	R
BA	First-gen EIA	0.31	NR	N	13.205	R	N	N	R1		518,454	9.13	R
BB	First-gen EIA	0.32	NR	N	0.16	CNR	N	N	N	N	31.076	2.91	R
BC	First-gen EIA	0.65	NR	P	>13.943	R	N	N	R1		13,204	77.69	R
BD	First-gen EIA	0.5	NR	P	10.17	R	N	N	R1		18,871,099	430.59	R
BE	First-gen EIA	0.38	NR	I	0.243	NR	N	N	N	N	>10,000,000	133.66	R
BF	First-gen EIA	0.54	NR	P	5.443	R	N	N	R1		>10,000,000	430.59	R
BG	First-gen EIA	5.69	R	P	>13.514	R	N	N	R1		460,809	12.66	R
BH	First-gen EIA	0.35	NR	N	0.332	NR	N	N	N	N	9,280,006	80.34	R
BI	First-gen EIA	2.03	R	P	10.544	R	N	N	R1		355	3.69	R
BJ	First-gen EIA	0.346	NR	I	0.123	NR	N	N	N	N	9.855	0.71	NR
BK	First-gen EIA	0.284	NR	N	0.403	NR	N	N	N	N	0.826	0.48	R
BL	First-gen EIA	5.13	R	P	>14.403	R	N	N	R1		792	109.51	R
BM	First-gen EIA	0.326	NR	N	0.345	NR	N	N	N	N	1,580,913	31.66	R
BN	First-gen EIA	2.059	R	I	1.18	NR	N	N	R1		4,971,267	48.38	R
BO	First-gen EIA	0.12	NR	N	0.188	NR	N	N	N	N	1,511	11.62	R
BP	First-gen EIA	1.015	NR	N	4.139	R	N	N	R1		>10,000,000	136.32	R
BQ	First-gen EIA	0.15	NR	N	0.539	CNR	N	N	N	N	3,427,863	14.92	R

Figure 3: Ability of different generation HIV assays to detect HIV infection in acute phase sera. This table is using Abbott's test from Pandori et al. 2009 J Clin Micro 47(8):2639-42 (8).

## Conclusions

- 17% of indeterminate specimens were followed up with a re-test.
- 10% of these were tested within 6 months of the initial test and 7% were tested after 6 months.
- In a study by Pandori et al, the 4<sup>th</sup> generation assay picked up 89% of the acute HIV positive specimens while the 3<sup>rd</sup> generation assay only picked up 42%.
- Most of the discrepant specimens between 3<sup>rd</sup> generation and 4<sup>th</sup> generation have viremia between 500,000 and 10,000,000 copies/mL.
- NAT testing would possibly resolve the 257 cases before they leave the laboratory.
- The Aptima NAT assay and the 4<sup>th</sup> generation assay have unique CPT codes which allow for higher reimbursement rates when compared to the 3<sup>rd</sup> generation assay.

## Recommendation

We suggest moving to 4<sup>th</sup> generation testing and centralizing NAT RNA HIV-1 testing for Public Health Laboratories by region in order to control cost for this important public health issue.

## References

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