Global Journal of Medicine & Public Health

Validation of a new testing algorithm for syphilis in a small developing country

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GJMEDPH 2019; Vol. 8, issue 6

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Conflict of Interest—none

Funding—none

ABSTRACT

Objective

To evaluate new test kits and a reverse algorithm for the diagnosis of syphilis at the Queen's Park Counselling Centre and Clinic (QPCC&C), the national reference laboratory in Trinidad and Tobago, to determine feasibility for implementation.

Methods

The diagnostic testing for syphilis in Trinidad and Tobago involved the use of the traditional algorithm. The QPCC&C in collaboration with the Centers for Disease Control and Prevention STI & International Laboratory Branches (CDC-STI), Atlanta, developed a reverse diagnostic syphilis algorithm that allows specimens to be screened with a treponemal test (ELISA) and non-reactive samples are reported as negative for syphilis. ELISA reactive specimens were reflexively screened with the quantitative VDRL test to detect present or past treated exposure to syphilis. Samples with discordant test results were subsequently screened using the Treponema Pallidum Particle Agglutination (TPPA) as a confirmatory test. To validate the algorithm, 5mls of blood from 40 high risk HIV and STI patients in Trinidad were obtained, the sera separated and split equally to allow for the simultaneous testing and review of results using the reverse testing algorithm at both the QPCC&C and CDC STI laboratories. Ethical approval was granted for this process by the Ministry of Health, Trinidad and Tobago.

Results

The results obtained showed 100% concordance between laboratories which resulted in the validation of the algorithm.

Conclusion

The validation of these test kits and reverse algorithm will allow the national STI reference laboratory to increase its throughput and offer an improved service to the population.

Keywords: Validation, Algorithm, Diagnosis, Syphilis, Trinidad and Tobago

INTRODUCTION

Syphilis and its complications is one of the oldest and best-characterized sexually transmitted infections in Western medicine, with reference to this disease dating to the late 15th century.[1] Generations of medical students have been taught the maxim attributed to Sir William Osler: "He who knows syphilis, knows medicine". Despite this, there is at yet no consensus regarding the optimal method of laboratory diagnosis of this disease, with a least three algorithms in current use.[2]

For many years, the mainstay of diagnosis was the traditional algorithm, consisting of two tests that

were first developed over half a century ago: an initial screening test with a nontreponemal assay [Rapid Plasma Regain (RPR) or Venereal Disease Research Laboratory (VDRL) assay], with reactive samples undergoing confirmation with a treponemal-specific assay [e.g. Treponema Pallidum Particle Agglutination (TPPA) assay.[3] This approach, however, has known disadvantages, including the propensity for false-positive and false negative results caused by variety of conditions, the necessity for the assays to be performed manually, and subjectivity in the interpretation of results.[4] Recent evidence has also suggested it would fail to diagnose syphilis in a significant proportion of patients living with HIV.[5]

Newer diagnostic methods have been developed, centered around modern and automated *T. pallidum*-specific immune-based techniques (e.g. enzyme immunoassay, EIA) as the initial screening test, followed by confirmatory nontreponemal testing. This approach is called the reverse-sequence algorithm and confers the advantage of greater potential throughput due to automation, and greater sensitivity.[6] The potential drawback of this technique has been the greater probability of false-positive results in populations with a low-prevalence of syphilis when compared to the traditional algorithm[7,8] but this effect is mitigated with increasing prevalence of disease.[9]

In most of the Americas, the laboratory diagnosis of syphilis is still based on the traditional algorithm, with 77% of responding laboratories across 30 countries in the region indicating such in a 2014 survey conducted by the Pan American Health Organisation.[10] Trinidad and Tobago is no exception. The reference laboratory for syphilis testing in the public sector is housed at the Queen's Park Counselling Centre and Clinic (QPCC&C), which is a network of 11 clinics, with two main sites in the north and south of the island, and 9 satellite clinics across both islands. The clinic functions as a referral centre for the diagnosis and management of sexually transmitted infections, and as such, the population it serves would be expected to have rates of syphilis higher than the general population.

Given the increasing rates of syphilis among some key populations living with HIV,[4] the global initiative for the elimination of mother to child transmission of HIV and syphilis[11] and the need to offer access to updated, accurate diagnostic services for syphilis, a validation exercise using three kits new to Trinidad and Tobago (Table 1) was performed in collaboration with a reference laboratory [the International Laboratory Branch (ILB), Centers for Disease Control and Prevention (CDC), Atlanta. The kits were selected based on their availability in the country and their stated sensitivity and specificity, and would comprise the proposed reverse sequence algorithm in Trinidad and Tobago.

Table 1 Description of Kits

Name of kit	What the kit detects	Type of sample to be used	Sensitivity	Specificity	Collection medium	Objective of test
BD VDRL Antigen with Buffered Saline	Reagin antibodies	Serum, CSF	93.3% – 95.2%	92.7% - 98.9%	Red top tubes	Confirmation, monitoring of clinical response
Bio-Rad Syphilis EIA II	Treponema pallidum antibodies	Serum, plasma	100%	99.4%	Red top, sodium citrate, heparin or EDTA tubes	Screening
Serodia TPPA	Treponema pallidum antibodies	Serum, plasma	90%	100%	Red top, sodium citrate, heparin or EDTA tubes	Confirmation

METHOD AND MATERIALS

Ethical approval for conducting the study was obtained from the Ethics Committee of the Ministry of Health of the Government of Trinidad and Tobago. The three kits selected for the proposed algorithm were the Bio-Rad Syphilis EIA II test kit (Bio-Rad Laboratories, Hercules, California, USA), the BD VDRL Antigen test kit (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and the Serodia TPPA test kit (Fujireibo Inc., Tokyo, Japan.) During the period February 2012 – July 2012, 122 samples were collected from randomly selected individuals presenting to the QPCC&C, and the Medical Research Foundation of Trinidad and Tobago, which is the largest treatment site for persons living with HIV in the country. All samples were anonymized, and no clinical information was forwarded to the personnel performing the assays.

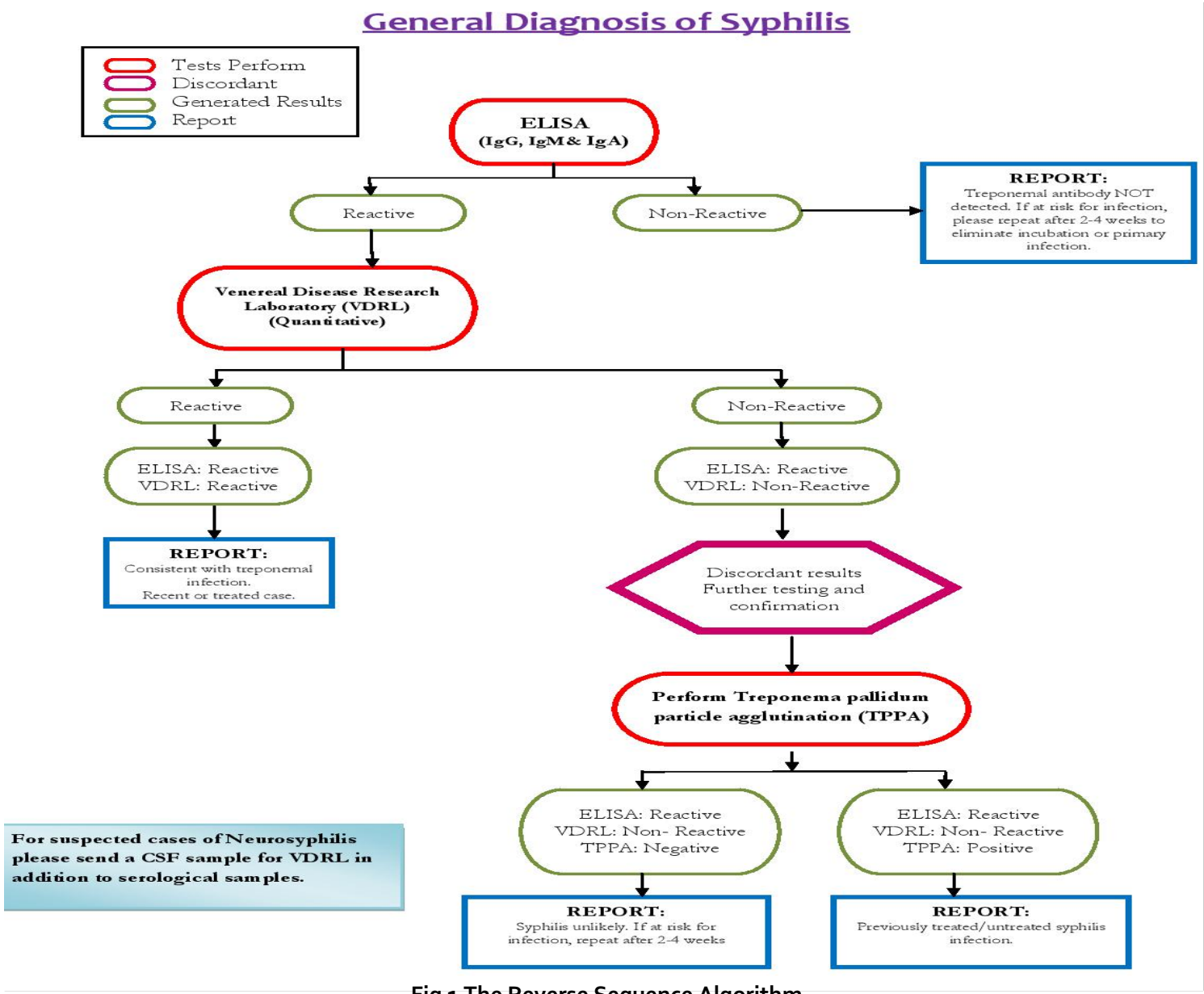

Fig 1 The Reverse Sequence Algorithm

The reverse sequence algorithm was applied to all 122 samples, consisting of an initial screening enzyme-linked immunoassay (EIA) for antibodies against *T. pallidum* using the Bio-Rad Syphilis EIA II Test Kit. EIA-reactive samples had confirmatory qualitative and quantitative testing with the BD VDRL Antigen test, and samples with discordant results EIA/VDRL results were further tested with the Serodia TPPA assay (Figure 1).

At the request of the CDC, forty (40) of those samples were sent to the ILB, consisting of 11 samples testing positive by EIA and either VDRL or TPPA, and 29 samples testing negative by EIA. The samples in both groups were selected randomly. They were re-tested employing a similar algorithm, using the kits in use at the CDC: the Trep-Sure EIA, Rapid Plasma Reagin (RPR) and TPPA assays. At the ILB, TPPA assays were performed on all EIA-positive samples, regardless of the VDRL/RPR result.

RESULTS

There was 100% agreement in the final diagnostic outcomes of the reverse sequence algorithm at the QPCC&C Laboratory as compared to the International Laboratory Branch, CDC. All 40 samples had similar screening EIA, qualitative RPR/VDRL, and where indicated, TPPA results. Additionally, the difference between quantitative RPR titres (CDC) and the corresponding quantitative VDRL titres (QPCC&C) was within 1 dilution (i.e. a twofold change) except for one sample, where an eight-fold difference in titre was observed (Table 2). Of 11 EIA-positive samples, five had negative VDRL results but a positive TPPA test, indicating either previous, treated or latent syphilis.

Table 2 Comparison of Results of EIA-Positive Samples

	International Laboratory Branch, CDC, Atlanta				QPCC&C Lab, Port-of-Spain, Trinidad			
Specimen#	Trep-Sure EIA	RPR	RPR titre (dils)	TPPA	Bio-Rad EIA	VDRL	VDRL titre (dils)	Serodia TPPA
1	+	R	1	+	+	WR		
2	+	R	32	+	+	R	64	
3	+	NR		+	+	NR	-	+
4	+	R	32	+	+	R	64	
5	+	NR		+	+	NR		+
6	+	R	32	+	+	R	256	
7	+	NR		+	+	NR		+
8	+	R	8	+	+	R	8	
9	+	NR		+	+	NR		+
10	+	NR		+	+	NR		+
11	+	R	1	+	+	WR		

Legend: R = Reactive, WR = Weakly reactive, NR = Non-reactive

DISCUSSION

Based on the perfect correlation between the QPCC&C laboratory and the ILB, the exercise succeeded in demonstrating the selected kits were able to give accurate results on patients from Trinidad and Tobago, and under local conditions. The exercise also was successful in illustrating one of the major advantages of the reverse sequence algorithm over the traditional algorithm, in that 5 of the 11 samples testing positive using the reverse sequence algorithm would have been missed using the traditional algorithm, as their VDRL result (the initial screening step in that algorithm) was negative, and as such, depending on the clinical scenario, the patient from whom those samples were collected may not have been recalled for further assessment. Additionally, it was also noted that there were no false positive results (i.e. a positive screening EIA but negative VDRL and TPPA), which is a potential limitation of the reverse sequence algorithm, as stated previously. These findings, though preliminary, support the

introduction of these kits as part of a revised algorithm for the laboratory diagnosis of syphilis in Trinidad and Tobago.

Introducing the reverse algorithm as the preferred method for syphilis diagnosis would offer greater sensitivity when compared to the traditional algorithm, and the use of automated EIA techniques as the initial screening test will result in decreased turnaround time, especially in settings with a high turnover of samples. Given the potential for fewer missed or late diagnoses, there are also direct implications of this on transmission of syphilis and HIV, the safety of the blood supply,[12] pre-transplant donor screening[13] and the elimination of mother-to-child transmission of HIV and syphilis. It may also have the effect of reducing labour costs,[14] and potentially reduce occupational hazard to laboratory staff, as the burden of manually processing large numbers of samples, as is necessary with the traditional algorithm, would be significantly reduced.

Two studies evaluated the sexually transmitted infection (STI) patterns among persons living with HIV (PLHIV) attending the STI Clinic in Trinidad[15, 16]. In both studies,[15, 16] there was a high prevalence of STIs among PLHIV and the most common STI was syphilis. In addition, there was a high prevalence of STIs among men who have sex with men (MSM) and the most commonly diagnosed STI in this group of patients was syphilis.[16] Both studies concluded that more frequent STI screening and targeted STI/HIV prevention interventions were urgently required among PLHIV in Trinidad.[15,16] The findings of an increased prevalence of syphilis among MSN in Trinidad are similar to the trends of syphilis in MSM in Western Europe and the United States,[17] hence the introduction of the reverse testing algorithm for syphilis is timely given its ability to accurately detect more patients with early or latent syphilis as compared to the traditional algorithm.[18]

The results of this exercise, however encouraging, are limited by the relatively small sample size and the lack of a direct comparison between the performance of the traditional and reverse sequence algorithms in the Trinidad and Tobago population. However, the previously cited reports are consistent in demonstrating higher sensitivity of the reverse sequence algorithm.[2, 4-9] Secondly, this exercise did not evaluate the issue of the eventual cost effectiveness of revising the approach to syphilis diagnosis, a consideration while outside the scope of this paper, is nonetheless important in resource-constrained settings and merits future consideration. A broader evaluation of the results from the complete 122-sample set, with modelling based on known patterns of testing and treatment, may go some distance in this regard.

In order to effect any future transition to a new screening algorithm, leadership from Ministry of Health will be necessary in order to implement the changes to procurement, laboratory infrastructure, and technician training. Furthermore, medical staff will need to be sensitized on the interpretation of the new algorithm, in order to avoid anxiety from both patients and caregivers regarding the interpretation of results, and to prevent overtreatment.

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