

Comparing the 3rd and 4th Generation HIV Algorithms in a High-risk Population

Brian Nefzger¹, Gracia Johnson¹, Jennifer Palm¹, Ramani S. Wonderling², Sara M. Vetter¹ ¹Minnesota Department of Health, Public Health Laboratory, St. Paul, MN 55112; ²Abbott Diagnostics, Abbott Park, IL 60064

Figure 3. 3rd Generation Algorithm

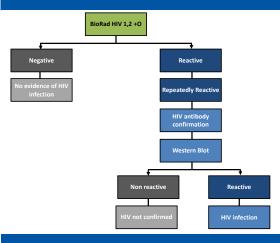
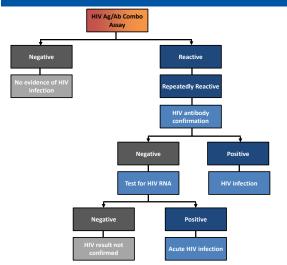


Figure 2. 4th Generation Algorithm



HIV testing at MDH-PHL (2009-2011)

- Serve metro area STD clinics
- ~ 2000-3000 tests/year
- 3.4% -4.8% Reactive
- Converted to 4th generation algorithm July 2012

Study Objective:

- Compare new 4th generation algorithm to the established 3rd generation algorithm
- Parameters examined Assay performance
- Sensitivity/Specificity
- o Ease of use
- Bench time
- Cost comparison

Introduction: As public health laboratories and clinical laboratories consider switching from the 3rd generation to the 4th generation algorithm for HIV testing, it is important to consider the impact on workflow and cost as well as the benefits of earlier detection in the infection process that newer technologies may produce. Objective: The Minnesota Department of Health-Public Health Laboratory (MDH-PHL) provides testing for local STD clinics that serve a high-risk subset of the population in the metropolitan area. We compared the use of two 4th generation combo immunoassays (IA), The Bio-Rad HIV Combo Ag/Ab enzyme immunoassay (EIA) and the Abbott ARCHITECT HIV chemiluminescent miroparticle immunoassay (CMIA), as part of the 4th generation HIV testing algorithm, with the Bio-Rad HIV 1,2 + O EIA using the 3rd generation algorithm. We assessed performance, cost, and tech time for each IA along with the entire algorithm. Methods: Specimens were tested with the 1,2+O EIA and both Combo IAs. For the 3rd generation algorithm, specimens that were Repeatedly Reactive (RR) with the 1,2+O EIA were confirmed with both the Bio-Rad Multispot HIV-1,HIV-2 rapid test for differentiation, and Bio-Rad HIV-1 Western Blot (WB). For confirmation with the 4th generation algorithm, RR specimens on either Combo IA were performed with the Multispot test only. Testing costs were tracked so the expense incurred following both algorithms could then be assessed. Results: MDH-PHL tested 658 specimens in parallel with the 1,2+O EIA and the two Combo IAs. Using the 3rd generation algorithm as a 'gold standard,' 33/658 (5.01%) of the specimens were positive for HIV antibodies. The 4th generation algorithm showed 100% agreement with the 3rd generation algorithm. The ARCHITECT CMIA had one false positive that did not confirm as true with supplemental testing. Two specimens tested initially reactive with the 1,2+O EIA, but did not confirm as true positives after completing the remainder of the confirmatory algorithm. These two specimens were also non-reactive on both combo lAs. To get a better idea about specificity of the combo lAs, seven previous false positive specimens (samples that were RR with 1,2 + O EIA, but negative Multispot and negative WB) were also tested with both combo IAs. All seven were non-reactive on the combo IA suggesting the combo IAs are more specific. Reagent cost for the combo IAs was greater than the cost of the 1,2 + O EIA reagents. However, the total cost to perform all of the testing in the 3rd generation algorithm using the BioRad HIV 1,2+O kit and confirming with the BioRad Western Blot kit was greater than the cost of performing the 4th generation algorithm using either combo kit followed by the Bio-Rad Multispot differentiation test. Actual "hands on" time was monitored. Both bench top EIAs required approximately 24 minutes tech time for 10 samples while the Architect platform only took about 9 minutes for the same amount of samples. The Mulitspot assay required 10 minutes tech time for 3 samples and the Western Blot assay used approximately 27 minutes for 3 samples. Conclusions: Both the Bio-Rad Ag/Ab Combo EIA and the ARCHITECT HIV CMIA outperformed the Bio-Rad 1,2+O EIA due to improved specificity. Using either combo IA in conjunction with the 4th generation algorithm yields a cost savings over the 3th generation algorithm. The ARCHITECT platform has a faster turnaround time (28 minutes/assay) for each test and will reduce labor costs compared to the Bio-Rad Combo test (2 hours/assay). However, the increased cost of purchasing the ARCHITECT platform may be prohibitive for laboratories that do not test a high volume of samples. Laboratories will need to consider their testing volumes and available budget in order to decide which platform best fits their needs

Methods

- · Every specimen was tested with 3 immunoassays
- BioRad 1 2+0
- BioRad Ab/Ag Combo HIV EIA
- Abbott ARCHITECT CMIA
- · Any specimen testing 'Repeatedly Reactive' on any assay was confirmed with BioRad Multispot HIV-1, HIV-2 rapid test and with BioRad Western Blot
- Cost estimates were based on
- o The Reagent cost for each assay based on either actual use or quoted costs (Table 1)
- The number of tests it took to complete testing using either the 3rd or 4th testing algorithm with either combo IA
- o Time per test was calculated based on hands on time and total time to complete each

Table 1. Estimated Cost per Sample for Each Test **Used in Algorithms**

	Cost/sample
BioRad 1,2 +O *,**	7.33
BioRad Ag/Ab Combo EIA **	9.21
Abbott ARCHITECT HIV CMIA #	15.39
BioRad Multispot *	48.00
BioRad Western Blot *	90.00

- * Cost estimates are based on three years of cost analysis ** Costs for IA are based on 10 samples run/plate plus controls, consumables and equipment
- # Cost estimates are based on quote from Abbott for reagents, consumables, and equipment

Table 2. Estimated Time per Sample for Each Test and Each Algorithm

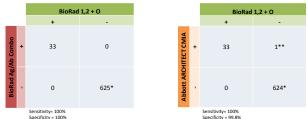
	Minutes/Sample		
	Hands on time	Total time	
BioRad 1,2 +O*	2.4	12	
BioRad Ag/Ab Combo EIA*	2.4	12	
Abbott ARCHITECT HIV CMIA*	0.9	2.4	
BioRad Multispot**	3.3	5	
BioRad Western Blot**	9	40	

- 658 specimens were tested (Table 3)
- Using the 3rd generation algorithm as a gold standard, 33 specimens were positive for HIV antibodies, and 625 were negative

Results

- o BioRad Ag/Ab Combo had 100% sensitivity and specificity
- Abbott ARCHITECT had 100% and 99.% sensitivity and specificity, respectively.
- All but 3 samples were in agreement on all platforms. (Table 4)
- o The 2 exceptions were initially reactive on the BioRad 1,2+O but nonreactive on both combo assays
- o One sample was repeat reactive on the ARCHITECT but negative on the other EIAs and confirmatory tests (Table 4)
- Previous false positives using the BioRad 1,2+O assay were non-reactive upon retesting with one of the Combo IAs (Table 5)
- Reagent cost to complete testing for all samples tested in the study for each algorithm (33 positive and 625 negative) were estimated. The cost of performing the 4th generation algorithm using BioRad Ag/Ab Combo EIA is estimated to be 15.3% lower than following the 3rd generation algorithm using western blot testing. Using the Abbott ARCHITECT CMIA, reagent costs are estimated to be 25.0% higher than the 3rd generation algorithm (Table 6).

Table 3. Performance of Combo IAs Compared to 3rd Generation Algorithm



Using the BioRad 1.2 + O EIA as the gold standard, the performance of each combo EIA was compared.

Table 4. Complete Results of any Specimen with <100% Agreement

Specimen		BioRad 1,2, + O		Bio	oRad Ag/Ab Com	bo	Abbott ARCHITECT CMIA		Multispot	Western Blot	NAAT	
	Run1	Run2	Run3	Run1	Run2	Run3	Run1	Run2	Run3			
3443	Reactive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	N/A
3563	Reactive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	N/A
5083	Negative	Negative	Negative	Negative	Negative	Negative	Reactive	Reactive	Reactive	Negative	Negative	Negative*

Aptima RNA HIV-1 qualitivative assay was used for NAAT testing

Table 5. Previous False Positives Using the BioRad 1,2 + O EIA

Specimen	BioRad 1,2 + O EIA	Retest
6179	Repeatedly Reactive	Negative*
4823	Repeatedly Reactive	Negative*
9657	Repeatedly Reactive	Negative*
8485	Repeatedly Reactive	Negative*
3292	Repeatedly Reactive	Negative*
3180	Repeatedly Reactive	Negative*
4411	Repeatedly Reactive	Negative**

Previous false positive specimens were retested using either the BioRad Ab/Ag Combo EIA* or the Abbott ARCHITECT CMIA**. In this context the Combo IAs show better specificity and would result in less testing. False positive samples were defined as samples testing Repeatedly Reactive on the EIA, but negative with the multispot and western blot assays. There was not enough specim

Table 6. Estimated Reagent Cost of each Algorithm for the 658 Samples Tested in the Study

3rd generation algorithm						
BioRad 1,2 + O						
EIAs	Multispot	Western Blots	Total			
5574.80	1584.00	2970.00	10128.80			
	4th generation algorithm					
BioRad Ag/Ab Combo						
EIAs	Multispot		Total			
7059.00	1518.00		8577.04			
Abbott ARCHITECT CMIA						
CMIAs	Multispot		Total			
11142.00	1518.00		12660.36			

Table 7. Estimated Hours to Complete all Testing in the Study Based on Each Algorithm

	Total hours to con	Total hours to complete testing		
	Hands on	Total time		
3rd generation algorithm				
BioRad 1,2 +O EIA*	35.71	169.55		
4th generation algorithm				
BioRad Ag/Ab Combo EIA*	30.76	147.55		
Abbott ARCHITECT HIV CMIA*	12.66	31.71		

^{*}Time estimates based on batches of 10 sample

. Hands on time and total time to complete all testing for the samples were calculated for each algorithm. The 4th generation algorithm using the Abbott ARCHITECT took the least amount of time to complete with only 12 total hours of hands on time and 31.71 hours for total testing time. The 4th generation algorithm using BioRad 1,2 + O and the 3rd generation algorithm took 30.76 and 35.71 hours, respectively, of hands on time to complete testing and 147.55 and 169.55 hours, respectively, of total time to complete testing.

Conclusions

- Despite what appears to be one false positive using the ARCHITECT CMIA, both Combo IAs performed well having sensitivities and sensitivities >99% matching package insert
- Testing previous false positives from the 3rd generation EIA with the 4th generation IAs suggest increased specificity with 4th generation FIAs.
- In regards to cost, the 4th generation algorithm using the BioRad Ag/Ab EIA is most cost effective, based on a volume of 2000 specimens a year.
- The 4th generation algorithm using the Abbott ARCHITECT utilizes the least amount of time.
- . With a larger volume of samples, the cost savings with the much less testing time utilizing the Abbott ARCHITECT could be greater than the increased reagent cost
- Smaller volume laboratories (<2000 samples a year) will have to consider batch sizes. purchasing options (reagent rentals vs. equipment purchase), and other testing that could be done using the ARCHITECT system when deciding the most appropriate system for their programs

· Abbott Diagnostics provided testing reagents and partial salary funds for this study

^{* 2} specimens were initially reactive on the BioRad 1,2, + 0 but were not repeatedly reactive
**Specimen is most likely a true negative based on confirmatory testing (Table 4).