Serum and OMT EIA repeat reactivity and signal-to-cutoff ratios as predictors of Western blot positivity

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INTRODUCTION

The MA State Laboratory Institute performs HIV antibody testing according to current standard testing algorithms. Specimens with a reactive EIA result are retested in duplicate, and repeatedly reactive specimens are tested by WB. A second specimen is needed to resolve indeterminate WB results. We examined laboratory data collected during routine testing with the following objectives:

1. To determine the likelihood of repeatedly reactive EIA test results following an initially reactive EIA
2. To determine the predictive value of signal-to-cutoff ratio in an HIV testing algorithm
3. To assess the effectiveness of testing a second specimen for resolution of indeterminate WB results.

METHODS

Routine testing methods

- All serum and oral mucosal transudate (OMT) specimens specimens are tested with the BioRad HIV-1/HIV-2 Plus O EIA. Repeatedly reactive specimens are tested by HIV-1 WB (bioMérieux for OMT, BioRad for serum).
- Specimens repeatedly reactive by EIA and WB indeterminate are assigned an "**indeterminate**" result.
- Specimens repeatedly reactive by EIA and WB-negative are assigned an "**inconclusive**" result.
- Follow-up serum samples are requested for all indeterminate and inconclusive results

Study methods

- Serum and OMT specimens received between April 1, 2006 and October 11, 2007 were included.
- EIA results on initial and repeat testing were compared to WB result.
- For specimens reactive on initial EIA, the signal-to-cutoff (SCO) ratio for the initial EIA was calculated by dividing the optical density of the patient sample by the cutoff value for positivity.
- Test results of specimens received in follow-up of an indeterminate or inconclusive result were compared to initial test results.
- If a follow-up specimen was not received within four weeks, the counseling and testing site was contacted to determine the patient's re-test status.

RESULTS

Objective 1: Determine the likelihood of repeatedly reactive EIA test results following an initially reactive EIA.

Between April 1, 2006 and October 11, 2007, 21,549 OMT and 21,113 serum specimens were tested (*Table 1*). An additional 297 sera were received for confirmation of a reactive rapid test performed at the point of care.

- Of 296 initially EIA-reactive OMT and 341 serum specimens, 210 (71%) and 305 (89%), respectively, were repeatedly reactive.
- Of 210 OMT and 305 serum specimens that were repeatedly reactive, 168 (80%) and 252 (83%), respectively, were positive by WB.
- Among samples reactive on 2 of 3 EIAs, 2 of 21 OMT, 0 of 2 serum, and 2 of 2 rapid test follow-up sera were WB-positive.

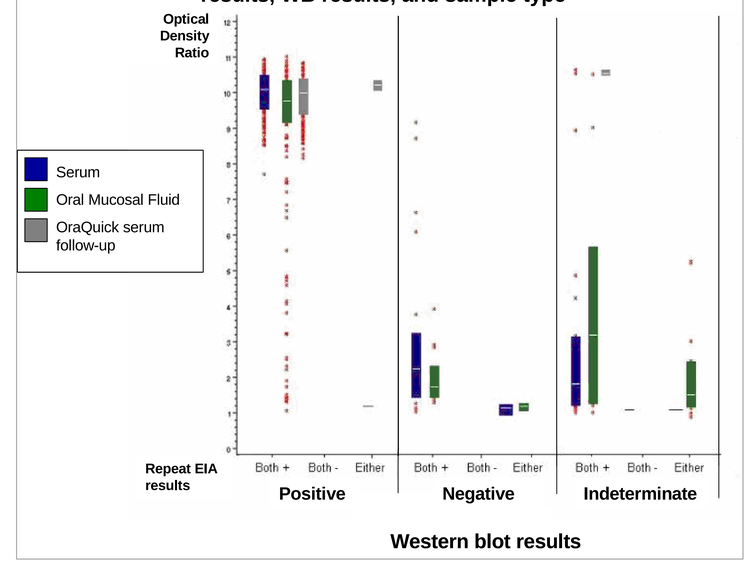
	Total samples	Initially reactive EIA	Initially reactive EIA with repeat EIA in duplicate						Repeatedly reactive EIA with positive WB	
			R/R		R/NR		NR/NR			
OMT	21,549	296	189	64%	21	7%	86	29%	168	80%
Serum	21,113	341	302	89%	3	1%	36	11%	252	83%
OraQuick follow-up serum	297	248	246	99%	2	1%	0	0%	246	99%

Table 1. Repeat EIA testing following initial reactive EIA and prediction of Western blot positivity.

Objective 2: Determine the predictive value of signal-to-cutoff ratio in an HIV testing algorithm.

- SCO ratios were calculated for initial reactive EIA specimens, including 296 OMTs, 341 sera, and 248 sera submitted in follow-up to a reactive rapid test. Initial EIA SCO values are plotted, by repeat EIA result, WB result, and specimen type, in *Figure A*.
- For sera, SCO ratios for WB-positive specimens were tightly clustered, with a median of 10.13 (n=252, range 7.75-10.99).
- For OMT, SCO ratios for WB-positive specimens have a similar median (9.77), but wider range (n=168 1.01-11.03).
- Among 260 serum and 145 OMT specimens with an initial EIA with SCO ratio ≥7, 252 (97%) and 143 (99%), respectively, were positive by WB.

Figure A: Signal to cutoff ratios of initial reactive EIA, by repeat EIA results, WB results, and sample type



Objective 3: Assess the effectiveness of testing a second specimen for resolution of indeterminate WB results.

For 86 patients with indeterminate and inconclusive specimens received between April 1, 2006 and August 31, 2007:

- 13 (15%) had no identified follow-up status
- 33 (38%) were known to be "lost to follow-up"
- 40 (47%) had follow-up testing

Results of follow-up testing, by initial result and specimen type, appear in *Table 2*.

	Serum		OMT		OraQuick serum follow-up		Total	%
Final Result	Ind	Inc	Ind	Inc	Ind	Inc		
Positive	1	0	3	0	1	0	5	13%
Negative	7	6	2	10	4	0	29	73%
Indeterminate	4	1	0	1	0	0	6	16%
Total	10	7	5	11	5	0	40	

Table 2. Follow-up testing results by specimen type.
Inc=inconclusive, Ind=indeterminate

DISCUSSION

Objective 1:

- 29% and 11% of initially EIA-reactive OMT and serum specimens, respectively, are not repeatedly reactive.
- 20% and 17% of EIA repeatedly reactive OMT and serum specimens, respectively, are not positive by WB.
- The practice of repeating initially reactive EIA substantially decreases the number of WB performed.

Objective 2:

- HIV EIA SCO ratio ≥7 appears to predict WB-positivity at least as well as an HCV EIA SCO ratio ≥3.8 predicts RIBA-positivity.
- Using CDC guidelines for HCV testing as a model, it may be possible to consider specimens with a high initial HIV EIA SCO ratio to be positive, without WB confirmation.
- However, because 1-3% of HIV-positives identified by high SCO ratio alone would be false positives, an effective counseling message, conveying a high but not absolute likelihood of HIV, would need to be developed.
- Specimens with initial HIV EIA SCO ratio <7 would need confirmatory testing.

Objective 3:

- In spite of active follow-up of patients with inconclusive/indeterminate results, only 47% of patients submitted a second specimen for retesting.
- Ideally, HIV test results would not be dependent on testing follow-up specimens.
- Further examination of similar data would be needed to determine whether our results can be generalized to different test settings.

FURTHER INFORMATION:
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