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ABSTRACT

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Treponemal Based Screening for Syphilis - Detecting Latent Cases

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Background: With the advent of national plans to eliminate syphilis from the United States and Canada it becomes vital that all cases (new and old) be identified and where necessary treated. Most laboratories in North America use non-treponemal based screening tests for syphilis. We asked how many cases of "unknown" syphilis infection were being missed using this, the oldest of all syphilis assays.

Methods: We selected 557 "high risk" (from STD Clinics) specimens from our routine syphilis submissions which were non-reactive in our standard non-treponemal test [Rapid Plasma Reagin (RPR)] and re-tested them using a new automatable EIA. The EIA (Trep-Chek™ - Phoenix Bio-Tech Corp) utilizes recombinant antigens from *Treponema pallidum*. All specimens were also tested using a microhemagglutination assay for *T. pallidum* (MHA-Tp) and EIA positives were confirmed using a *T. pallidum* specific Western blot (WB). Sensitivity of the new assay was checked against 15 patients with proven early primary syphilis (direct fluorescence confirmed, dark-field positive smears from primary lesions) and a panel of 16 treated, confirmed syphilis patients. A panel of 119 "biological false positive" specimens (RPR reactive but MHA-Tp non-reactive) was also tested.

Results: The panel of 16 treated, confirmed patients were all positive by EIA. Of the 15 early primary syphilis cases 14 were positive by EIA. Of the 119 BFP specimens 108 were negative by EIA but 10 of the remaining 11 were either positive or equivocal in the WB (thus not true BFP's). Thus the initial sensitivity and specificity for the new EIA was 97% and 99% respectively. In the "high risk" population there were 16 positive and 11 indeterminate EIA cases (4.85% of those tested). Of these only 9 were MHA-Tp reactive. However fully 24 of these 27 EIA positives were either positive or equivocal in the WB (our Gold Standard assay). Most importantly 20 of these 24 "confirmed" positive syphilis patients were not previously identified in our 20 yr database thus potentially new cases.

Conclusions: Standard non-treponemal based assays are clearly under sensitive (as apparently are some treponemal assays like the MHA-Tp) potentially allowing some syphilis cases to go undetected and untreated.

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INTRODUCTION

Syphilis is a disease that if left untreated can lead to devastating consequences for the individual. Sequellae could include neuro-syphilis, as well as cardiovascular and gummatous syphilis. While the latter two are relatively rare in North American populations, neurologic manifestations of tertiary syphilis is not. Studies have also demonstrated that infectious lesions can occur in both early and late latent phases of the infection thus potentially allowing for transmission to new individuals. Furthermore women with untreated latent syphilis can sometimes pass on the disease to their unborn children.

The Centers for Disease Control and Prevention (CDC) announced in late 1999 that they had the goal of eliminating syphilis from the United States within five years (2005). Elimination was defined as the absence of sustained transmission and would be reflected in having a national rate of primary and secondary infections of 0.4 cases per 100,000 population. Health Canada has stated that their national goal is less than 0.5 cases of "infectious syphilis" per 100,000 population. Given the fact that *Treponema pallidum*, the causative organism for syphilis, has not developed resistance to penicillin, an inexpensive and readily available drug, the stated national goals of both the United States and Canada are likely achievable.

However the ability to detect and treat syphilis in individuals is in large part dependant on laboratory tests. Yet many published studies have indicated that the sensitivity of non-treponemal screen tests decreases significantly with the stage of the disease. We asked how many otherwise undetected cases of syphilis (any stage) might be detected using a treponemal-based screening assay.

METHODS

Specimens: Five hundred and fifty-seven specimens submitted to our Public Health Laboratory for syphilis testing were selected as being "high-risk" based on their originating from clinics specialized for sexually transmitted diseases. These specimens were all non-reactive by our standard screen test, the rapid plasma reagin (RPR) test. One hundred specimens used to establish sensitivity of the EIA were selected based on being reactive in the RPR screen test as well as being reactive in our standard confirmatory test the microhemagglutination assay for antibodies to *T. pallidum* (MHA-Tp). We also used fifteen patients with early primary syphilis based on direct fluorescence confirmed, dark-field positive smears from primary lesions. A panel of 119 "biological false positive" specimens (RPR reactive but MHA-Tp non-reactive) was used in a preliminary evaluation of specificity of the EIA.

Serologic Tests: Our standard screen test is the rapid plasma reagin (RPR) 18 mm circle card test. Standard confirmatory testing employs the microhemagglutination assay for antibodies to *T. pallidum* (MHA-Tp). The serologic gold standard in our laboratory is the Western Blot assay where a positive IgG consists of reactions at 15.5, 17 and 47 kilodaltons. Positive IgM blots have bands at any two of these three molecular weights. Equivocal Western Blot results for either IgG or IgM have reactivity at any one of these significant bands. The EIA (Trep- Chek™ - Phoenix Bio-Tech Corp*) utilizes recombinant antigens from *T. pallidum*. This assay makes use of internal positive and negative controls as well as a cut-off control which we ran in triplicate on each plate. Positive specimens were those whose calculated ratio (OD of specimen divided by mean OD of cut-off control multiplied by the cut-off factor) were greater than 1.1. A grey-zone (indeterminate) was reserved for specimens with calculated ratios of 0.9-1.1 while negative specimens had ratios less than 0.9.

* Bob Notenboon developed this test, and provided the test to Neal & Margaret at the OPHL on Resources Rd. It has a "K" number.

RESULTS

All one hundred confirmed reactive specimens, by standard testing, were also positive in the new treponemal EIA. Of the 15 early primary cases of syphilis (confirmed dark field positive) 14 were positive by EIA. All were also positive by IgG Western blot (WB). Preliminary sensitivity of this new treponemal EIA was calculated to be 99%.

Utilizing 119 specimens sent in for syphilis testing and which tested positive in the RPR screen test but unreactive in the MHA-Tp confirmatory test we determined 108 to be negative by EIA. All of the remaining eleven, which were either positive or equivocal by EIA, were further examined by Western Blot. Five were strongly positive on IgG WB with positive reactions not only with the three "significant" bands but also with numerous other proteins extracted from whole *T. pallidum*. Five other sera were classified as "equivocal" by WB with two specimens having two of the three significant bands on IgG blots (and were also reactive with numerous other bands). The other three sera had only one significant band on the IgM blot, two of which also had "extra" reactions with other bands (see Table 1). One specimen was equivocal by EIA but non-reactive by all other treponemal tests albeit the specimen did react with three non-significant bands on Igm WB. Taking all sera that were either positive or equivocal by WB as being syphilitic then the specificity of the new EIA is calculated to be 99%.

Testing 557 "high risk" patients, who were non-reactive in our standard RPR screen test, using the EIA resulted in 16 clear positiveand 11 equivocal specimens (4.85% of those tested). All of these were subsequently tested using MHA-Tp and both IgG and IgM WB for *T. pallidum*. Results are shown in Table 2.

Analysis revealed ten IgG WB positives, each of which had multiple extra bands as well. Only nine of these were also reactive by MHA-Tp. There were a further 11 IgG WB equivocal results, seven of which had two significant bands and all of which had additional reactions with other *T. pallidum* proteins. None of these were reactive by MHA-Tp. The remaining six specimens were negative on the three significant antigens by IgG but four had reactions with other proteins extracted from *T. pallidum*. Within these six IgG negative specimens, three were IgM equivocal each with one significant band and multiple (four or more) other reactions with other *T. pallidum* proteins (see Table 2).

DISCUSSION

Sensitivity of this new Trep-Chek™ EIA only looked at fifteen early primary and 100 routine, unclassified specimens, but appears to be excellent at 99%. However further work is necessary to establish sensitivity in defined stages of infection, including more primary, secondary, early latent, late latent, neuro-syphilis, and congenital infections. Specificity of this new assay was only examined with specimens that would normally be classified as "biological false positives." Using these 119 highly selected specimens and using the Western blot as the "Gold Standard" the test had a calculated specificity of 99%. However as stated above significant more work is required to establish a more broadly based specificity rating including the use of sera from infections that may have cross reactive antigens with *T. pallidum* such as *Borrelia burgdorferi* (Lyme disease).

Examining the 557 "high risk" patients the new EIA was reactive with 27 specimens. This represents 4.85% of those tested. Of these fully 24 were either positive or equivocal by Western blot. Not all of these are necessarily from syphilitic patients, especially some of the Western blot IgM equivocal specimens. However, even a very conservative approach to these 24 specimens would recommend either further testing or consultation with the primary care giver, none of which would occur if these specimens were tested using conventional means.

Utilizing our twenty year database for T. pallidum testing we could only identify four of these 24 specimens as being previously identified as syphilis patients. This suggests that those patients detected by this new EIA that are true syphilitic patients may be new syphilis patients and possibly requiring treatment.

An unexpected finding from our study revealed at least six sera which were indisputably syphilitic (strongly positive on IgG Western blot) but which were non-reactive by MHA-Tp. This suggests that new work may be warranted in determining the sensitivity of this well established treponemal-based assay (the MHA-Tp).

In conclusion we find that treponemal-based screening may be essential if we are to achieve our national goal of eliminating syphilis.

Table 1. Western Blot Results on "Biological False Positive" Specimens

Specimen #	Ratio	TrepChek™ Result	RPR	MHA-Tp	IgG Western Blot					IgM Western Blot				
					15.5	17	47	Extra	Result	15.5	17	47	Extra	Result
308543	1.186	Pos	1:16	NR	+	+	+	5	Pos	-	-	+	1	Equiv
29254	1.053	Equiv	1:4	NR	-	-	-	0	Neg	-	-	-	3	Neg
35163	1.091	Equiv	1:2	NR	-	-	-	0	Neg	-	-	+	0	Equiv
54817	1.147	Pos	1:8	NR	-	-	-	0	Neg	-	-	+	1	Equiv
22499	1.279	Pos	1:4	NR	+	+	+	3	Pos	-	-	+	2	Equiv
49777	1.310	Pos	1:8	NR	-	-	-	1	Neg	-	-	+	4	Equiv
75449	1.712	Pos	1:4	NR	-	+	+	5	Equiv	-	-	+	3	Equiv
158854	2.458	Pos	1:4	NR	+	+	+	6	Pos	-	-	+	2	Equiv
169277	2.602	Pos	1:2	NR	-	+	+	5	Equiv	-	-	+	2	Equiv
159965	2.708	Pos	1:4	NR	+	+	+	4	Pos	-	-	-	1	Neg
182668	3.448	Pos	1:4	NR	+	+	+	4	Pos	+	+	+	4	Pos

Table 2. Western Blot Results on Trep-Chek™ Reactive Specimens

Specimen #	Ratio	TrepChek™ Result	RPR	MHA-Tp	IgG Western Blot					IgM Western Blot				
					15.5	17	47	Extra	Result	15.5	17	47	Extra	Result
327528	2.607	Pos	NR	R	Previous Western Blot IgG Positive; Not Re-Run									
33227[redacted]	3.100	Pos	NR	NR	-	-	-	0	Neg	-	-	-	0	Neg
333281	2.505	Pos	NR	R	+	+	+	7	Pos	-	-	-	0	Neg
335308	1.159	Pos	NR	NR	+	-	-	2	Equiv	-	-	-	0	Neg
336230	1.215	Pos	NR	NR	-	-	-	1	Neg	-	-	-	0	Neg
336238	2.102	Pos	NR	R	Previous Western Blot IgG Positive; Not Re-Run									
336240	1.031	Equiv	NR	NR	-	-	-	0	Neg	-	-	-	0	Neg
337557	1.385	Pos	NR	NR	-	-	+	2	Equiv	-	-	-	0	Neg
338247	1.969	Pos	NR	R	+	+	+	5	Pos	-	-	+	2	Equiv
338264	1.104	Pos	NR	NR	-	-	-	1	Neg	-	-	+	4	Equiv
338982	4.084	Pos	NR	R	+	+	+	7	Pos	-	-	+	2	Equiv
339080	3.371	Pos	NR	R	+	+	+	9	Pos	-	-	-	4	Neg
345085	0.947	Equiv	NR	NR	-	+	+	1	Equiv	-	-	-	4	Neg
345309	0.990	Equiv	NR	NR	+	-	+	2	Equiv	-	-	+	1	Equiv
347909	1.218	Pos	NR	NR	+	-	+	1	Equiv	-	-	+	2	Equiv
349012	0.901	Equiv	NR	NR	+	-	+	5	Equiv	-	-	+	4	Equiv
351028	0.965	Equiv	NR	NR	-	-	-	2	Neg	-	-	+	5	Equiv
351030	0.961	Equiv	NR	NR	-	-	+	2	Equiv	-	-	+	3	Equiv
353245	1.034	Equiv	NR	NR	+	-	+	3	Equiv	-	-	+	5	Equiv
353248	1.234	Pos	NR	R	+	+	+	6	Pos	-	-	+	4	Equiv
353792	0.984	Equiv	NR	NR	+	-	+	1	Equiv	-	-	+	4	Equiv
353798	4.373	Pos	NR	R	+	+	+	10	Pos	-	-	+	5	Equiv
355854	1.050	Equiv	NR	NR	-	-	+	2	Equiv	-	-	+	3	Equiv
357187	0.904	Equiv	NR	NR	-	-	-	4	Neg	-	-	+	4	Equiv
357774	5.570	Pos	NR	R	Previous Western Blot IgG Positive; Not Re-Run									
357787	2.376	Pos	NR	NR	+	+	+	3	Pos	-	-	-	2	Neg
358327	0.924	Equiv	NR	NR	+	-	+	6	Equiv	-	-	+	4	Equiv