Evaluation of the Elecsys Syphilis electrochemiluminescence immunoassay as a first-line screening test in the reverse algorithms for syphilis serodiagnosis

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ABSTRACT

Objectives: With the development of the automated treponemal test, new syphilis serodiagnosis algorithms, reverse algorithm, and European Centre for Disease Prevention and Control (ECDC) algorithm have been recommended recently. We investigated the efficacy of an electrochemiluminescence immunoassay (ECLIA) as an initial screening test in the reverse and ECDC algorithms.
Methods: Samples from 4,771 subjects were included in this study. We performed rapid plasma reagin (RPR), ECLIA, and *Treponema pallidum* particle agglutination (TPPA) according to these three algorithms. The fluorescent treponemal antibody absorbed (FTA-ABS) test was additionally applied for discordant cases between the RPR and ECLIA results. The FTA-ABS results and the consensus of three algorithms were considered a gold standard.
Results: A total of 208 subjects were diagnosed with syphilis. The traditional algorithm had a sensitivity of 25.96%, specificity of 100%, and accuracy of 96.77%. Both the reverse and ECDC algorithms showed the same diagnostic performance, sensitivity of 95.19%, specificity of 99.96%, and accuracy of 99.75%. The agreements between the traditional algorithm and the other algorithms were 96.9% with a kappa value of 0.415.
Conclusions: The diagnostic accuracy of the reverse and ECDC algorithms using the ECLIA as a first-line screening test was superior to that of the traditional algorithm.

Introduction

Syphilis caused by *Treponema pallidum* is a chronic bacterial infection. Syphilis remains a public health concern worldwide, although it can be successfully controlled by public health measures owing to the availability of a highly sensitive diagnostic test and a highly effective and affordable treatment (Lin et al., 2011; Lipinsky et al., 2012). A study reported a prevalence of 0.2% for syphilis in the Korean general population (Cho et al., 2003). This study reported that the syphilis prevalence in Korea has decreased since 1977. A recent study in Korea reported that the incidence rate of syphilis had decreased steadily from 49.2/100,000 person-years to 44.4/100,000 person-years from 2009 to 2014 (Joo et al., 2016). A global pooled analysis reported that the prevalence of syphilis in the general population appears to be declining in all regions (Smolak et al., 2018). However, the incidence of syphilis in the U.S. has increased recently (Centers for Disease Control and Prevention, 2017). The incidence rate of syphilis was 11.2 cases per 100,000 population in 2000, the lowest rate since 1941, and increased to 27.4 cases per 100,000 population in 2016. In addition, there is a marked increasing trend of syphilis incidences in China (Tucker et al., 2010).

T. pallidum cannot be cultured *in vitro*, and new molecular tests for syphilis are unlikely to replace serology in the short term because they are fairly expensive and require sophisticated equipment (Larsen et al., 1995). Therefore, antibody detection by non-treponemal and treponemal tests is still regarded as the mainstay for diagnosing syphilis and monitoring treatments

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(Workowski et al., 2010). Syphilis serologic assays are divided into non-treponemal and treponemal tests. Non-treponemal tests include the Venereal Disease Research Laboratories (VDRL) test, the rapid plasma reagin (RPR) test, and toluidine red unheated serum test (Janier et al., 2014). These assays detect antibodies against lipoidal antigens during early and active infections. The non-treponemal test is used to monitor disease activity and efficacy of treatment, because values of the non-treponemal test reflect disease activity. In contrast, treponemal tests detect antibodies for treponemal antigens, such as Tp15, Tp17, Tp45, and Tp47. There are many treponemal tests, such as *T. pallidum* hemagglutination (TPHA) test, *T. pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption (FTA-ABS) test, IgG immunoblot test for *T. pallidum*, enzyme immunoassay (EIA), and chemiluminescence immunoassay (CLIA). The last two methods are the most widely used treponemal assays. Any single serologic assay does not have diagnostic performance enough for syphilis serodiagnosis. Therefore, a combination of serologic assays is used for syphilis serodiagnosis according to a specific sequence of methods called an algorithm.

At present, three algorithms have been suggested for syphilis serodiagnosis. The first is the traditional algorithm, which is designed to detect active infection. Its screening step is performed with a non-treponemal test and the screening result is confirmed using a treponemal test (Workowski et al., 2010). The availability of automatable EIA and CLIA have led laboratories to validate such automated treponemal methods for use as syphilis screening tests followed by a non-treponemal test, which is a reverse sequence algorithm (Janier et al., 2014). The U.S. Centers for Disease Prevention and Control (CDC), the Association of Public Health Agency, and the United Union Kingdom Health Protection Agency have also offered the reverse algorithm in addition to the traditional screening algorithm for syphilis (Centers for Disease Control and Prevention, 2011; Egglestone and Turner, 2000; Loeffelholz and Binnicker, 2012). This reverse algorithm starts with a screening treponemal test, and a positive treponemal test is followed by a non-treponemal assay. The reverse algorithms could detect not only current infection and but also past infection that would be undetected with the traditional algorithm. In cases with reactive treponemal test results and non-reactive non-treponemal test results, a second and different treponemal assay needs to be performed to resolve the discordance (Loeffelholz and Binnicker, 2012). Another drawback of the reverse algorithm is its inability to distinguish between treated and untreated infections (Sena et al., 2010). Most recent guidelines suggest that in cases of a reactive second treponemal test, treatment should be pursued if patients have not been treated previously. For patients with prior treatment, no additional therapy is recommended unless sexual history suggests re-exposure (Ghanem, 2015). The European Centre for Disease Prevention and Control (ECDC) also suggested another modified reverse algorithm: a reactive treponemal screening test is followed by a second and different treponemal test but is not accompanied by a non-treponemal test which detects active infection (Janier et al., 2014). There is no generally recognized diagnostic algorithm.

In this study, we investigated the diagnostic performance of the reverse and ECDC algorithms using the electrochemiluminescent immunoassay (ECLIA) as an initial screening test in comparison with the traditional algorithm for syphilis serodiagnosis.

Materials and methods

Study subjects

We collected blood samples from the subjects who were tested by the RPR test between September 2016 and April 2017. After duplicate cases were excluded, a total of 4,771 residual serum specimens were consecutively obtained and preserved at $-70\,^{\circ}C$ until analysis. The specimens were obtained from the subjects referred for preoperative evaluations, diagnostic work up for syphilis, or routine health checkup. This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (KBSMC 2016-08-021).

Serologic tests

As a non-treponemal test, a quantitative RPR (Mediace rapid plasma reagin, Sekisui Chemical, Osaka, Japan) test was performed on the Roche P800 (Roche Diagnostics, Mannheim, Germany). The RPR cutoff was 1.0 RU. As a treponemal test, the Elecsys Syphilis assay (Roche Diagnostics, Mannheim, Germany), which is an automated ECLIA, was performed using the Roche e602 platform (Roche Diagnostics, Mannheim, Germany) and interpreted according to 1.0 cutoff index (COI). The automated ECLIA simultaneously detects anti-treponemal IgG and IgM antibodies using recombinant TP antigens such as TpN15, TpN17, and TpN47. Specimens with reactive results in ECLIA and those with discrepant results between RPR and ECLIA were tested using TPPA (Fujirebio, Tokyo, Japan). All specimens were tested according to the manufacturer's instructions for each assay. The FTA-ABS IgG and IgM (Scimedx, Denville, NJ, USA) were also tested in the specimens showing discordance among the RPR, ECLIA, and TPPA results for confirmation. We regarded the FTA-ABS results as a gold standard regardless of the algorithms. In the remaining cases, a consensus among three algorithms was regarded as the gold standard.

Traditional, reverse, and ECDC algorithms

In the traditional algorithm, specimens were screened by RPR test and the RPR-reactive specimens were checked by ECLIA. If the ECLIA also showed a reactive result, the specimen was considered as positive for syphilis serodiagnosis. In the reverse algorithm, specimens were screened by ECLIA, and the ECLIA-reactive specimens were tested by the RPR test. If the RPR test was reactive, the specimen was regarded as positive for syphilis. When a discordance between ECLIA and RPR was obtained, the specimen was confirmed by a second different treponemal assay, TPPA test. In the ECDC algorithm, specimens were screened by ECLIA, and the ECLIA-reactive specimens were subjected to the TPPA test. It has the same composition and order of treponemal tests with the reverse algorithm. Therefore, the ECDC algorithm in this study is not independent but subsidiary information.

Data analysis

We used the $\chi 2$ test to compare proportions and Mann–Whitney test to compare median values. The seroprevalence of syphilis between algorithms was compared using McNemar's test for paired proportions. The κ coefficient was calculated to determine the agreement among algorithms. The strength of agreement according to the κ coefficient was categorized as very good (0.81–1.00), good (0.61–0.80), moderate (0.41–0.60), fair (0.21–0.40), or poor (≤ 0.20) (Altman, 1991). The Kruskal-Wallis test was used to compare the COI values of ECLIA according to the TPPA and FTA-ABS results, and the Bonferroni correction method with Mann–Whitney test was used as a post-hoc analysis. A receiver operating characteristic (ROC) curve analysis of the ECLIA results was performed for the prediction of the TPPA and FTA-ABS results of the discordant cases in the reverse algorithm, and the area under the curve (AUC) with 95% confidence interval (CI) was calculated. Statistical analyses were performed using MedCalc for

Windows, version 18.5 (MedCalc Software, Ostend, Belgium). *P* values of <0.05 were considered to be significant.

Results

Characteristics of study subjects

A total of 4,771 subjects had a median age of 54 years, and females accounted for 52.7% of the study subjects (Table 1). Of the 4,771 subjects, 208 (4.4%) showed positive serodiagnosis according to the final interpretation. This positive serodiagnosis group showed slight male predominance and older age than the negative group. The RPR and ECLIA results were significantly different between two groups.

Serologic test results according to the three syphilis serodiagnosis algorithms

The serologic test results according to the three different algorithms are illustrated in Figure 1. Among the 4,771 specimens, 92 (1.9%) were reactive in the RPR test in the traditional algorithm. Of the 92 RPR-reactive specimens, 54 (58.7%) were also reactive by ECLIA and were suggestive of syphilis. In the reverse algorithm, 212 (4.4%) specimens were reactive by ECLIA and 54 of 212 (25.5%) were reactive by RPR, suggestive of syphilis. Among the remaining 158 ECLIA+/RPR− specimens, 146 (92.4%) were positive or indeterminate by TPPA. In the ECDC algorithm, 212 (4.4%) specimens with reactive results by ECLIA were followed by TPPA, and 200 were TPPA-positive or indeterminate.

The differences in seroprevalence between the traditional and the other algorithms were significant (1.1% *vs.* 4.2%, *P* < 0.0001). As a first-line serologic test, RPR was reactive in 92 (1.9%) specimens in the traditional algorithm, and ECLIA was reactive in 212 (4.4%) specimens in the other two algorithms. In the perspective of the initial screening step, the traditional screening showed a higher false-reactive rate (0.8%; 38/4,771) than the reverse screening (0.3%; 12/4,771) (*P* = 0.0003). The corresponding κ value between the traditional and reverse algorithm was 0.415 (95% CI, 0.339–0.491), which indicated a moderate agreement. Very good agreement (κ = 1.0; 95% CI, 1.000–1.000) was observed between the ECDC and reverse algorithms (Table 2).

Diagnostic performance of the three syphilis serodiagnosis algorithms

In the traditional algorithm, 54 of 4,563 (1.1%) subjects were positive for syphilis serodiagnosis (Table 3). However, 154 positive syphilis serodiagnosis cases were not detected, even though there were no false-positive results. The traditional algorithm showed a

low sensitivity of 25.96% (95% CI, 20.14%–32.48%), perfect specificity of 100.00% (95% CI, 99.92%–100.00%), and acceptable accuracy of 96.77% (95% CI, 96.23%–97.26%). The reverse and ECDC algorithms showed identical results. They showed two false-positive cases and ten false-negative cases (Table 4). Two false-positive cases showed discordant results of ECLIA+/RPR−, and were confirmed by TPPA+ with high titers. Of ten false-negative cases, seven showed ECLIA+/RPR−/TPPA−, and three showed ECLIA−/RPR+/TPPA−. The sensitivity, specificity, and accuracy of these two algorithms were 95.19% (95% CI, 91.34%–97.67%), 99.66% (95% CI, 99.84%–99.99%), and 99.75% (95% CI, 99.56%–99.87%), respectively.

Analysis of discordant results (ECLIA+/RPR−) in the reverse algorithm

Among the 4,771 cases, 158 showed discordant serologic results of ECLIA+/RPR−. According to the results of the second treponemal test, we divided the discordant results into three groups: TPPA-negative, -indeterminate, and -positive groups. There were no significant differences in age and sex among the three groups (Table 5). In contrast, the median ECLIA COIs showed significant differences among all three groups. The proportion of reactive FTA-ABS IgG results showed significant differences only between the TPPA-negative and -positive groups. On the 158 ECLIA+/RPR− specimens, ECLIA COI values of the TPPA-positive group was significantly higher than those of the TPPA-indeterminate and negative groups (Figure 2A). The TPPA-indeterminate group also had significantly higher COI values than the TPPA-negative group. The FTA-ABS IgG-reactive group also had higher ECLIA COI values than the FTA-ABS IgG-weakly reactive and non-reactive groups (Figure 2B). However, there were no significant differences of ECLIA COI values between the FTA-ABS IgG-weakly reactive and non-reactive groups. For the prediction of TPPA results, the optimal ECLIA COI value was 3.6, which had the best diagnostic efficacy with a sensitivity of 91.8% and specificity of 91.7% (Figure 3A). The same COI value of ECLIA could predict the FTA-ABS IgG results with the best diagnostic efficacy, showing a sensitivity of 88.7% and specificity of 88.7% (Figure 3B).

Discussion

This study indicated that the seroprevalence (4.2%) of the reverse syphilis algorithm was higher than that (1.1%) of the traditional algorithm. Nah et al. reported seroprevalences of 5.4% and 0.6% for the reverse and traditional algorithms, while Huh et al. reported very low seroprevalence of 0.6% and 0.1%, respectively (Huh et al., 2016; Nah et al., 2017). Both studies included subjects undergoing routine health checkups, but they showed different

Table 1
Basic characteristics of the 4,771 subjects.

Characteristic	Total (n = 4,771)	Negative syphilis serodiagnosis (n = 4,563)	Positive syphilis serodiagnosis (n = 208)	*P* value
Age (years)				
Median (interquartile range)	54 (35–68)	53 (35–68)	66 (57–74)	<0.0001
Sex				
Female, n (%)	2,512 (52.7)	2,440 (53.5)	72 (34.6)	<0.0001
Male, n (%)	2,259 (47.3)	2,123 (46.5)	136 (65.4)	
RPR				
Non-reactive, n (%)	4,679 (98.1)	4,528 (99.2)	151 (72.6)	<0.0001
Reactive, n (%)	92 (1.9)	35 (0.8)	57 (27.4)	
ECLIA (COI)				
Median (interquartile range)	0.077 (0.074–0.084)	0.077 (0.074–0.083)	22.84 (7.97–51.56)	<0.0001

Abbreviations: RPR, rapid plasma reagin; ECLIA, electrochemiluminescence immunoassay; COI, cutoff index.
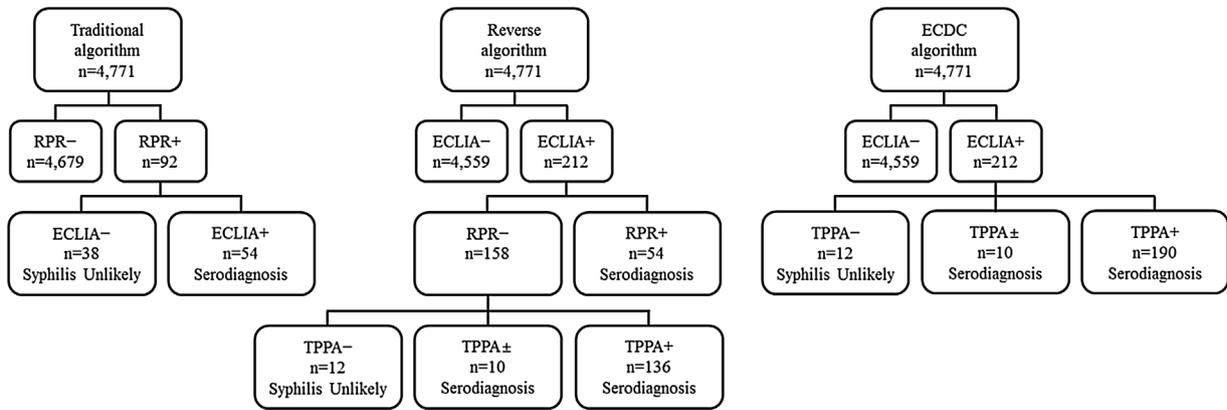
Figure 1. Serologic test results according to the traditional, reverse, and ECDC algorithms for syphilis serodiagnosis.
Abbreviations: ECDC, European Centre for Disease Prevention and Control; RPR, rapid plasma reagin; ECLIA, electrochemiluminescence immunoassay; TPPA, *Treponema pallidum* particle agglutination.

Table 2
The concordance among traditional, reverse, and ECDC algorithms for syphilis serodiagnosis.

Algorithms	Reverse algorithm		Total	Agreement (%)	Kappa value (95% CI)
	Positive	Negative			
Traditional algorithm					
Positive	54	0	54	96.9	0.415
Negative	146	4571	4,717		(0.339–0.491)
ECDC algorithm					
Positive	200	0	200	100	1.000
Negative	0	4571	4,571		(1.000–1.000)

Abbreviations: ECDC, European Center for Disease Prevention and Control; CI, confidence interval.

seroprevalences. The subjects of the former study had a skewed male-to-female ratio (9.9, 90.8% *vs.* 9.2%) and this male predominance could result in a relatively high seroprevalence of syphilis in spite of the health screening setting (Ndeikoundam Ngangro et al., 2018; Stone et al., 2018). In addition, the study subjects of the former study and our study resided in a big city, while those of the latter study resided in a medium-sized city. Many previous studies consistently reported higher syphilis seroprevalences by the reverse algorithm than that by the traditional algorithm: 11.4% *vs.* 8.7% in a Chinese study (Tong et al., 2014), 0.86% *vs.* 0.43% in a retrospective U.S. study (Dunseth et al., 2017), 0.9% *vs.* 0.4% in a prospective U.S. study (Binnicker et al., 2012), and 1.98% *vs.* 0.46% in a Canadian study (Mishra et al., 2011). In HIV-infected individuals, the difference in seroprevalence between two algorithms was more remarkable (24.9% *vs.* 14.2%) (Chen et al., 2017). These

findings might be caused by increasing serodiagnosis of primary, latent, and tertiary syphilis using the reverse algorithm (Tong et al., 2014). Especially, latent syphilis substantially accounts for syphilis and is a major target of the reverse algorithm.

In this study, we investigated the diagnostic performances of three algorithms based on the gold standards. There have only been a few studies that compared the diagnostic performances of algorithms based on the gold standards such as clinical diagnosis and FTA-ABS results. The diagnostic performance of the reverse and ECDC algorithms was superior to that of the traditional algorithm. Diagnostic performance is mainly determined by the performances of the initial screening and confirmatory tests or seroprevalence. The initial screening test of the reverse and ECDC algorithms in our study, Elecsys Syphilis, showed the best diagnostic accuracy, a sensitivity of 99.4%, and specificity of 100%, when compared with the other five automated treponemal assays (Park et al., 2016). The second treponemal test in our study, TPPA, is generally recommended as a confirmatory test because of its excellent performance, sensitivity of 99.1%, and specificity of 100%, which is the best among the agglutination assays (Cole et al., 2007). With the traditional algorithm, a low sensitivity of 25.96% and a high missed-diagnosis rate of 74.04% were observed in our study, which may pose a threat to public health due to underestimation of seroprevalence. The RPR tests showed low sensitivities of 52.5% when compared to TPPA, and 65.0%–80.0% for latent syphilis when compared to clinical diagnosis (Lee et al., 2014; Noh et al., 2008). Because of the limited analytical sensitivity of the non-treponemal test, the traditional algorithm is expected to show a lower diagnostic sensitivity and higher

Table 3
Diagnostic performance of three syphilis serodiagnosis algorithms.

Algorithms	Gold standard[a]		Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	PLR (95% CI)	NLR (95% CI)	Accuracy, % (95% CI)
	Positive	Negative							
Traditional									
Positive	54	0	25.96	100.00	100.00	96.74	∞ (∼)	0.74	96.77
Negative	154	4,563	(20.14–32.48)	(99.92–100.00)	(93.40–100.00)	(96.47–96.98)		(0.68–0.80)	(96.23–97.26)
Reverse									
Positive	198	2	95.19	99.96	99.00	99.78	2171.81	0.05	99.75
Negative	10	4,561	(91.34–97.67)	(99.84–99.99)	(96.12–99.75)	(99.60–99.88)	(543.13–8684.36)	(0.03–0.09)	(99.56–99.87)
ECDC									
Positive	198	2	95.19	99.96	99.00	99.78	2171.81	0.05	99.75
Negative	10	4,561	(91.34–97.67)	(99.84–99.99)	(96.12–99.75)	(99.60–99.88)	(543.13–8684.36)	(0.03–0.09)	(99.56–99.87)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; ECDC, European Center for Disease Prevention and Control.
[a] The FTA-ABS results or consensus among the three algorithms were regarded as a gold standard.

Table 4
False-positive and false-negative cases via the reverse algorithm.

Syphilis serodiagnosis	Age	Sex	Results	ECLIA (COI)	RPR (RU)	TPPA (titer)	FTA-ABS IgG	FTA-ABS IgM
False positive (n = 2)	25	M	ECLIA+/RPR−/TPPA+	1.670	NR	R (1:160)	NR	NR
	74	M	ECLIA+/RPR−/TPPA+	38.730	NR	R (1:320)	NR	NR
False negative (n = 10)	52	M	ECLIA+/RPR−/TPPA−	2.400	NR	NR	R	NR
	69	F	ECLIA+/RPR−/TPPA−	11.540	NR	NR	R	NR
	25	M	ECLIA−/RPR+/TPPA−	0.095	R (1.6)	NR	R	R
	72	F	ECLIA+/RPR−/TPPA−	2.800	NR	NR	WR	NR
	41	M	ECLIA−/RPR+/TPPA−	0.077	R (7.0)	NR	WR	NR
	63	M	ECLIA+/RPR−/TPPA−	2.770	NR	NR	WR	NR
	68	F	ECLIA+/RPR−/TPPA−	1.140	NR	NR	WR	NR
	75	F	ECLIA+/RPR−/TPPA−	1.050	NR	NR	R	NR
	40	M	ECLIA−/RPR+/TPPA−	0.076	R (4.5)	NR	WR	NR
	62	F	ECLIA+/RPR−/TPPA−	1.990	NR	NR	R	NR

Abbreviations: ECLIA, electrochemiluminescence immunoassay; RPR, rapid plasma reagin; TPPA, Treponema pallidum particle agglutination; COI, cutoff index; RU, RPR units; FTA-ABS, fluorescent treponemal antibody absorbed; M; male; F, female; NR, nonreactive; R, reactive; WR, weakly reactive.

Table 5
Characteristics of the subjects with discordant ECLIA+/RPR− results in the reverse algorithm.

Characteristic	ECLIA+/RPR−/TPPA− (n = 12)	ECLIA+/RPR−/TPPA± (n = 10)	ECLIA+/RPR−/TPPA+ (n = 136)	P value
Age (years)				
Median (interquartile range)	70.5 (65.5–74.5)	69.5 (66–76)	67 (59–75)	0.4589
Sex				
Female, n (%)	6 (50.0)	5 (50.0)	45 (33.1)	0.3062
Male, n (%)	6 (50.0)	5 (50.0)	91 (66.9)	
ECLIA (COI)				
Median (interquartile range)	2.175 (1.165–2.785)	5.250 (2.730–8.950)	20.170 (8.080–40.575)	<0.0001[a]
FTA-ABS IgG				
Nonreactive, n (%)	5 (41.7)	0 (0.0)	2 (1.5)	<0.0001[b]
Reactive, n (%)	7 (58.3)	10 (100.0)	134 (98.5)	

Abbreviations: ECLIA, electrochemiluminescence immunoassay; RPR, rapid plasma reagin; TPPA, Treponema pallidum particle agglutination; COI, cutoff index; FTA-ABS, fluorescent treponemal antibody absorbed.
[a] Median COIs of ECLIA among three groups were significantly different in the post hoc analysis using the Bonferroni correction method.
[b] Proportions of reactive FTA-ABS IgG were significantly different between TPPA− and TPPA± groups in the post hoc analysis using the Bonferroni correction method.
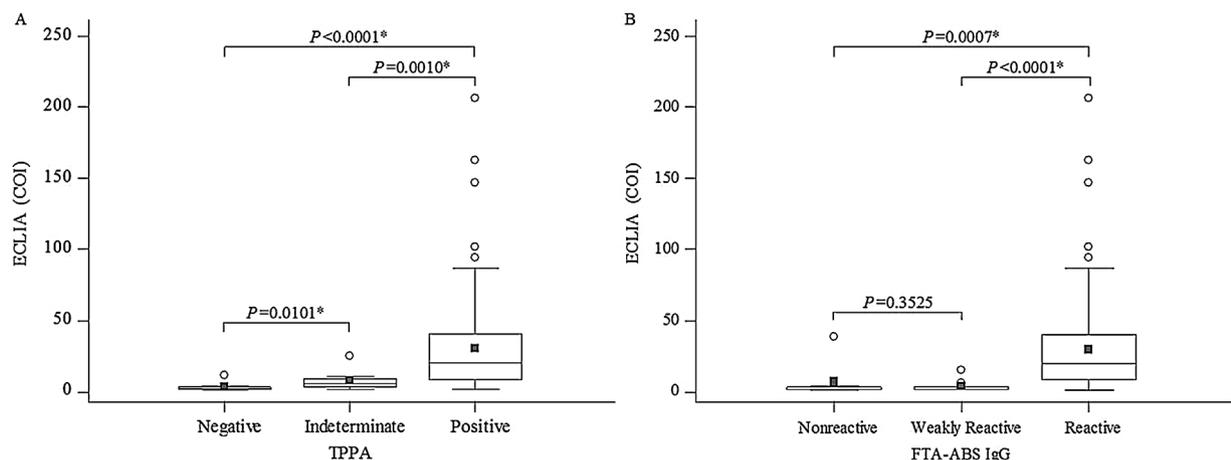

Figure 2. Distribution of cutoff index (COI) of ECLIA in specimens that were ECLIA+/RPR− by the reverse algorithm according to (A) TPPA results (TPPA-negative, n = 12; indeterminate, n = 10; positive, n = 136), and (B) FTA-ABS IgG results (non-reactive, n = 7; weakly reactive, n = 12; reactive, n = 139).
*Indicated significant in post hoc analysis of the Bonferroni correction method using Mann–Whitney test.
Abbreviations: ECLIA, electrochemiluminescence immunoassay; COI, cutoff index; TPPA, Treponema pallidum particle agglutination; FTA-ABS, fluorescent treponemal antibody absorbed; RPR, rapid plasma reagin.

missed-diagnosis rate than the reverse or ECDC algorithms (Binnicker et al., 2012; Dunseth et al., 2017; Huh et al., 2016; Mishra et al., 2011; Nah et al., 2017). Tong et al. evaluated the algorithms using clinical diagnosis as a gold standard, and reported a sensitivity of 75.81% and a missed-diagnosis rate of 24.19% by the traditional algorithm in a population with high

syphilis prevalence of 11.4%, while the reverse and ECDC algorithms had higher sensitivities of 99.38%–99.85% when compared to the traditional algorithm (Tong et al., 2014). This difference in diagnostic performance between the traditional and the reverse algorithm is contributed by reported intrinsic property of algorithms. While the traditional algorithm is
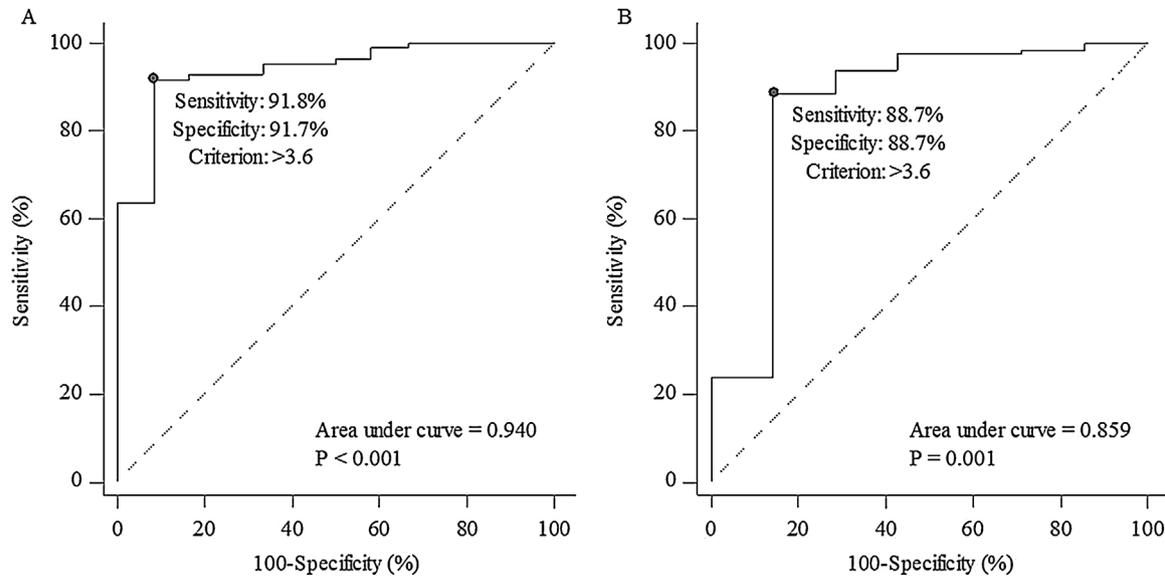
Figure 3. Receiver operating characteristic curves of ECLIA for prediction of (A) TPPA and (B) FTA-ABS results on ECLIA+/RPR− specimens (n = 158). Abbreviations: ECLIA, electrochemiluminescence immunoassay; TPPA, Treponema pallidum particle agglutination; FTA-ABS, fluorescent treponemal antibody absorbed.

designed to detect only current infection, the reverse algorithm could detect both current and past infection. In this study, the corresponding κ value between the traditional and reverse algorithm was 0.415, which indicated moderate agreement. The other study reported a κ value of only 0.191, indicating a poor agreement in the routine health checkup setting (Nah et al., 2017), while a study in China reported a κ value of 0.668 in HIV-infected individuals (Chen et al., 2017). These differences may contribute to the different screening methods in each algorithm, characteristics of subjects, and distribution of syphilis stages. The ECDC algorithm showed a performance equivalent to the reverse algorithm in this study, and this result suggests that a second treponemal assay alone and skipping the non-treponemal assay as a confirmatory test would be sufficient for syphilis serodiagnosis. This same diagnostic performance of the two algorithms reflects that the specimens with ECLIA+/RPR+ might predict positive TPPA results. Chen et al. also reported a good agreement (κ value of 0.994) between the reverse and ECDC algorithms in HIV-infected individuals (Chen et al., 2017), and a κ value of 0.999 was reported in low-prevalence populations (Peng et al., 2018). Tong et al. reported 18 inconsistent cases between reverse and ECDC algorithms among 2,756 initial screening positive cases due to the different results of second treponemal test from those of first-line treponemal test. Inconsistency between two algorithms could be more frequent in a higher prevalence population (Tong et al., 2014).

The reverse algorithm revealed 158 (3.3%) subjects with discordant results in this study. Nah et al. classified the discordant cases with positive treponemal and negative non-treponemal tests into two groups by the second treponemal test results, and reported that the second treponemal test-positive group tended to be older (Nah et al., 2017). However, there were no significant differences in age among the three groups according to the second treponemal test results in our study. It might be helpful to predict the second treponemal test results based on the first treponemal test results. ECLIA, Elecsys Syphilis, COI value of 3.6 was the most efficient, with a sensitivity of 91.8% and specificity of 91.7%, for predicting the TPPA results in this study. A previous study suggested the Architect Syphilis TP S/CO value of 3.1 with a sensitivity of 82.7% and specificity of 87.5% for the same purpose (Lee et al., 2013).

According to the College of American Pathologists report using voluntary questionnaires, most (63%; 1,205 of 1,911) of the laboratories that responded use the traditional algorithm (Rhoads et al., 2017). The reverse and both algorithms are used in about 16% and 2.5% of laboratories, respectively. There was no report for a trend of syphilis serodiagnosis algorithms in Korea, and most laboratories still use the traditional algorithm. In recent years, various automated treponemal assays with greater sensitivity and specificity have been developed (Morshed and Singh, 2015). Especially, CLIA using a recombinant TP-specific antigen has been increasingly used for its high throughput. Along with these changes, many laboratories have considered changing the diagnostic approach for a more efficient high-volume screening. Syphilis prevalence varies according to region, even within a country (Centers for Disease Control and Prevention, 2017; Tucker et al., 2010). Sexual behavior is also an important demographic factor. The significant increase in syphilis prevalence among men who have sex with men (MSM) was reported (Stamm, 2010; Tucker and Cohen, 2011). A recent report in the U.S. showed that MSM accounts for 58.1% of primary and secondary syphilis (Centers for Disease Control and Prevention, 2017). Therefore, it is important for laboratories to determine the algorithm depending on seroprevalence, cost, ease of use, suitability for automation, and patient demographics.

There have been controversies regarding the cost-effectiveness of the reverse syphilis algorithm. A study in Canada concluded that the reverse algorithm was more cost-effective than the traditional algorithm (Chuck et al., 2008). In contrast, a study in the U.S. reported that the reverse algorithm would be more expensive in terms of public health (Buono et al., 2018). Despite the slight increase in false-positive cases, the reverse algorithm can detect substantial cases missed by the traditional algorithm. A reduction in false-negatives with the reverse algorithm may have a positive impact on public health; therefore, it is necessary to investigate the cost-effectiveness of the reverse algorithm on public health.

This study has some limitations. First, we could not use the clinical diagnosis as a gold standard because of insufficient medical records. Instead, we used the final interpretation by FTA-ABS and consensus among the three algorithms as a reference. Therefore, we excluded the clinical diagnosis and limited the interpretation to the serodiagnosis. Especially, the cases with negative results in the

non-treponemal test and positive results in the treponemal test have probability for both latent syphilis and treated past infection. The serodiagnosis could be influenced by epidemiologic and clinical characteristics of study population. Therefore, the performance of the algorithms could be variable according to the population even with the same composition of assays. Second, we performed FTA-ABS tests only for the 196 cases with discrepant results among three syphilis tests (RPR, ECLIA, and TPPA). We additionally obtained treponemal test results of 237 out of 4,575 cases with concordant results by medical records review. Among 4,521 RPR−/ECLIA−/TPPA (not tested) cases, 212 showed non-reactive *treponema pallidum* latex agglutination (TPLA) and/or FTA-ABS results. Among 54 RPR+/ECLIA+/TPPA+ cases, reactive results of TPLA and/or FTA-ABS were observed in 25 cases. Although it is limited information, it suggests that the possibility of false positive or false negative cases might be low in the cases with concordant results. Third, the study population had different reasons for taking the syphilis serologic test. Some of them were referred to the diagnostic test for suspicious symptoms of syphilis, while others were tested during a routine health checkup or before surgery. Therefore, the study subjects were composed of heterogeneous groups, which could not accurately represent the general population. However, this study population could represent the seroprevalence of syphilis in a tertiary hospital in an urban area.

In conclusion, this study indicated that the reverse or ECDC algorithm using the ECLIA as a first-line screening test and the TPPA as a second treponemal test could detect more cases of missed serodiagnosis by the traditional algorithm. The reverse algorithm could contribute to the appropriate serodiagnosis of syphilis with superior diagnostic performance.

Ethical approval

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Conflict of interest

There is no conflict of interest.

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