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Comparison of three syphilis algorithms in West China

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ABSTRACT

Objective: The study was designed to analyze three different algorithms with the implementation of treponemal tests for detecting suspected syphilis in west China where no syphilis algorithms guideline exists.
Methods: We retrospectively collected data to reanalyze three syphilis testing algorithms: the classical reverse algorithm, and two options of syphilis screening in European syphilis guideline. The kappa (κ) coefficients were used to compare the concordance between algorithms using different syphilis assays during two periods. A receiver operating characteristic curve was used to determine the optimal S/CO ratios of InTec EIA or Lumipulse® G TP-N assay (CLIA) to predict confirmatory TPPA results.
Results: The agreements between the reverse algorithm and the EU option 1 algorithms were perfect irrespective of EIA or CLIA used (all $\kappa > 0.9$). But the agreements between the EU option 2 algorithms and reverse or EU option 1 were not good ($\kappa < 0.4$). > 50% cases confirmed syphilis infections by reverse or EU option 1 algorithm were missed by EU option 2. There is no very need for a confirmatory TPPA assay in EU Option 1 when S/CO is above 6.12 for CLIA (3.67 for EIA). The false-positive rate was 0.26% above this cutoff level.
Conclusions: EU Option 1, involving a reactive TT, followed by another TT of a different type and a quantitative NTT if second TT is positive, which is the same as the 2015 UK syphilis algorithm, is recommended. We also propose that when S/CO of the CLIA or EIA is high enough, TPPA confirmation can be omitted from the testing algorithm, and costs would be significantly reduced.

1. Introduction

Syphilis, a sexually transmitted disease (STD) caused by *Treponema pallidum* subspecies *pallidum (TP)*, is a health problem of increasing incidence in recent years. Generally, TP is revealed serologically in the framework of the diagnostic algorithm comprised of treponemal immunoassays (TTs) and non-treponemal tests (NTTs). In 2014, European (EU) guideline provide three options for serodiagnosis of syphilis infection [1]. EU Option 1 involves a reactive TT, followed by another TT of a different type and a quantitative NTT if second TT is positive, which is the same recommendation as 2015 UK syphilis guideline [2]. Obviously, EU option 1 is different from the classical reverse algorithm, the procedure of which involves a reactive TT followed by a reactive NTT and confirmed by another TT. A quantitative NTT is followed by confirmation using a TT, which is also called the traditional algorithm (EU option 2). Both a TT and a NTT are used as an initial screening at the same time (EU option 3), which is only wise in case of suspicion of very early syphilis and not talked about here. Despite that there is a 1.0% false-reactive rate, the reverse syphilis algorithm detected 21 patients with possible latent syphilis that may have gone undetected by the traditional algorithm [3]. Katz, A. R et al. reported a RPR (Rapid plasma reagin) positive case report of a false-negative syphilis treponemal enzyme immunoassay test result in an HIV-infected male [4]. The traditional algorithm had a missed serodiagnosis rate of 30.0% in high-prevalence populations in Slovenia [5]. The identified syphilis rate and overall false-positive rate of the traditional algorithm were lower than those of the reverse algorithm [6]. A survey showed that the majority of laboratories still perform the traditional algorithm, but a significant minority has implemented the reverse algorithm [7].Although treponemal-first algorithms identify a higher percentage of screen-reactive patients than the traditional algorithm, it presents a unique challenge-significantly higher number of follow-ups and over-treatment, particularly when applied to low-prevalence populations [8]. Hence, caution is needed before shifting from one algorithm to another algorithm.

The prevalence of syphilis in China vary greatly from 0.65% in urban citizens [9], to 10.9% in men sex with men [10].The diagnosis of syphilis may cause greater individual and social stress for a patient

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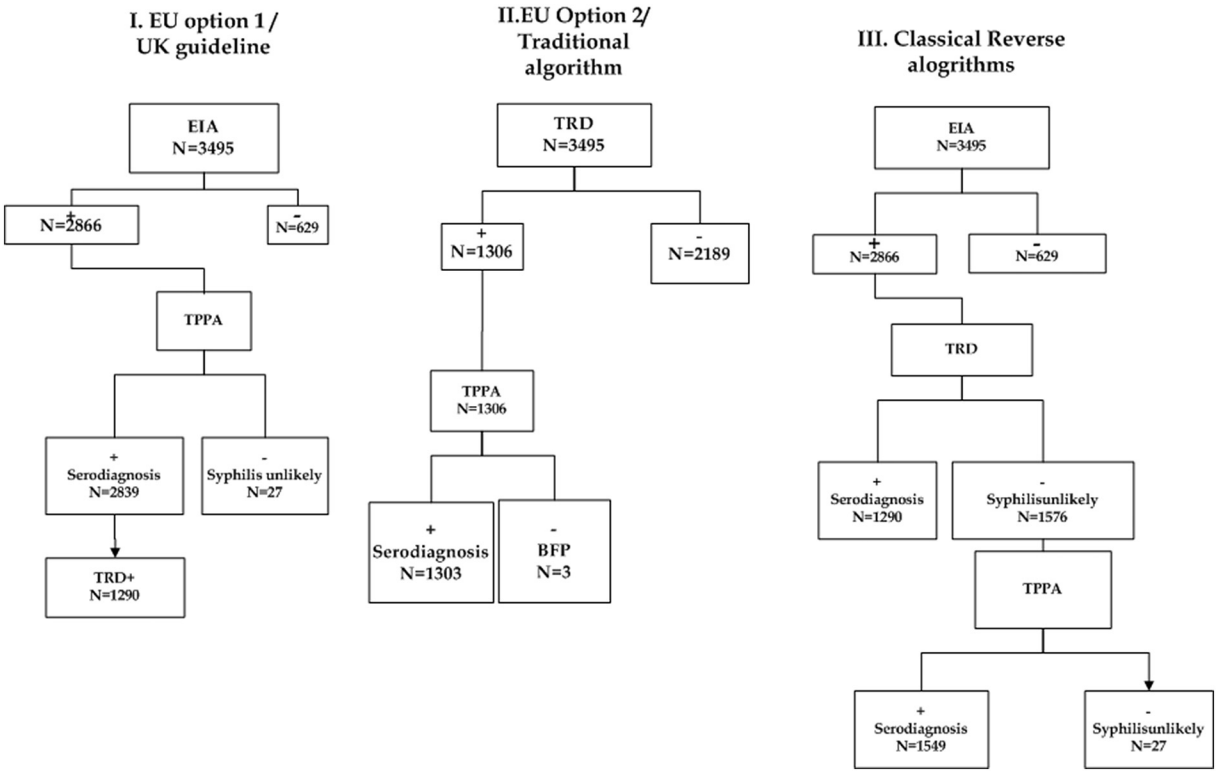

Fig. 1. Syphilis testing algorithms using different assay order during 2012–1014.
+, positive; −, negative.
Algorithm 2 in EU option 1 is exactly the same as UK guidelines.
Abbreviations: BFP, biological false positive; CLIA, chemiluminescence immunoassay; EIA, enzyme immunoassay; TRD, the toluidine red unheated serum test quantification; TPPA, Treponema pallidum particle agglutination
K values:EU option 1 vs EU option 2 = 0.229; EU option 1 vs reverse algorithm = 1.0; EU option 2vs reverse algorithm = 0.229; All *P* values were < 0.05.

because of the country's reserved sexual culture. Therefore, the clinical diagnosis performance is of great importance for syphilis screening. Based on the Dagnosis of Syphilis of China (WS 273-2007, http://www.nhfpc.gov.cn/zhuz/s9491/201410/a72a7387653842b09c0c0f10fdfdf9b7.shtml), syphilis was clinically diagnosed by combining serodiagnosis and personal history (including clinical characteristics and/or the patient's sexual history. Up to 1000 patients before operation and/or transfusion blood products are compulsively screened for anti-TP (TP antibodies) every workday in our hospital. Syphilis screening can begin with InTec enzyme immunoassay (EIA) (Intec, Xiamen, China), the toluidine red unheated serum test (TRUST) (Rongsheng, Shanghai, China), or the combination of *T. pallidum* particle agglutination assay (TPPA) (Fujirebio, Tokyo, Japan) and TRUST according to clinical requests as described in our previous report [11]. A chemiluminescence assay (CLIA), Lumipulse G TP-N Syphilis (Fujirebio, Tokyo, Japan), is used as the screening assay instead of the Intec EIA assay after 2014. The goal of this study was to compare commercially available total antibody treponemal assays: a conventional TPPA test with an automated CLIA assay or EIA assay. The study was designed to analyze three different algorithms (EU option 1, EU option 2 and classical reverse algorithms) with the implementation of treponemal tests for detecting suspected syphilis in west China.

2. Materials and methods

We retrospectively collected testing data without personal identifiers from serum or plasma specimens from West China Hospital from April 2012 to June 2014. During this period, 3495 samples, which were performed using TRUST, TPPA and InTec EIA simultaneously, were enrolled, after duplicate tests were excluded. Another 4210 samples from July 2014 to June 2016, which were measured using TRUST, TPPA and Lumipulse CLIA simultaneously, were enrolled, after duplications were excluded. All those data were reanalyzed according to the EU option 1(UK algorithms), the EU option 2(traditional algorithms) and the classical reverse algorithms (Fig. 1&Fig. 2).

The percentages of agreement and κ coefficients were calculated to determine the agreements among algorithms. The agreement of the results according to their κ values was categorized according to the Tong's report [12]. A receiver operating characteristic curve (ROC) analysis was used to study the relationship between the S/CO ratios of the reactive samples and the TPPA confirmatory test results. The diagnostic sensitivity, diagnostic specificity, positive predictive value (PPV), and optimal cutoff point of the S/CO ratio were identified from analyses of the ROC curves and associated data. The statistical analysis was performed using SPSS software, version 17 for Windows.

3. Results

3.1. Result of TTs and NTTs

The top three result patterns of three assays-EIA/TPPA/TRUST were TRUST + /TPPA + /EIA + (36.9%),TRUST-/TPPA + /EIA + (44.3%),and TRUST-/TPPA-/EIA-(17.4%). These three similar patterns were also common seen in CLIA/TPPA/TRUST results. Overall, 6(0.1%) TRUST-/TPPA + /CLIA- cases and 6(0.2%) TRUST-/TPPA + /EIA- subjects without the presence of the prozone phenomenon of TRUST may be considered to acute or early infection, which also needs further confirmation. These 27(0.8%) TRUST-/EIA + /TPPA- cases indicated the high sensitivity of EIA assay. In total, 220 (5.2%) TRUST-/CLIA + /TPPA- cases suggested high false reaction of CLIA. TPPA + /CLIA- and TPPA + /EIA- suggested the false negative of EIA (0.39%) and CLIA (0.67%). Obviously, discordant results between TPPA and EIA (or CLIA)

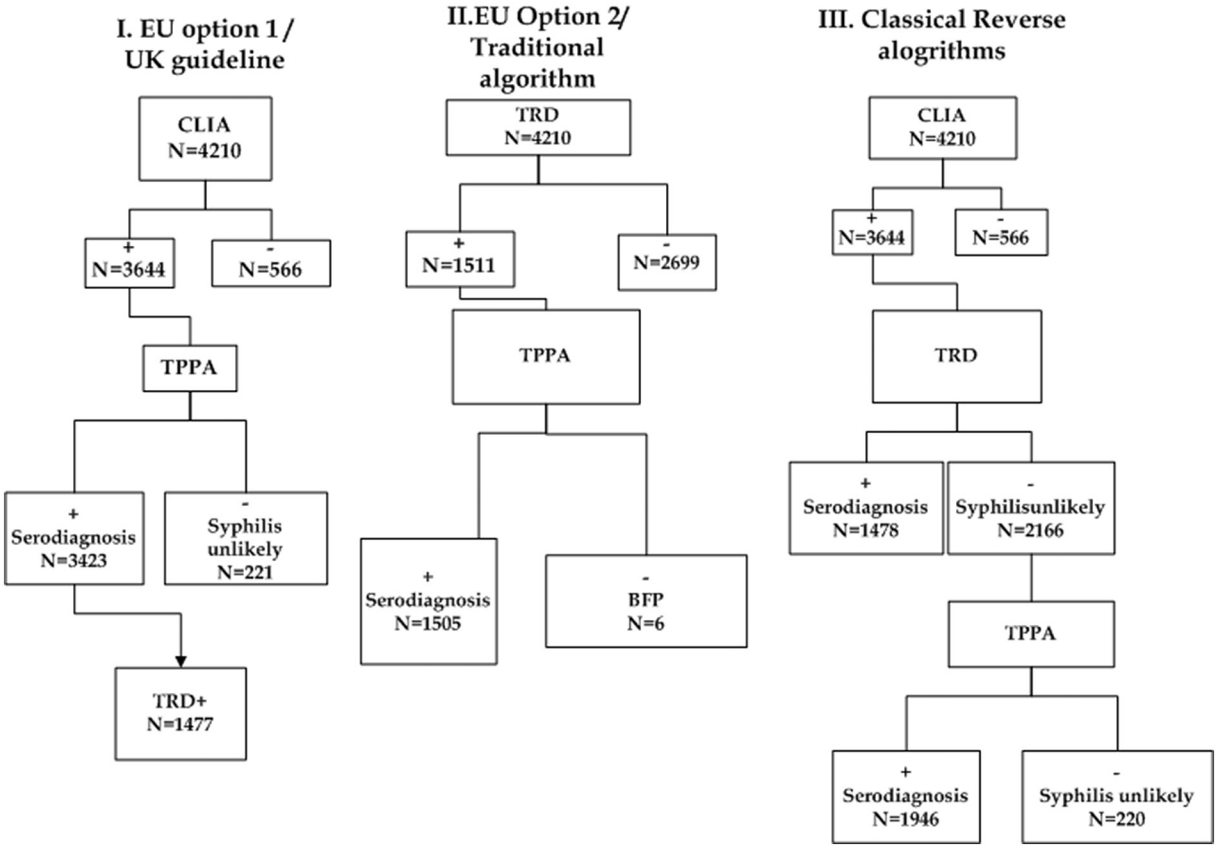

Fig. 2. Syphilis testing algorithms using different assay order during 2014–2016.
K values:EU option 1 vs EU option 2 = 0.204; EU option 1 vs reverse algorithm = 0.999; EU option 2vs reverse algorithm = 0.204; All *P* values were < 0.05.
+, positive; −, negative.
Abbreviations: BFP, biological false positive; CLIA, chemiluminescence immunoassay; EIA, enzyme immunoassay; TRD, the toluidine red unheated serum test quantification; TPPA, Treponema pallidum particle agglutination

Table 1
Results of NTTs and TTs during two periods.

Period 1(2012–2014)				Period 2(2014–2016)			
EIA	TPPA	TRUST	n (%)	CLIA	TPPA	TRUST	n (%)
−	−	+	3(0.08%)	−	−	+	5(0.1%)
−	+	+	13(0.4%)	−	+	+	28(0.7%)
−	−	−	607(17.4%)	−	−	−	527(12.5%)
+	−	−	27(0.8%)	+	−	−	220(5.2%)
−	+	−	6(0.2%)	−	+	−	6(0.1%)
+	+	−	1549(44.3%)	+	+	−	1946(46.2%)
+	+	+	1290(36.9%)	+	+	+	1477(35.1%)
+	−	+	0(0%)	+	−	+	1(0.02%)

Note: All results of assays combination during two stages were displayed.
+, positive; −, negative.

were common. More detailed information listed in Table 1.

3.2. Comparison of the agreement of three serodiagnosis algorithms

The perfect agreements were found between the reverse algorithm and the EU option 1 algorithms irrespective of EIA or CLIA used (all $\kappa > 0.9$). The fair agreements between the EU option 2 algorithms and reverse or EU option 1 were found ($\kappa < 0.4$). > 50% cases confirmed syphilis infections by reverse or EU option 1 algorithm were missed by EU option 2.

3.3. Improved UK algorithm using S/CO ratios of Lumipulse G TP-N syphilis or InTec EIA assays

There is a strong trend that the number of cases with TP infection increased was in relation to the S/CO ratio (Table 2). According to ROC analysis, we set the specificity as 88.7% by setting artificial cutoff value as 3.54 for CLIA (Table 3). Accordingly, the PPV could increase to 99.2%. Likewise, we set the artificial cutoff value as 6.12 to set the specificity as 95.0% for the CLIA assay according to the ROC curves and PPV accordingly increased to 99.6%. The false-positive rate was 0.30% above this cutoff level, indicating that when the S/CO ratio is 6.12 or greater, there is not very need for a confirmatory TPPA. Similarly, for InTec EIA assay, when the S/CO ratio is 3.67 or greater; the false-positive rate was near zero (0.14%) above this cutoff level.

4. Discussion

The InTec ELISA test assay for TP available in our lab may be the bottleneck in shortening the turn-around time (TAT) for its batch test mode. With the heavier burden of the syphilis screening, there is a critical demand for simple, rapid, and high-throughput detection of syphilis. The Lumipulse G TP-N Syphilis assay, a new chemiluminescent enzyme immunoassay (CLIA) which can be fully automated, demonstrated the specificity of 99.37–100% [13], comparable to those of InTec assay for TP (99.3–100.0%) [11] and Serodia TPPA (95.3–100%) [14]. Chen et al. [15] proved directly Lumipulse G TP-N Syphilis has better specificity and comparable sensitivity than InTec assay. The TPPA test currently is considered to be the most suitable confirmatory treponemal test [16,17], although the FTA-ABS test has been

Table 2
TPPA results in relation to S/CO ratios of InTec EIA assay and Lumipulse® G TP-N assay.

InTec EIA	n	TPPA positive		Lumipulse G TP-N assay		TPPA positive	
S/CO		%	n	S/CO	n	%	n
< 1	629	3.02	19	< 1	566	6.01	34
1–3	281	92.52	260	1–2	335	56.41	189
3–4	112	97.3	109	2–3	202	82.67	167
4–6	208	100	208	3–5	256	89.45	229
6–8	206	99.02	204	5–10	348	98.28	342
8–10	224	100	224	10–50	1354	99.70	1350
10–20	1646	99.88	1644	50–100	601	99.50	598
≥20	189	100	189	≥100	548	100	548

Note: The number of cases with TP infection increased in relation to the S/CO ratios.

Table 3
Performances of two assays in reactive samples according to S/COs.

S/CO	False positive	Sensitivity	Specificity	Positive Predictive Value(PPV)
Lumipulse® G TP-N assay				
1	221	100% (99.9%, 100%)	0% (0.0%, 1.7%)	93.94% (93.1%, 94.7%)
3.54	25	87.7% (86.5%, 88.8%)	88.7% (83.8%, 92.5%)	99.2% (98.8%, 99.5%)
6.12	11	79.8% (78.4%, 81.2%)	95.0% (91.3%, 97.5%)	99.6% (99.3%, 99.8%)
InTec EIA assay				
1	27	100% (99.9%, 100.0%)	0% (0.0%, 12.8%)	99.1% (98.6%,99.4%)
3.67	4	88.1% (86.8%, 89.2%)	85.19% (66.27%, 95.81%)	99.84% (99.59%, 99.96%)
10.13	1	63.7% (61.9%, 65.5%)	96.3% (81.0%, 99.9%)	99.9% (99.7%, 100.0%)

Note: The PPVs approaching to 100% as S/CO of two assays increasing in reactive samples.

considered the gold standard treponemal test traditionally. Table 1 indicated that there was a high degree of consistency between the CLIA (or EIA) test and the TPPA test. Usually, except for biological false positive reactions, the TRUST test was positive, and then the TPPA test would certainly be positive. Totally, 9 subjects were TRUST + /TPPA-, 1 CLIA + , 3 EIA- and 5 CLIA-, which were also seen in Tong's report [12]. The discordant results between assays emphasize the importance of disease history including clinical characteristics and/or the patient's sexual history, as well as syphilis algorithms used.

The perfect agreements were found between the reverse algorithms and the EU option 1 algorithms irrespective of EIA or CLIA used (all $\kappa > 0.9$). Most importantly, the fair or poor agreements between the tradition algorithms and reverse or EU option 1 algorithms were found ($\kappa < 0.4$). These discordances were overestimated due to the inflated constituent ratio of syphilis infectors in this study. The prevalence of anti-TP-positive rate is about 2–3% in our hospital [11,13]. The CLIA assay has been adopted as an initial screening test for its automation, and TPPA is used as a confirmatory test [17]. Hence, considering the volume of samples for syphilis testing per day and labor costs, we recommend the EU option 1, which is also recommended by UK syphilis guideline [2]. We further demonstrated a strong correlation between the S/CO ratios of the Lumipulse® G TP-N assay (or InTec EIA assay) and TPPA positive results. Dai et al., proposed a cost-effective reverse screening algorithm based on S/CO ratios [18], obviating the need for

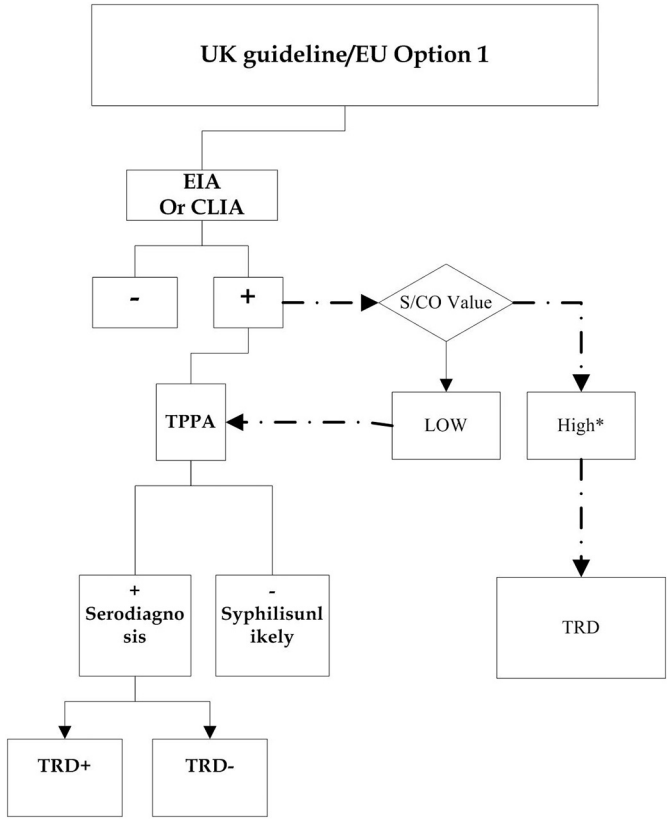

Fig. 3. The UK syphilis algorithm and improved UK algorithm.
*The definition of High S/CO of EIA or CLIA may be calculated where the lab adopt the improved version.
Abbreviations: CLIA, chemiluminescence immunoassay; EIA, enzyme immunoassay; TRD, the toluidine red unheated serum test quantification; TPPA, Treponema pallidum particle agglutination

the secondary treponemal testing in 65.2% of the screening-reactive samples. Here, we also modified EU option 1(UK algorithm) based on S/CO ratios to avoid some unnecessary TPPA confirmation. According to ROC analysis, for InTec EIA assay, the false-positive rate was near zero (0.14%) above this cutoff level, indicating that when the S/CO ratio is 3.67 or greater, there is no need for a confirmatory TPPA assay. We also set the artificial cutoff value as 6.12 to set the specificity as 95.0% for the CLIA assay and PPV accordingly increased to 99.6%.There is no very need for a confirmatory TPPA assay in UK algorithms when S/CO is above 6.12. The false-positive rate was 0.30 above this cutoff level. When S/CO is high enough, TPPA confirmation could be omitted from the UK algorithm, costs would be significantly reduced.

In conclusion, EU Option 1, involving a reactive TT, followed by another TT of a different type and a quantitative NTT if second TT is positive, which is the same recommendation as 2015 UK syphilis guideline, is recommended. We also proposed that when S/CO is high enough, a second TT confirmation can be omitted from modified UK algorithm (Fig. 3), and costs would be significantly reduced. However, there are some limitations: 1) the data enrolled in this study cannot reflect the true prevalence of syphilis. Not all patients screened for TP infection were performed using TRUST, TPPA and InTec EIA (or CLIA) simultaneously. Therefore, discordances between the algorithms may be overestimated. PPVs calculation should take into consideration of true TP prevalence. 2) the modified UK syphilis algorithm was not evaluated in this study.

Ethic statements

The Ethics Committee of West China Hospital of Sichuan University

approved this study. No extra materials or tissue were need in this study. For researchers, all personal or private information were blind.

Conflict of interest

The authors declare that they have no competing interests.

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