Clinical Biochemistry 63 (2019) 121–125

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Strategy for performing treponemal tests in reverse-sequence algorithms of syphilis diagnosis

Joowon Yi, Woongryong Choi, Seonhyeon Shin, Juhee Choi, Hanah Kim, Hee-Jung Chung, Hee-Won Moon*, Mina Hur, Yeo-Min Yun

Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
Syphilis
Reverse-sequence algorithm
EIA
TPLA
TPPA
ICA

ABSTRACT

Objectives: In South Korea, automated *T. pallidum* Latex Agglutination (TPLA) based on turbidoimmunoassays and immunochromatographic assay (ICA) are widely used for syphilis diagnosis. However, there is sparse data on the validation of these assays in the reverse-sequence algorithm setting.

Methods: We assessed 551 specimens submitted for syphilis testing. We compared varying reverse-sequence algorithms using combinations of the Cobas Syphilis EIA (Roche Diagnostics, Mannheim, Germany), Mediace TPLA (Sekisui Medical Co., Tokyo, Japan), TPPA (Fujirebio Inc., Tokyo, Japan), and SD Bioline ICA (Standard Diagnostic, Yongin, Korea). We also evaluated modified algorithms incorporating a cut off of high specificity for EIA and TPLA using receiver operating characteristic curves.

Results: The agreement was almost perfect between EIA and TPLA (Kappa, 0.953) and strong between TPPA and ICA (Kappa, 0.887). Among TPPA positive and ICA negative specimens, 67% of the specimens were from individuals with syphilis histories. Compared to EIA/RPR/TPPA, the agreement with EIA/RPR/ICA, TPLA/RPR/TPPA and TPLA/RPR/ICA were almost perfect (Kappa, 0.930, 0.995 and 0.914, respectively). When a cut off of 95% specificity was applied, the number of TPPA tests could be reduced by 44% and 40% in EIA and TPLA, respectively.

Conclusions: TPLA showed almost perfect agreement with EIA and that it could be used in the site of EIA in a reverse sequence algorithm. ICA showed a lower detection rate than TPPA as a 2nd treponemal test and should be used with caution. With cut offs of higher specificity, more efficient reverse-sequence algorithms can be made possible.

1. Introduction

Treponema pallidum, which causes syphilis, is currently difficult to culture *in vitro* [1]. It remains a global public health care concern [2,3] and serologic tests are the favored methods for screening and diagnosis for syphilis. Serologic tests are divided into treponemal tests, which detect antibodies against individual or a mixture of specific *T. pallidum* antigens, and nontreponemal tests, which detect antibodies to lipoidal antigens [4]. Screening for syphilis has historically been done by performing non-treponemal tests and then performing treponemal tests on samples that were found to be positive using non-treponemal tests [5,6]. However, with the advent of automated treponemal assays, it is now feasible to utilize a reverse-sequence algorithm, especially in settings where high-volume testing is needed [7–12]. In the reverse-sequence algorithm recommended as an alternative to the traditional algorithm by the CDC, enzyme- and chemiluminescence immunoassays [EIA/CIA] are the first treponemal tests, followed by a nontreponemal test and *T. pallidum* particle agglutination (TPPA) in the case of discordant results [13]. In South Korea, automated *T. pallidum* Latex Agglutination (TPLA) based on turbidoimmunoassays and immunochromatographic assays (ICAs) are also widely used. However, there is sparse data on the validation of these assays in the reverse-sequence algorithm setting.

In this study, we compared varying reverse-sequence algorithms using combinations of EIA, TPLA, TPPA, and ICA. Then, in order to reduce the number of TPPA tests performed we first calculated the cut-off value at a specificity of 95% for Roche EIA (Roche Diagnostics, Mannheim, Germany) and Mediace TPLA (Sekisui Medical Co, Tokyo, Japan).

* Corresponding author at: Department of Laboratory Medicine, Konkuk University School of Medicine, Konkuk University Hospital, 120-1, Neungdong-ro, Hwayang-dong, Gwangjin-gu, Seoul 05030, Republic of Korea.
E-mail address: hannasis@hanmail.net (H.-W. Moon).

https://doi.org/10.1016/j.clinbiochem.2018.09.013
Received 13 July 2018; Received in revised form 30 August 2018; Accepted 30 September 2018
Available online 02 October 2018

2. Material and methods

2.1. Study population

This study was approved by the Institutional Review Board (KUH1200072) of the Konkuk University Medical Center, Seoul, Korea, before collecting any specimens from any patients. A total of 551 sera submitted for syphilis testing were included between November 2017 and January 2018. The residual specimens were collected after routine blood testing, and then stored at −70 °C until they were used. The data were analyzed anonymously and this study required neither study-specific intervention nor any other intervention. Therefore, the need for written informed consent from the enrolled patients was exempted. The medical charts of patients positive for nontreponemal and/or treponemal tests were reviewed in order to collect clinical information.

2.2. Assays

We performed the Cobas Syphilis EIA (Roche Diagnostics, Germany) and Mediace TPLA (Sekisui Medical Co, Japan) as the first screening test. The Cobas Syphilis EIA is a one-step double-antigen sandwich assay that detects total IgG and IgM antibodies against bacterial TpN15, TpN17, and TpN47 antigens. The syphilis IgG and IgM levels are measured through chemiluminescence and the samples are deemed to be positive or negative depending on a predetermined cutoff index, with ≥1.0 being considered positive (Elecsy Syphilis Factsheet). Mediace TPLA is a latex turbidoimmunoassay using latex particles coated with *T. pallidum* antigens and performed on a Toshiba automated chemistry analyzer (Hitachi, Tokyo, Japan). The results of the Mediace TPLA ≥10.0 TU. were considered to be positive with a measurable range of 5–250 TU. TPPA (Fujirebio Inc., Tokyo, Japan) and the SD Bioline ICA (Standard Diagnostic, Yongin, Korea) were performed in the samples with a positive first treponemal test as per the manufacturer's instructions. While all other assays have cut-offs based on numeric values, the results of the TPPA assay can be interpreted as negative, weak positive, or positive, according to the manufacturers' information sheet. Negative results are when no agglutination is visible, positive results are when agglutination is obvious, and weak positive results are when agglutination has occurred but not completely. The rapid plasma reagin [RPR] test was performed using the Mediace RPR assay (Sekisui Medical Co., Tokyo, Japan).

2.3. Simulation of various algorithms

Using the two recommended treponemal tests in the CDC-recommended algorithm (EIA/RPR/TPPA) as the reference algorithm, we performed comparisons with three possible combinations of treponemal tests (EIA/RPR/ICA, TPLA/RPR/TPPA, and TPLA/RPR/ICA). We also evaluated the modified algorithms incorporating quantitative values of EIA and TPLA.

2.4. Statistical analysis

Data were expressed as median and interquartile range (IQR) or number and percentage. The agreements between assays were determined using Cohen's Kappa (agreement: < 0.20, none; 0.21–0.39, minimal; 0.40–0.59, weak; 0.60–0.79, moderate; 0.80–0.90, strong; > 0.90, almost perfect) [14]. The receiver operating characteristic (ROC) curve was used for predicting TPPA positivity. The optimal cutoff was determined using the Youden maximum index value, which assigns equal weight to sensitivity and specificity, and also by fixing specificity to ≥95% (cut off$_{95}$). Statistical analysis was performed using MedCalc Statistical Software (version 15.8, MedCalc Software, Mariakerke, Belgium) and IBM SPSS Statistics 22.0 (IBM Corporation, Armonk, NY, USA). *P* values < 0.05 were considered to be statistically significant.

Table 1
Agreement between first and second treponemal assays.

1st treponemal assays					
		TPLA		Concordance (95% CI)	Kappa (95% CI)
		Positive	Negative		
EIA	Positive	138	1	98.20% (96.7–99.0%)	0.953 (0.924–0.982)
	Negative	9	403		
2nd treponemal assays					
		TPPA		Concordance (95% CI)	Kappa (95% CI)
		Positive	Negative		
ICA	Positive	115	10	96.00% (94.5–97.6%)	0.887 (0.841–0.933)
	Negative	12	414		

Abbreviations: EIA, enzyme immunoassay; TPLA, *Treponema pallidum* Latex Agglutination; CI, confidence interval; ICA, immunochromatographic assay; TPPA, *T. pallidum* particle agglutination.

3. Results

3.1. Agreement in the first treponemal and second treponemal tests

The agreement was almost perfect between EIA and TPLA (Kappa, 0.953, 95% confidence interval (CI), 0.924–0.982) and strong between TPPA and ICA (Kappa, 0.887, 95% CI, 0.841–0.933) (Table 1). Details of the discrepant results between the first and second treponemal tests were shown in Table 2. There were nine patients with specimens that were EIA-negative but TPLA-positive, but there were no patients with any history of syphilis or who were positive in any other treponemal test (TPPA or ICA). A single EIA-positive and TPLA-negative patient was found to have undergone syphilis treatment in the past. Twelve specimens were ICA-negative but TPPA-positive, and upon investigation, it was revealed that eight specimens (67%) were from patients who were either suspected of having syphilis, had active syphilis, or had latent syphilis (Table 2). On the other hand, ten specimens were ICA-positive but TPPA negative, but only two were from patients who had a history of syphilis treatment. There were also seven patients that showed weak positive for TPPA (Table 3). All seven patients were positive for EIA, TPLA, and ICA and there were five patients who were negative for RPR and one patient who had a prior history of syphilis infection.

3.2. Agreement of various algorithms with reference algorithm

Compared to EIA/RPR/TPPA, the agreement with EIA/RPR/ICA, TPLA/RPR/TPPA and TPLA/RPR/ICA were almost perfect (Kappa, 0.930, 0.995 and 0.914, respectively). There were 4 EIA-screened specimens and 6 TPLA-screened specimens that were missed in ICA-

Table 2
Detailed findings in discrepancy samples between first and second treponemal assays.

EIA	TPLA	Total no.	RPR (+)	TPPA (+)	ICA (+)	Syphilis history
+	−	1	1 (100%)	0 (0%)	0 (0%)	1 (100%)
−	+	9	0 (0%)	0 (0%)	0 (0%)	0 (0%)

TPPA	ICA	Total no.	RPR (+)	EIA (+)	TPLA (+)	Syphilis history
+	−	12	7 (58%)	11 (92%)	11 (92%)	8 (67%)
−	+	10	1 (10%)	10 (100%)	10 (100%)	2 (20%)

Abbreviations: see Table 1. RPR, rapid plasma reagin.

Table 3
Laboratory and clinical findings for samples that showed weak positive for TPPA (N = 7).

EIA (+)	TPLA (+)	RPR (+)	ICA (+)	syphilis history
7 (100%)	7 (100%)	2 (28.6%)	7 (100%)	1 (14.3%)

Abbreviations: see Table 2.

Table 4
Agreement between different screening algorithms.

Compared to EIA/RPR/TPPA					
		Positive	Negative	Concordance (95% CI)	Kappa (95% CI)
EIA/RPR/ICA	Positive	123	10	97.46%	0.930
	Negative	4	414	(95.7–98.8%)	(0.893–0.966)
TPLA/RPR/TPPA	Positive	126	0	99.82%	0.995
	Negative	1	424	(99.1–100%)	(0.985–1.0)
TPLA/RPR/ICA	Positive	121	11	96.92%	0.914
	Negative	6	413	(95.3–98.5%)	(0.874–0.954)

Abbreviations: see Table 1.

confirmed algorithms compared to the EIA screened and TPPA-confirmed algorithms. There was only one discrepant specimen between two TPPA-confirmed algorithms (EIA/RPR/TPPA and TPLA/RPR/TPPA, Table 4).

3.3. Estimation of high specific cut off for predicting TPPA positivity in Roche EIA and Mediace TPLA

ROC curve analysis for prediction of TPPA positivity was performed with Roche EIA and Mediace TPLA quantitative values. AUC values were 0.977 for EIA (95% CI, 0.961–0.988) and 0.961 for TPLA (95% CI, 0.941–0.975). The cut offs showing ≥95% specificity for TPPA positivity were 21.45 COI for EIA and 96.52 TU for TPLA, respectively. The number of tests and TPPA results in the different strategies based on pre-defined cut-off and cut off of 95% specificity were shown in Table 5. When cut off of 95% specificity was applied in the CDC recommended algorithm, the number of TPPA tests performed on the RPR-negative specimens could be reduced by 44% (43 samples to 24) and 40% (53 samples to 32) in EIA and TPLA, respectively. The remaining 19 and 21 samples for EIA and TPLA respectively have higher values than the cut off_{95} values and do not need additional tests (Table 5). Detailed results for samples that were TPPA negative while being ≥ cut off_{95} of EIA or TPLA were described in Table 6. There were nine specimens with ≥cut off_{95} for either EIA or TPLA while also having negative TPPA results. Of these nine specimens, eight showed positive results for at least two out of three treponemal tests administered in this study.

4. Discussion

Initial reports suggested that the reverse algorithm had lower specificity, but later reports have demonstrated that to be untrue [6,15]. Another fact in favor of the reverse algorithm is that the sensitivity of the RPR assay is decreased during primary syphilis [6,8]. However, because treponemal antibodies usually persist for life, immunologic assessment of reinfection is difficult and requires attention to the patient's history and use of a nontreponemal assay. Another factor to consider is that laboratory monitoring of syphilis treatment and recovery can only be done with a nontreponemal assay [11]. Thus, the question of which algorithm to use is one that has not yet been solved. Reflecting this fact is that a College of American Pathologists survey carried out in 2015 indicated that only 18.4% of laboratories utilized the reverse-sequence algorithm alone or alongside the traditional algorithm [11].

In South Korea, automated TPLA based on turbidoimmunoassays and ICA are also widely used and 11.3% and 43.9% of laboratories performing TPLA and ICA, respectively, according to the report by a Korean external quality assessment program in 2015 [16]. Whereas TPLA is usually used as screening method in large-volume laboratories, ICA is manually performed and used as the second test or also as a screening test in small-volume laboratories. However, its use as the first or second treponemal test in the reverse-sequence algorithm is not discussed in recent guidelines and there is a lack of relevant validation data [13]. Due to the lack of such data, we began by validating TPLA and ICA by comparing them with EIA and TPPA, respectively (Table 1). Of note, since TPPA is not an automated test and requires additional costs, several studies suggested the use of quantitative values of the first treponemal test in the reverse-sequence algorithm in order to reduce the number of TPPA confirmation tests [6,17–19]. Another downside of the automation of treponemal assays is that the number of false positive results increases, especially in a low prevalence setting [4–6,20]. Due to this potential increase in false positive results, recently published syphilis diagnosis guidelines recommend confirmation using TPPA assays [13]. However, TPPA assays are individually done manually, so each test performed increases the workload and turnaround time, and it is cumbersome to perform these tests randomly. Previous studies have attempted to reduce the number of TPPA tests performed by applying a cut-off value from the 1st treponemal test with a high specificity, mostly with Architect or Bioplex platforms [17–19].

In this study, the agreement was almost perfect between EIA and TPLA. There were nine patient specimens that were EIA-negative but TPLA-positive (Table 2). Chart reviews indicated that all nine of these specimens were from patients who did not have syphilis and all specimens were discovered to be negative for syphilis when subjected to a

Table 5
The results of RPR and TPPA test results in specimens with different values according to predefined and cut off_{95} (1.0 and 21.45 COI for EIA and 10 and 96.52 TU for TPLA, respectively) and the number of needed TPPA tests in different strategies.

	EIA (COI)			TPLA (TU)		
	≥1.0	≥1.0 < 21.45	≥21.45	≥10	≥10 < 96.52	≥96.52
Number	139	37	102	148	49	99
RPR negative	**43**	**24**	19	**53**	**32**	21
TPPA negative	11	8	3	20	11	9
	Predefined	Modified		Predefined	Modified	
No. of TPPA tests needed if applying cut offs	43	24[a]		53	32[a]	

Abbreviations: see Table 2.
[a] These specimens are RPR negative and have lower values than the cut off_{95} value (< 21.45 COI and < 96.52 TU, respectively).

Table 6
Detailed results for samples with ≥cut off$_{95}$ of EIA or TPLA and TPPA negative.

No.	EIA (COI)	TPLA (TU)	ICA	TPPA	RPR	Clinical findings
1	34.78	183.84	Positive	Negative	Non-reactive	Adenomyomatosis
2	47.80	96.52	Negative	Negative	Non-reactive	No specific history
3	54.94	340.31	Positive	Negative	Reactive	Past syphilis
4	57.54	137.80	Positive	Negative	Non-reactive	No specific history
5	0.08	96.52	Negative	Negative	Non-reactive	No specific history
6	20.32	100.77	Positive	Negative	Non-reactive	Arrythmia
7	20.58	144.51	Positive	Negative	Non-reactive	No specific history
8	21.18	128.60	Positive	Negative	Non-reactive	No specific history
9	6.64	144.70	Positive	Negative	Non-reactive	No specific history

Abbreviations: see Table 2.

second treponemal test, either TPPA or ICA. This finding means a more frequent false positive rate in TPLA. However, these samples were removed in the course of TPPA-confirmed algorithms, indicating that TPLA could be used in the site of EIA in reverse algorithms. When comparing ICA with TPPA, we found twelve specimens that were ICA-negative but TPPA-positive. This may be due to the low sensitivity of ICA or because the prozone phenomenon is more pronounced in ICA assays than in the TPPA assay [21]. Among 12 ICA-negative but TPPA-positive samples, 8 were discovered as being from patients who were either suspected of having syphilis, had active syphilis, or had latent syphilis (Table 2), indicating false negatives. However, in the absence of further characterization of the specimens in question using PCR and Western blot techniques, a clear answer to this problem is not yet available. On the other hand, only 20% of ten ICA-positive, TPPA negative specimens had a history of syphilis, suggesting that majority were possible false positives. Although line immunoassay such as INNO-LIA showed high specificity in high-risk population in a recent study, larger studies are recommended to better define the specificity in low prevalence populations [22].

Since TPPA can also result in a weak positive according to the manufacturer's insert, we noted each weak positive specimen for further investigation. There were seven specimens that showed a weak positive result in TPPA (Table 3). All seven specimens were positive for EIA, TPLA, and ICA. Out of the seven specimens, only one was found to have been from a patient with a past history of syphilis. Taken together, weak positive TPPA should not be ignored and needs to be confirmed by other tests.

We firstly calculated a high specific cut off of Roche EIA and Mediace TPLA. When the 95% cut-off value was applied, the number of required TPPA tests was reduced by 44% for EIA and 40% for TPLA, respectively (Table 5). Other studies that sought to reduce the number of secondary treponemal tests performed through applying different cut-off values for the initial treponemal test showed a reduction of roughly 60% [6,17]. A previous study that performed an analysis to determine the potential savings in using cut-off values to reduce the number of secondary treponemal tests indicated that TPPA tests were reduced by 65% [6]. A larger study done in the Los Angeles area calculated the incremental cost-effectiveness ratio which came out to US $39 per additional syphilis case detected [23]. Since syphilis diagnosis involves multiple tests, diagnosis takes precious time. So, it should be possible to reduce the number of tests performed and the time to a definitive diagnosis by attempting to find a threshold value for treponemal tests at which point performing additional tests. Because a syphilis diagnosis has such widespread consequences for a patient ranging from healthcare to social interactions, reducing the time needed for a definitive diagnosis would do much to reduce the anxiety experienced by a patient who has had a false positive result under the current testing algorithm. Another benefit would be a decrease in the number of tests performed, leading to increased laboratory efficiency. There were nine specimens that exceeded the cut off$_{95}$ for either EIA or TPLA while also having negative TPPA results (Table 6). Although these samples showed TPPA negativity, the majority of these samples showed positive results for at least two out of the other three treponemal tests, indicating possible true positives. There was only one sample (No. 5) that was ≥ TPLA cut off$_{95}$ but negative for all other tests, suggesting a false positive for TPLA. The TU of this sample was the same as the cut off$_{95}$ value (96.52).

There are several limitations in this study. The number of specimens that was EIA-positive and RPR-negative were small, so in the future a validation study will have to be performed with a larger number of specimens. Another limitation is an insufficient amount of clinical information. Many patients could not recognize primary infections or did not offer access to their syphilis treatment history.

In conclusion, TPLA showed almost perfect agreement with EIA and could be used in the site of EIA in a reverse sequence algorithm. ICA showed lower detection rate than TPPA as a 2nd treponemal test and should be used with caution. With a cut off of a higher specificity, a more efficient reverse-sequence algorithm could be possible in the future.

Conflicts of interest statement

This study was supported by Roche Diagnostics, Germany. The sponsor had no role in the study design, data collection and analysis, preparation of manuscript and decision to publish.

Acknowledgments

None.

References

[1] J.D. Radolf, R.K. Deka, A. Anand, D. Smajs, M.V. Norgard, X.F. Yang, *Treponema pallidum*, the syphilis spirochete: making a living as a stealth pathogen, Nat. Rev. Microbiol. 14 (2016) 744–759.
[2] L. Newman, J. Rowley, H.S. Vander, N.S. Wijesooriya, M. Unemo, N. Low, et al., Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting, PLoS One 10 (2012) e0143304.
[3] World Health Organization, Report on Global Sexually Transmitted Infection Surveillance, World Health Organization, Geneva, Switzerland, 2015.
[4] S.A. Larsen, B.M. Steiner, A.H. Rudolph, Laboratory diagnosis and interpretation of tests for syphilis, Clin. Micro. Rev. 8 (1995) 1–21.
[5] K.A. Workowski, S. Berman, Sexually transmitted diseases treatment guidelines, 2010, Morbidity and Mortality Weekly Report (MMWR), Recommendations and Reports. Centers for Disease Control and Prevention 59, 2010, pp. 1–110.
[6] G.J. Berry, M.J. Loeffelholz, Use of treponemal screening assay strenght of signal to avoid unnecessary confirmatory testing, Sex. Transm. Dis. 43 (2016) 737–740.
[7] M.J. Binnicker, D.J. Jespersen, L.O. Rollins, Direct comparison of the traditional and reverse syphilis screening algorithms in a population with a low prevalence of syphilis, J. Clin. Microbiol. 50 (2012) 148–150.
[8] J. Gratrix, S. Plitt, B.E. Lee, L. Ferron, B. Anderson, B. Verity, et al., Impact of reverse sequence syphilis screening on new diagnoses of late latent syphilis in Edmonton, Canada, Sex. Transm. Dis. 39 (2012) 528–530.
[9] M.J. Loeffelholz, M.J. Binnicker, It is time to use treponema-specific antibody screening tests for diagnosis of syphilis, J. Clin. Microbiol. 50 (2012) 2–6.
[10] S. Mishra, M.C. Boily, V. Ng, W.L. Gold, T. Okura, M. Shaw, et al., The laboratory

impact of changing syphilis screening from the rapid plasma reagin to a treponemal enzyme immunoassay: a case-study from the Greater Toronto Area, Sex. Transm. Dis. 38 (2011) 190–196.
[11] D.D. Rhoads, J.R. Genzen, C.P. Bashleben, J.D. Faix, M.Q. Ansari, Prevalence of traditional and reverse-algorithm syphilis screening in laboratory practice: a survey of participants in the college of american pathologists syphilis serology proficiency testing program, Arch. Pathol. Lab. Med. 141 (2017) 93–97.
[12] A.R. Rourk, F.S. Nolte, C.M. Litwin, Performance characteristics of the reverse syphilis screening algorithm in a population with a moderately high prevalence of syphilis, Am. J. Clin. Pathol. 146 (2016) 572–577.
[13] Centers for Disease Control and Prevention, Discordant results from reverse sequence syphilis screening–five laboratories, United States, 2006–2010, MMWR Morb. Mortal. Wkly Rep. 60 (2011) 133–137.
[14] M.L. McHugh, Interrater reliability: the kappa statistic, Biochem. Med. 22 (2012) 276–282.
[15] A. Marangoni, V. Sambri, S. Accardo, F. Cavrini, A. D'Antuono, A. Moroni, et al., Evaluation of LIAISON treponema screen, a novel recombinant antigen-based chemiluminescence immunoassay for laboratory diagnosis of syphilis, Clin. Diagn. Lab. Immunol. 12 (2005) 1231–1234.
[16] Y.J. Cha, J.H. Bae, S.J. Jung, S.L. Chae, Annual report on the external quality assessment scheme of viral markers and serological tests for syphilis in Korea [2015], J. Lab. Med. Qual. Assur. 38 (2016) 214–224.
[17] S. Dai, P. Chi, Y. Lin, X. Zheng, W. Liu, J. Zhang, et al., Improved reverse screening algorithm for Treponema pallidum antibody using signal-to-cutoff ratios from chemiluminescence microparticle immunoassay, Sex. Transm. Dis. 41 (2014) 29–34.
[18] S. Jonckheere, M. Berth, M. Van Esbroeck, S. Blomme, K. Lagrou, E. Padalko, Evaluation of different confirmatory algorithms using seven treponemal tests on Architect Syphilis TP-positive/RPR-negative sera, Eur. J. Clin. Microbiol. Infect. Dis. 34 (2015) 2041–2048.
[19] K. Lee, H. Park, E.Y. Roh, S. Shin, K.U. Park, M.H. Park, E.Y. Song, Characterization of sera with discordant results from reverse sequence screening for syphilis, Biomed. Res. Int. (2013) 269347, , https://doi.org/10.1155/2013/269347.
[20] H.J. Huh, J.W. Chung, S.Y. Park, S.L. Chae, Comparison of automated treponemal and nontreponemal test algorithms as first-line syphilis screening assays, Ann. Lab. Med. 36 (2016) 23–27.
[21] L.L. Liu, L.R. Lin, M.L. Tong, H.L. Zhang, S.J. Huang, Y.Y. Chen, et al., Incidence and risk factors for the prozone phenomenon in serologic testing for syphilis in a large cohort, Clin. Infect. Dis. 59 (2014) 384–389.
[22] I.U. Park, Y.F. Fakile, J.M. Chow, K.J. Gustafson, H. Jost, J.M. Schapiro, et al., Performance of treponemal tests for the diagnosis of syphilis, Clin. Infect. Dis. (2018) (Epub ahead of print).
[23] S.A. Buono, R. Basurto-Davila, H.A. Godwin, N.M. Green, Economic assessment of reverse algorithm syphilis screening in a high prevalence population, Sex. Transm. Dis. (2018) (Epub ahead of print).