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Original article

Comparison of the automated rapid plasma reagin (RPR) test versus the conventional RPR card test in syphilis testing

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0 Figures, 5 Tables, and 16 References

ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: Method comparative study using clinical remnant specimens in a single institute

Paticipants: A total of 112 serum samples including 59 TPPA- (Treponemal pallidum particle agglutination) positive and 53 TPPA-negative specimens were collected for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the auto RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed higher positivity than the automated RPR test, whereas the automated RPR test showed higher seronegative changes (43.5%, 10/23) than the RPR card test (4.3%, 1/23) after syphilis treatment.

Conclusions: The automated RPR test showed a lower sensitivity compared to the manual RPR test when comparing TPPA, but showed higher seronegative conversion after treatment

than the conventional RPR card test. The automated RPR test may be more useful to monitor treatment response than the conventional RPR card test.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.
- The automated RPR test showed a lower sensitivity compared to the manual RPR test when comparing TPPA.
- The automated RPR showed higher seronegative conversion after treatment than the conventional RPR card test. The automated RPR test may be more useful to monitor treatment response than the conventional RPR card test.
- Limitations of this study should be considered, including its small sample size and not categorized patients group according to the stage of syphilis infection.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have reached a plateau.[1] Still, syphilis is an important infectious disease because it can cause serious health problems including neurosyphilis and congenital infection. Therefore, appropriate screening, confirmation, and follow-up protocols [2-4] should be well established. Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponemal pallidum* particle agglutination (TPPA) test, and fluorescent treponemal antibody absorption test (FTA-ABS) have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with large scale samples, minimal person-to-person variation, simple automated procedures, and rapid reports with good analytical performances.

The aim of this study was to evaluate possible benefits of an automated RPR test compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 year, Male to Female = 25:34) and 53 non-syphilic controls (45±17 year, Male to Female = 27:26)were collected from November 2012 to April 2013 and preserved at -70℃ until analyses. Patients were not categorized according to syphilis stage due to the infrequency of syphilis patients. HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared with Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical histories of syphilis treatment.

Serologic tests

Conventional RPR card test

Briefly, the principle of the conventional manual RPR card test is as follows: the Macro-Vue RPR Card test uses cardiolipin antigen with a carbon particle to detect reagin, an antibody-like substance present in the serum or plasma of syphilis patients. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic flocculation. Controls were established in each day's testing to confirm optimal reactivity of the antigen. Each step of the test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. 15-μL serum samples were used for analysis and then 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) were reacted within 10 seconds. The absorption of reaction at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR that were equal to or greater than 1.0 RPR unit (R.U.) indicated a reactive result to syphilis. A CA-400 (Furuno Electric Co., Ltd. Nishinomiya, Japan) photometric analyzer was used for the automated procedure and analysis.

***Treponemal pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100-μL sample of diluent and 25 μL of test specimen were mixed first, then 2-fold serial dilutions were made with 25 μL of sample diluent. The 3rd well of the test plate was mixed with unsensitized particles, while sensitized particles from 4th well were serially mixed in the next wells with a plate mixer for 30 seconds. After a 2-hour incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted to compare the agglutination patterns of reagents and positive and negative controls. Sensitized particles (1:80 final dilution or greater) were considered to indicate a positive reaction. For quantitative results, the antibody titer was determined as being the final dilution showing a positive pattern.

Statistical analyses

The percent agreement, kappa coefficient, between automated RPR test and manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by IBM SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered as statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be moderate according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two kinds of RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were 4 results with discrepancies between both of the RPR tests and the

TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the auto RPR and manual RPR card tests was fair (kappa value: 0.296 (59 TPPA positive results); 0.293 (53 TPPA negative results)) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seronegative conversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Treponemal tests cannot discriminate between past infections, active disease, treated patients, and non-treated patients. Patients who are treponemal test-reactive have positive results for this test for the remainder of their lives, regardless of treatment or disease activity.[10] In contrast, non-treponemal tests can discriminate between patients who have been treated during the primary or secondary stage of the disease. Usually, the titer showed a 2-dilution decline after treatment.[7] Therefore, a guaranteed non-treponemal test is important for clinical settings to manage syphilitic patients.

We compared an automated RPR test with a conventional RPR card test including a TPPA test. The TPPA test was reported to be as sensitive as the FTA-ABS test in primary syphilis and as useful as the RPR test for monitoring therapy. The TPPA test is also less subjective

than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied in CSF to diagnose neurosyphilis.[12] Although the BD Macro-Vue RPR card test showed a better positivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test may be useful for monitoring treatment response, especially if treponemal-specific tests are used for first-line detection of syphilis as a reverse algorithm of syphilis screening. Recently, this reverse algorithm for syphilis screening has been suggested as a first-line screening for syphilis because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

The automated non-treponemal test has an additional important advantage over the conventional manual non-treponemal test in addition to those previously described. Our study presented that the automated RPR test showed earlier seronegative conversion than conventional card RPR test in discrimination after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be ideal in that the treponemal tests screen sensitively first and then the non-treponemal tests accurately show negative in the treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seronegative conversion more effectively after treatment.[2 13 14]

Unfortunately, this study had a limited number of syphilic patients due to low prevalence rate of syphilis and not been classified as each syphilis stage. Further, well designed studies will

be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and some evaluations comparing RPR tests to VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from those of the automated serological testing methods.[16] In the current study, we also noted moderately consistent results between auto RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is more effectively applicable in a reverse syphilis screening algorithm. Through the reverse syphilis screening algorithm, we could increase the detection sensitivity of syphilis screening, and the relatively low sensitivity of the automated RPR test may be compensated for by its rapid seronegative conversion after treatment.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. However, the automated RPR test may be more helpful to monitor the seroconversion responses to syphilis treatment, especially in the reverse syphilis screening algorithm. Further large-scale studies including patients well-categorized by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all stages of the study. Jong-Han Lee participated in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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No additional data are available.

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56
Number of observed agreements: 88 (78.6% of the observations)			
Kappa= 0.565			
95% confidence interval: 0.422 to 0.709			

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.

[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.

Table 2 Summary of the four patients showing discrepant results to treponemal test

Case No.	Age /Sex	RPR card test	Auto RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Table 3 Comparison of non-treponemal RPR tests according to Treponemal pallidum particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	31	20
	Negative	0	8
Number of observed agreements: 39 (66.1% of the observations)			
Kappa= 0.296			
95% confidence interval: 0.118 to 0.474			

TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	1	2
	Negative	2	48
Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.293			
95% confidence interval: -0.212 to 0.798			

Table 4 Overall Results of non-treponemal RPR tests

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1	0	1:5120	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

STARD checklist for reporting of studies of diagnostic accuracy
(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5-6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5-6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	5-6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	N/A
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	N/A
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	7
	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	8-10

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Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: Method comparative study using clinical specimens in a single institute

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the auto RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed higher seronegative changes (43.5%, 10/23) than the manual RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to manual RPR test based on treponemal test, TPPA, probably due to higher seronegative conversion after treatment than the conventional RPR card test. So, the automated RPR test might be

more useful to monitor treatment response than the conventional RPR card test, especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower sensitivity compared to the manual RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher seronegative conversion after treatment than the conventional manual RPR. So, the automated RPR test may be more useful to monitor treatment response than the conventional manual RPR, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis infection in Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Even though, syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. So, appropriate screening, confirmation, and follow-up protocols are well established. [2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with large scale samples, minimal person-to-person variation, simple automated procedures, and rapid reports with good analytical performances.

The aim of this study was to evaluate the possible benefits of an automated RPR test

compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34) and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26) were collected after treponemal test completed from November 2012 to April 2013 in a university hospital in Korea. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. There was a report that only 1,424 cases are registered in Korean Centers for Control and Prevention (KCDC) in 2007.

The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic

flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2 hours incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. And there were two false positive cases in HiSens Auto RPR LTIA test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were

4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the auto RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seronegative conversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between the past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-

treponemal test is important for managing the syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the confirmed patients' sera with TPPA. The TPPA test was reported to be as sensitive as the FTA-ABS test in all the stages of syphilis and as useful as the RPR test for monitoring therapy. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. But, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could be used as a useful monitoring marker of treatment response, especially if treponemal-specific tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and began to adopt because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

The automated non-treponemal test has an additional important advantage over the conventional manual non-treponemal test in addition to those previously described. Our study presented that the automated RPR test showed earlier seronegative conversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be ideal in that the treponemal tests screen sensitively first and then the

non-treponemal tests accurately show negative in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seronegative conversion more effectively after treatment.[2 13 14] Unfortunately, our study had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-trponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between auto RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is more effectively applicable in a reverse syphilis screening algorithm. Through the reverse syphilis screening algorithm, we could increase the detection sensitivity of syphilis screening, and the automated RPR test may be useful after treatment for its rapid seronegative conversion. The automated RPR could be more useful in the treated cases though it's sensitivity is lower after treatment.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. However, the automated

RPR test may be more helpful to monitor the seroconversion responses in treated syphilis patients, especially in the reverse syphilis screening algorithm. Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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No additional data are available.

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56
Number of observed agreements: 88 (78.6% of the observations)			
Kappa= 0.565			
95% confidence interval: 0.422 to 0.709			

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.

[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.

Abbreviations: RPR, rapid plasma regain.

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Auto RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR		TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative			Positive	Negative
BD Macro-Vue RPR card	Positive	31	20	BD Macro-Vue RPR card	Positive	1	2
	Negative	0	8		Negative	2	48
Number of observed agreements: 39 (66.1% of the observations)				Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.296				Kappa= 0.293			
95% confidence interval: 0.118 to 0.474				95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

Original article

Comparison of anthe automated rapid plasma reagin (RPR) test toversus the conventional RPR card test in syphilis testing

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Participants:~~**Paticipants:**~~ A total of 112 serum samples including 59 TPPA-(*Treponema* ~~(Treponemal~~ *pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included~~collected~~ for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the auto RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed higher seronegative changes (43.5%, 10/23) than the manual RPR card test (4.3%, 1/23) in treated patients ~~after syphilis treatment.~~

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Conclusions: The automated RPR test showed overall~~a~~ lower sensitivity compared to ~~the~~ manual RPR test based on treponemal test,~~when comparing~~ TPPA, probably due to~~but showed~~ higher seronegative conversion after treatment than the conventional RPR card test. So, the~~The~~ automated RPR test might~~may~~ be more useful to monitor treatment response than the conventional RPR card test, especially in the reverse screening algorithm in syphilis testing.~~test.~~

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared~~but variable results were reported when comparing~~ the automated test to conventional RPR card tests.
- The automated RPR ~~test~~ showed overall~~a~~ lower sensitivity compared to the manual RPR ~~test~~ when comparing to treponemal test, TPPA.
- The automated RPR showed higher seronegative conversion after treatment than the conventional manual RPR. So, the~~ card test. The~~ automated RPR test may be more useful to monitor treatment response than the conventional manual RPR, especially in the reverse screening algorithm for syphilis testing.~~RPR card test.~~
- ~~■~~ Limitations of this study could~~should~~ be considered, including ~~its~~ small sample size and the patient groups could not accurately categorized ~~patients group~~ according to the stage because of low prevalence of syphilis infection in Korea.~~.~~

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INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] ~~reached a plateau.~~[1] Even though, ~~Still,~~ syphilis is an important infection ~~infectious disease~~ because it can cause serious health problems including neurosyphilis and congenital infection. So, ~~Therefore,~~ appropriate screening, confirmation, and follow-up protocols are[2-4] ~~should be~~ well established.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema~~l~~ pallidum* particle agglutination (TPPA) test, ~~and~~ fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC

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to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first--line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with large scale samples, minimal person-to-person variation, simple automated procedures, and rapid reports with good analytical performances.

The aim of this study was to evaluate the possible benefits of an automated RPR test compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 years-old,~~year,~~ Male to Female = 25:34) and 53 non-syphilic controls (45±17 years-old,~~year,~~ Male to Female = 27:26) ~~)~~were collected after treponemal test completed from November 2012 to April 2013 in a university hospital in Korea. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis.~~analyses.~~ Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. There was a report that only 1,424 cases are registered in Korean Centers for Control and Prevention (KCDC) in 2007.~~-~~

The automated~~HiSens Auto~~ RPR test~~LTIA (HBI Co., Ltd, Anyang, Korea)~~ was compared

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with manual card~~Macro-Vue~~ RPR Test~~Card Tests~~ (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA)~~Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan)~~ according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history~~histories~~ of syphilis treatment.

Serologic tests

Conventional RPR card test

The~~Briefly, the principle of the conventional manual RPR card test is as follows: the~~ Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin.~~reagin, an antibody-like substance present in the serum or plasma of syphilis patients.~~ Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic flocculation. Controls were established in each ~~day's~~ testing to confirm optimal reactivity of the antigen. The~~Each step of the~~ test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with~~used for analysis and then~~ 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-

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400 photometric analyzer was used for the automated procedure and analysis. The absorbance~~were reacted within 10 seconds. The absorption of reaction~~ at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR ~~that were~~ equal to or greater than 1.0 RPR unit (R.U.) were considered as~~indicated a~~ reactive RPR. The upper detection limit~~result to syphilis. A CA-400 (Furuno Electric Co., Ltd. Nishinomiya, Japan) photometric analyzer~~ was 20 R.U.. ~~used for the automated procedure and analysis.~~

***Treponema~~l~~ pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 -µL sample of diluent and 25 µL of test specimen were mixed first and~~, then~~ 2-fold serial dilutions were made with 25 µL of sample diluent. The ~~3rd well of the test plate was mixed with unsensitized particles, while~~ sensitized particles ~~from 4th well~~ were serially mixed in the next wells with a plate mixer for 30 seconds. After ~~a~~ 2 hours~~ hour~~ incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by~~to compare~~ the agglutination patterns with ~~of reagents and~~ positive and negative controls. ~~Sensitized particles (1:80 final dilution or greater) were considered to indicate a positive reaction. For quantitative results, the antibody titer was determined as being the final dilution showing a positive pattern.~~

Statistical analyses

The percent agreement, kappa coefficient, of~~between~~ automated RPR test with~~and~~ manual RPR card test was calculated. The overall sensitivity and specificity of each test were

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calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by ~~IBM~~ SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered ~~as~~ statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two ~~kinds of~~ RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. And there were two false positive cases in HiSens Auto RPR LTIA test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test.

There were 4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the auto RPR and manual RPR card tests was "fair" (kappa value: 0.296, ~~(~~59 TPPA positive results;~~results);~~ 0.293, ~~(~~53 TPPA negative results)~~)~~ according to TPPA

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results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seronegative conversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity.

Treponemal tests cannot discriminate between the past infections, active disease, treated patients, and non-treated patients.[10] ~~Patients who are treponemal test reactive have positive results for this test for the remainder of their lives, regardless of treatment or disease activity.~~[10] In contrast, non-treponemal tests can discriminate between patients who have been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test ~~Usually, the~~ titer should show~~showed~~ a 2-dilution decline after treatment, usually within 6 months.[7] ~~treatment.~~[7]

Therefore, ~~a guaranteed~~ non-treponemal test is important for managing the~~clinical settings to~~

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~~manage~~ syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the confirmed patients' sera with~~cluding a~~ TPPA~~. test.~~ The TPPA test was reported to be as sensitive as the FTA-ABS test in all the stages of ~~primary~~ syphilis and as useful as the RPR test for monitoring therapy. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to ~~in~~ CSF samples to diagnose neurosyphilis.[12]

In our study,~~Although~~ the BD Macro-Vue RPR card test showed ~~a~~ better sensitivity~~positivity~~ than did the HBI HiSens Auto RPR LTIA test in syphilis screening. But,~~screening,~~ the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could~~may~~ be used as a useful ~~for~~ monitoring marker of treatment response, especially if treponemal-specific tests are used for first-line screening~~detection~~ of syphilis as a reverse algorithm of syphilis testing.~~screening.~~ Recently, this reverse algorithm for syphilis testing~~screening~~ has been suggested and began to adopt~~as a first-line screening for syphilis~~ because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

The automated non-treponemal test has an additional important advantage over the conventional manual non-treponemal test in addition to those previously described. Our study presented that the automated RPR test showed earlier seronegative conversion than

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conventional card RPR test ~~in discrimination~~ after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be ideal in that the treponemal tests screen sensitively first and then the non-treponemal tests accurately show negative in ~~the~~ treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seronegative conversion more effectively after treatment.[2 13 14]

Unfortunately, our~~this~~ study had a limited number of syphilitic~~syphilis~~ patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to~~as each~~ syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-trponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further ~~Further,~~ well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and ~~some~~ evaluations comparing conventional RPR tests and~~to~~ VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from ~~those of~~ the automated serological testing methods.[16] In this~~the current~~ study, we noticed~~also noted~~ moderately consistent results between auto RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is more effectively applicable in a reverse syphilis screening algorithm. Through the reverse syphilis screening algorithm, we could increase the detection sensitivity of syphilis screening, and the ~~relatively low sensitivity of the~~ automated RPR test may be useful after

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treatment~~compensated~~ for ~~by~~ its rapid seronegative conversion. The automated RPR could be more useful in the treated cases though it's sensitivity is lower after treatment.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. However, the automated RPR test may be more helpful to monitor the seroconversion responses in treated~~to~~ syphilis patients~~treatment~~, especially in the reverse syphilis screening algorithm. Further large-scale studies including ~~patients~~ well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this ~~the~~ study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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No additional data are available.

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56
Number of observed agreements: 88 (78.6% of the observations)			
Kappa= 0.565			
95% confidence interval: 0.422 to 0.709			

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.

[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.

Abbreviations: RPR, rapid plasma regain.

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Table 2 Discrepant RPR~~Summary of the four patients showing discrepant~~ results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Auto RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

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Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	31	20
	Negative	0	8
Number of observed agreements: 39 (66.1% of the observations)			
Kappa= 0.296			
95% confidence interval: 0.118 to 0.474			

TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	1	2
	Negative	2	48
Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.293			
95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

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Table 4 Performance characteristics~~Overall Results~~ of ~~non-treponemal~~ RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

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Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1	0	1:5120	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

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STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5-6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5-6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	5-6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	N/A
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	N/A
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	7
	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	8-10

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Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: A comparative study of lab methods using clinical specimens in a single institute.

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the automated RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed more seroconversion changes (43.5%, 10/23) than the manual RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to conventional RPR test based on treponemal test. But, the automated RPR test showed more seroconversion after treatment than the conventional RPR card test. The automated RPR test

might be used to monitor treatment response, especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower sensitivity compared to the conventional RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher seroconversion after treatment than the conventional manual RPR. So, the automated RPR test may be used to monitor treatment response, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis in Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Despite these low rates, syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. So, appropriate screening, confirmation, and follow-up protocols are well established.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with a large number of samples, minimal person-to-person variation, and simple automated procedures.

The aim of this study was to evaluate the possible benefits of an automated RPR test

compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34, ratio = 0.7) and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26, ratio = 1) after treponemal test were collected from November 2012 to April 2013 in a university hospital in Korea. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. There was a report that only 1,424 cases are registered in Korean Centers for Control and Prevention (KCDC) in 2007.
The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic

flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2 hours of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were 4 results with discrepancies between both of the RPR tests and the

TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the automated RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seroconversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-

treponemal test is important for managing syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the sera confirmed by TPPA. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. Though, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could be used as a monitoring marker of treatment response, especially if treponemal tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and has been adopted in many jurisdictions because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] in a low prevalence area and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

Our study presented that the automated RPR test showed earlier seroconversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be used that the treponemal tests screen sensitively first and then the non-treponemal tests accurately show negative change in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seroconversion more effectively after treatment.[2 13 14] Unfortunately, our study

had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-trponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between automated RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is effectively applicable. Through the reverse syphilis screening algorithm, we can increase the detection sensitivity of syphilis screening by treponemal test screening initially and the automated RPR test may be used after treatment for its rapid seroconversion, though the sensitivity of automated RPR is lower than manual RPR.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to be earlier seroconversion response in treated cases than those of conventional RPR card test. If reverse algorithm is applied, sensitive treponemal tests were used in first

line, and then the automated RPR might be used as adjunct to detect earlier seroconversion in treated patients.

Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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No additional data are available.

No data sharing

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56

Number of observed agreements: 88 (78.6% of the observations)
Kappa= 0.565
95% confidence interval: 0.422 to 0.709

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.
[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.
Abbreviations: RPR, rapid plasma reagin.

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Automated RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR		TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative			Positive	Negative
BD Macro-Vue RPR card	Positive	31	20	BD Macro-Vue RPR card	Positive	1	2
	Negative	0	8		Negative	2	48
Number of observed agreements: 39 (66.1% of the observations)				Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.296				Kappa= 0.293			
95% confidence interval: 0.118 to 0.474				95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

STARD checklist for reporting of studies of diagnostic accuracy
(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5-6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5-6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	5-6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	N/A
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	N/A
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	7
	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	8-10

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Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: A comparative study of lab methods using clinical specimens in a single institute.

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the automated RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed more seroconversion changes (43.5%, 10/23) than the manual RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to conventional RPR test based on treponemal test. But, the automated RPR test showed more seroconversion after treatment than the conventional RPR card test. The automated RPR test

might be used to monitor treatment response, especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower sensitivity compared to the conventional RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher seroconversion after treatment than the conventional manual RPR. So, the automated RPR test may be used to monitor treatment response, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis in Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Despite these low rates, syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. So, appropriate screening, confirmation, and follow-up protocols are well established.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with a large number of samples, minimal person-to-person variation, and simple automated procedures.

The aim of this study was to evaluate the possible benefits of an automated RPR test

compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34, ratio = 0.7) and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26, ratio = 1) after treponemal test were collected from November 2012 to April 2013 in a university hospital in Korea. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. This study targeted to evaluate the same RPR tests with ethically protected remnant specimens. This case was Institutional Review Board (IRB) exempted in our institution.

The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test

antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2 hours of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and

negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the

TPPA test. There were 4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the automated RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seroconversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should

show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-treponemal test is important for managing syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the sera confirmed by TPPA. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. Though, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could be used as a monitoring marker of treatment response, especially if treponemal tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and has been adopted in many jurisdictions because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] in a low prevalence area and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

Our study presented that the automated RPR test showed earlier seroconversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be used that the treponemal tests screen sensitively first and then the non-treponemal tests accurately show negative change in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the

patients to see seroconversion more effectively after treatment.[2 13 14] Unfortunately, our study had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-trponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between automated RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is effectively applicable. Through the reverse syphilis screening algorithm, we can increase the detection sensitivity of syphilis screening by treponemal test screening initially and the automated RPR test may be used after treatment for its rapid seroconversion, though the sensitivity of automated RPR is lower than manual RPR.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to be earlier seroconversion response in treated cases than those of conventional

RPR card test. If reverse algorithm is applied, sensitive treponemal tests were used in first line, and then the automated RPR might be used as adjunct to detect earlier seroconversion in treated patients.

Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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Data sharing statement

No additional data are available.

No data sharing

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56

Number of observed agreements: 88 (78.6% of the observations)
Kappa= 0.565
95% confidence interval: 0.422 to 0.709

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.
[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.
Abbreviations: RPR, rapid plasma reagin.

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Automated RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR		TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative			Positive	Negative
BD Macro-Vue RPR card	Positive	31	20	BD Macro-Vue RPR card	Positive	1	2
	Negative	0	8		Negative	2	48
Number of observed agreements: 39 (66.1% of the observations)				Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.296				Kappa= 0.293			
95% confidence interval: 0.118 to 0.474				95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: AMethod comparative study of lab methods using clinical specimens in a single institute.

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the automated RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed more seroconversionhigher seronegative changes (43.5%, 10/23) than the manual RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to conventional~~manual~~ RPR test based on treponemal test. But, the automated RPR test showed more sero~~test, TPPA, probably due to higher seronegative~~ conversion after treatment than the conventional RPR card test. The~~So, the~~ automated RPR test might be used~~more useful~~ to monitor treatment response~~, than the conventional RPR card test,~~ especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower sensitivity compared to the conventional~~manual~~ RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher sero~~seronegative~~ conversion after treatment than the conventional manual RPR. So, the automated RPR test may be used~~more useful~~ to monitor treatment response~~, than the conventional manual RPR,~~ especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis in~~fection in~~ Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Despite these low rates,~~Even though,~~ syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. So, appropriate screening, confirmation, and follow-up protocols are well established.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with a large number of~~scale~~ samples, minimal person-to-person variation, and simple automated procedures.~~procedures, and rapid reports with good analytical performances.~~

The aim of this study was to evaluate the possible benefits of an automated RPR test

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compared to a conventional RPR card test in clinical application.

METHODS

Subjects

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34, ratio = 0.7)~~25:34)~~ and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26, ratio = 1)~~27:26) were collected~~ after treponemal test were collected~~completed~~ from November 2012 to April 2013 in a university hospital in Korea. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. This study targeted to evaluate the same RPR tests with ethically protected remnant specimens. This case was Institutional Review Board (IRB) exempted in our institution.~~There was a report that only 1,424 cases are registered in Korean Centers for Control and Prevention (KCDC) in 2007.~~

The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2

hours of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. ~~And there were two false-positive cases in HiSens Auto RPR LTIA~~

test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2 cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were 4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the automated RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seroseronegative conversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between the past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have

been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-treponemal test is important for managing ~~the~~ syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the sera confirmed by~~patients' sera with~~ TPPA. ~~The TPPA test was reported to be as sensitive as the FTA-ABS test in all the stages of syphilis and as useful as the RPR test for monitoring therapy.~~ The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. Though,~~But,~~ the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts.

Also, we noticed that the automated RPR test could be used as a ~~useful~~ monitoring marker of treatment response, especially if treponemal ~~specific~~ tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and has been adopted in many jurisdictions~~began to adopt~~ because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] in a low prevalence area and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

~~The automated non-treponemal test has an additional important advantage over the~~

~~conventional manual non-treponemal test in addition to those previously described.~~ Our study presented that the automated RPR test showed earlier sero~~seronegative~~ conversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be used~~ideal in~~ that the treponemal tests screen sensitively first and then the non-treponemal tests accurately show negative change in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see sero~~seronegative~~ conversion more effectively after treatment.[2 13 14] Unfortunately, our study had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-trponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between automated RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is ~~more~~ effectively applicable~~. in a reverse syphilis screening algorithm.~~ Through the reverse syphilis screening algorithm, we can~~could~~ increase the detection sensitivity of

syphilis screening by treponemal test screening initially~~,~~ and the automated RPR test may be used~~useful~~ after treatment for its rapid seroconversion,~~seronegative conversion. The automated RPR could be more useful in the treated cases~~ though the ~~it's~~ sensitivity of automated RPR is lower than manual RPR.~~after treatment.~~

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In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to~~test may~~ be earlier ~~more helpful to monitor the~~ seroconversion response~~s~~ in treated cases than those of conventional RPR card test. If~~syphilis patients, especially in the~~ reverse ~~syphilis screening~~ algorithm is applied, sensitive treponemal tests were used in first line, and then the automated RPR might be used as adjunct to detect earlier seroconversion in treated patients.

~~.~~Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae

Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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Competing interests

None.

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Data sharing statement

No additional data are available.

No data sharing

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56
Number of observed agreements: 88 (78.6% of the observations)			
Kappa= 0.565			
95% confidence interval: 0.422 to 0.709			

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.
[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.
Abbreviations: RPR, rapid plasma reagin ~~regain.~~

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Automated RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

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Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR		TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative			Positive	Negative
BD Macro-Vue RPR card	Positive	31	20	BD Macro-Vue RPR card	Positive	1	2
	Negative	0	8		Negative	2	48
Number of observed agreements: 39 (66.1% of the observations)				Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.296				Kappa= 0.293			
95% confidence interval: 0.118 to 0.474				95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

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STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5-6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5-6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	5-6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	N/A
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	N/A
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	7
	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	8-10

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Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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0 Figures, 5 Tables, and 16 References

ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: A comparative study of lab methods using clinical specimens in a single institute.

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the automated RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed more seroconversion changes (43.5%, 10/23) than the conventional RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to conventional RPR test based on treponemal test. But, the automated RPR test showed more seroconversion after treatment than the conventional RPR card test. The automated RPR test

might be used to monitor treatment response, especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower positivity compared to the conventional RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher seroconversion after treatment than the conventional manual RPR. So, the automated RPR test may be used to monitor treatment response, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis in Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Despite these low rates, syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. Appropriate screening, confirmation, and follow-up protocols are required.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with a large number of samples, minimal person-to-person variation, and simple automated procedures.

The aim of this study was to evaluate the possible benefits of an automated RPR test

compared to a conventional RPR card test in clinical application.

METHODS

Subjects

All positive sera for syphilis by one or more tests from November 2012 to April 2013 from a university hospital were included along with matched controls. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. This study targeted to evaluate the same RPR tests with ethically protected remnant specimens. This case was Institutional Review Board (IRB) exempted in our institution. All the study processes were followed by the World Medical Association Declaration of Helsinki. The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic

flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2 hours of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34, ratio = 0.7) and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26, ratio = 1) after treponemal test were collected from November 2012 to April 2013 in a university hospital in Korea.

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be “moderate” according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2

cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were 4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the automated RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seroconversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have

been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-treponemal test is important for managing syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the sera confirmed by TPPA. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. Though, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could be used as a monitoring marker of treatment response, especially if treponemal tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and has been adopted in many jurisdictions because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] in a low prevalence area and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

Our study presented that the automated RPR test showed earlier seroconversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be used that the treponemal tests screen sensitively first and then the non-

treponemal tests accurately show negative change in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seroconversion more effectively after treatment.[2 13 14] Unfortunately, our study had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-treponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between automated RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is effectively applicable. Through the reverse syphilis screening algorithm, we can increase the detection sensitivity of syphilis screening by treponemal test screening initially and the automated RPR test may be used after treatment for its rapid seroconversion, though the sensitivity of automated RPR is lower than manual RPR.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that

automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to be earlier seroconversion response in treated cases than those of conventional RPR card test. If reverse algorithm is applied, sensitive treponemal tests are used as a first line screening test, and then the automated RPR might be used as an adjunct to detect earlier seroconversion in treated patients.

Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56
Number of observed agreements: 88 (78.6% of the observations)			
Kappa= 0.565			
95% confidence interval: 0.422 to 0.709			

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.

[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.

Abbreviations: RPR, rapid plasma reagin.

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Automated RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR		TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative			Positive	Negative
BD Macro-Vue RPR card	Positive	31	20	BD Macro-Vue RPR card	Positive	1	2
	Negative	0	8		Negative	2	48
Number of observed agreements: 39 (66.1% of the observations)				Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.296				Kappa= 0.293			
95% confidence interval: 0.118 to 0.474				95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
HBI HiSens Auto RPR	Positive	31	3
	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

Original article

Comparison of an automated rapid plasma reagin (RPR) test to the conventional RPR card test in syphilis testing

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ABSTRACT

Objective: We compared the automated non-treponemal reagin (rapid plasma reagin, RPR) test to the conventional RPR card test for usefulness in clinical applications.

Setting: A comparative study of lab methods using clinical specimens in a single institute.

Participants: A total of 112 serum samples including 59 TPPA-(*Treponema pallidum* particle agglutination) positive and 53 TPPA-negative specimens were included for this evaluation.

Outcome measures: HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) was compared to Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan). The percent agreement, kappa value, and overall sensitivity and specificity were compared between the two RPR tests. Also, seroconversion rates after treatment were compared by each RPR test.

Results: The agreement of both RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709). Sensitivity and specificity of the automated RPR test to TPPA was 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed more seroconversion changes (43.5%, 10/23) than the conventional RPR card test (4.3%, 1/23) in treated patients.

Conclusions: The automated RPR test showed overall lower sensitivity compared to conventional RPR test based on treponemal test. But, the automated RPR test showed more seroconversion after treatment than the conventional RPR card test. The automated RPR test

might be used to monitor treatment response, especially in the reverse screening algorithm in syphilis testing.

Key words: Syphilis, Automated RPR (Rapid plasma reagin), RPR card, Agreement

Strengths and limitations of this study

- Automated rapid plasma regain (RPR) tests have been introduced in clinical laboratories, so we compared the automated test to conventional RPR card tests.
- The automated RPR showed overall lower positivity compared to the conventional RPR when comparing to treponemal test, TPPA.
- The automated RPR showed higher seroconversion after treatment than the conventional manual RPR. So, the automated RPR test may be used to monitor treatment response, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study could be considered, including small sample size and the patient groups could not accurately categorized according to the stage because of low prevalence of syphilis in Korea.

INTRODUCTION

Positive rates for syphilis have rapidly decreased since the 1970s in Korea, consistent with the global trend. In 2000, approximately 0.2% of the general Korean population was estimated to be syphilis-positive, and since that time, levels have appeared to have decreased, and the prevalence rate is still very low.[1] Despite these low rates, syphilis is an important infection because it can cause serious health problems including neurosyphilis and congenital infection. Appropriate screening, confirmation, and follow-up protocols are required.[2-4] Serological analysis of non-treponemal reagin tests such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), and treponemal tests such as the *Treponema Pallidum* hemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption test (FTA-ABS) and Treponema-specific antibody test have been used to diagnose and monitor syphilis infections. Recently, there have been issues regarding selection of the best algorithm for initial screening and follow-up by either non-treponemal or treponemal-specific tests.[2 5 6] A non-treponemal reagin test is still recommended by the CDC to be used as a first line diagnostic approach.[2] Two kinds of non-treponemal test have been widely used, the VDRL and RPR methods. RPR is the most common first-line non-treponemal test used to screen for syphilis infection.[7] Recently, automated RPR tests have been introduced but variable results were reported when comparing the automated test to conventional RPR card tests.[8] The automated RPR test has some advantages over the conventional RPR card test such as greater capacity to deal with a large number of samples, minimal person-to-person variation, and simple automated procedures.

The aim of this study was to evaluate the possible benefits of an automated RPR test

compared to a conventional RPR card test in clinical application.

METHODS

Subjects

All positive sera for syphilis by one or more tests from November 2012 to April 2013 from a university hospital were included along with matched controls. Remnant sera from requested treponemal test after confirmation were included and preserved at -70℃ until analysis. Patients were not categorized according to the syphilis stage due to the infrequency of syphilis infection. True syphilis patients were very rare because of its low prevalence in this country. This study targeted to evaluate the same RPR tests with ethically protected remnant specimens. This case was Institutional Review Board (IRB) exempted in our institution. All the study processes were followed by the World Medical Association Declaration of Helsinki. The automated RPR test was compared with manual card RPR Test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA). A confirmatory treponemal-specific test was performed by a *Treponema pallidum* particle agglutination assay (TPPA) according to the manufacturer's instructions. Seroconversion rates of each non-treponemal RPR tests were evaluated with 23 syphilic patients who had medical history of syphilis treatment.

Serologic tests

Conventional RPR card test

The Macro-Vue RPR Card test (Becton Dickinson BD Microbiology Systems, Sparks, MD, USA) uses cardiolipin antigen with a carbon particle to detect reagin. Reagin binds to the test antigen, which consists of cardiolipin-lecithin-cholesterol particles, causing macroscopic

flocculation. Controls were established in each testing to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer's instructions.

Automated RPR

HiSens Auto RPR LTIA (HBI Co., Ltd, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particle reacts with the reagin in the serum of syphilis patients. The 15 μL serum samples were reacted with 120 μL Hisens auto RPR LTIA R1 (buffer) and 60 μL Hisens auto RPR LTIA R2 (latex reagent containing cardiolipin-lecithin-cholesterol 1.0 mg/mL) in CA-400 autoanalyzer (Furuno Electric Co., Ltd. Nishinomiya, Japan). The CA-400 photometric analyzer was used for the automated procedure and analysis. The absorbance at 600 nm was read after 5.3 seconds and 10 seconds at room temperature, in duplicate. Results of the HiSens auto RPR equal to or greater than 1.0 RPR unit (R.U.) were considered as reactive RPR. The upper detection limit was 20 R.U..

***Treponema pallidum* particle agglutination (TPPA)**

The Serodia TPPA assay (Fujirebio, Inc., Tokyo, Japan) is based on the agglutination of colored gelatin particles that have been sensitized (coated) with *T. pallidum* (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL of test specimen were mixed first and 2-fold serial dilutions were made with 25 μL of sample diluent. The sensitized particles were serially mixed in the next wells with a plate mixer for 30 seconds. After 2 hours of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted by the agglutination patterns with positive and negative controls.

Statistical analyses

The percent agreement, kappa coefficient, of automated RPR test with manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the data from TPPA results. Kappa values were used to categorize results as very good (0.81 to 1.0), good (0.61 to 0.8), moderate (0.41 to 0.6), fair (0.21 to 0.4), or poor (0 to 0.2).[9] The McNemar test was used to compare the seroconversion rate between the automated RPR test and the conventional manual RPR card test and was performed by SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 112 serum samples from 59 syphilis patients (48±21 years-old, Male to Female = 25:34, ratio = 0.7) and 53 non-syphilic controls (45±17 years-old, Male to Female = 27:26, ratio = 1) after treponemal test were collected from November 2012 to April 2013 in a university hospital in Korea.

The percent agreement of the two RPR tests was 78.6% (kappa: 0.565; 95% CI: 0.422-0.709, Table 1). The strength of agreement between the automated RPR test and manual RPR card test was considered to be "moderate" according to the kappa value scale. The specimens of both RPR tests positive results (n=32) showed 96.9% (31/32) TPPA-positive and both RPR negative results (n=56) showed 85.7% (48/56) TPPA-negative results.

There were 24 discrepant results (21.4%) between the two RPR tests, including 22 negative cases of HBI HiSens Auto RPR LTIA test results that showed positive results by the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 cases were TPPA-positive and 2

cases were TPPA-negative, while 2 cases were positive in the HBI HiSens Auto RPR LTIA test but negative in the BD Macro-Vue RPR card test. These 2 cases were negative in the TPPA test. There were 4 results with discrepancies between both of the RPR tests and the TPPA assay which were due to conditions other than syphilis infections (Table 2). The strength of agreement between the automated RPR and manual RPR card tests was "fair" (kappa value: 0.296, 59 TPPA positive results; 0.293, 53 TPPA negative results) according to TPPA results (Table 3).

The overall sensitivity and specificity of the HBI HiSens Auto RPR LTIA test based on TPPA results were 52.5% (95% CI: 39.1%-65.7%) and 94.3% (95%CI: 84.3%-98.8%), respectively. The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI: 75%-93.9%) and 94.3% (95% CI: 84.3%-98.8%), respectively (Table 4). Automated RPR gave a higher seroconversion rate after syphilis treatment, with a value of 43.5% (10/23), than that of the conventional RPR card test, which was 4.3% (1/23, p=0.004) by McNemar test. Detailed comparison results of treated syphilis cases are described in Table 5.

DISCUSSION

Manual RPR test has been used for decades, and recently the automated RPR test method was launched and has been used because of its convenience in clinical settings. However, there has been the need to thorough inspection and comparing results of this new automated test to conventional manual RPR test in the diagnostic approaches. The treponemal test results will not change even after treatment, and the patients live with positive results for the remainder of their lives regardless of treatment or disease activity. Treponemal tests cannot discriminate between past infections, active disease, treated patients, and non-treated patients.[10] In contrast, non-treponemal tests can discriminate between patients who have

been treated during the primary or secondary stage of the disease. When the primary or secondary stage of a first *T. pallidum* infection is treated, nontreponemal test titer should show a 2-dilution decline after treatment, usually within 6 months.[7] Therefore, non-treponemal test is important for managing syphilitic patients.

We compared an automated RPR test with a conventional RPR card test in the sera confirmed by TPPA. The TPPA test is also known to be less subjective than the FTA-ABS and easier to read than the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP).[11] TPPA has also been suggested to be applied to CSF samples to diagnose neurosyphilis.[12]

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than did the HBI HiSens Auto RPR LTIA test in syphilis screening. Though, the automated RPR test does have some advantages in the clinical settings. For example, the automated RPR test reduced the workload and overall test turn-around time. It also can deal with greater test quantities in a given time than the manual RPR card test, and does not require test experts. Also, we noticed that the automated RPR test could be used as a monitoring marker of treatment response, especially if treponemal tests are used for first-line screening of syphilis as a reverse algorithm of syphilis testing. Recently, this reverse algorithm for syphilis testing has been suggested and has been adopted in many jurisdictions because this approach may be more sensitive and effective than the traditional algorithm [3 4 6] in a low prevalence area and could be automated. However, the Centers for Disease Control and Prevention (CDC) still recommend screening for syphilis with a non-treponemal test first, such as RPR.[2]

Our study presented that the automated RPR test showed earlier seroconversion than conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, it could be used that the treponemal tests screen sensitively first and then the non-

treponemal tests accurately show negative change in treated cases. In this situation we could use treponemal tests for first-line screening and non-treponemal tests for monitoring the patients to see seroconversion more effectively after treatment.[2 13 14] Unfortunately, our study had a limited number of syphilitic patients due to low prevalence rate of syphilis in our country and the number of samples was small and could not been classified according to syphilis stage. In fact, some late or latent syphilis cases were hard to interpret the results of non-treponemal test after initial treatment in our study (Case No. 8 or 9 in Table 5). So, further well designed studies will be needed to clarify the serologic responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have been recently introduced in clinical laboratories and evaluations comparing conventional RPR tests and VDRL tests were reported.[8 15] However, the results were variable. Tomohiko et al. also suggested that when the automated serological testing method is used in clinical settings, the same reagent should be consistently selected to evaluate the changes in antibody titers because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods.[16] In this study, we noticed moderately consistent results between automated RPR and manual RPR.

We found that the automated RPR has a greater processing capability within a limited time and is effectively applicable. Through the reverse syphilis screening algorithm, we can increase the detection sensitivity of syphilis screening by treponemal test screening initially and the automated RPR test may be used after treatment for its rapid seroconversion, though the sensitivity of automated RPR is lower than manual RPR.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared to the conventional manual RPR card test. Therefore, we thought that

automated RPR is not matched to use as initial screening of syphilis. However, the automated RPR seems to be earlier seroconversion response in treated cases than those of conventional RPR card test. If reverse algorithm is applied, sensitive treponemal tests are used as a first line screening test, and then the automated RPR might be used as an adjunct to detect earlier seroconversion in treated patients.

Further large-scale studies including well-categorized patients by syphilis stage are warranted to clarify the accurate diagnostic efficiency of the automated RPR test.

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Contributors

Hyon-Suk Kim designed and participated in all the stages of this study. Jong-Han Lee participated in the experiments and in statistical analyses and draft the manuscript. Chae Seung Lim and Min-Geol Lee helped to consultations of this study. All authors read and approved the final manuscript.

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No data sharing

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Table 1 Comparison of non-treponemal RPR tests for syphilis detection

		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	32	22[1)]
	Negative	2[2)]	56

Number of observed agreements: 88 (78.6% of the observations)
Kappa= 0.565
95% confidence interval: 0.422 to 0.709

[1)] The 20 cases were positive and 2 cases (Case No. 1, 2 in Table 2) were negative in TPPA test.
[2)] The 2 cases (Case No. 3, 4 in Table 2) were negative in TPPA test.
Abbreviations: RPR, rapid plasma reagin.

Table 2 Discrepant RPR results to treponemal test for diagnosis of syphilis

Case No.	Age /Sex	RPR card test	Automated RPR (RPR Unit)	TPPA	Clinical Diagnosis
1	28/F	1+	Negative	Negative	Atopic dermatitis, Anti-phospholipid syndrome
2	50/F	1+	Negative	Negative	Bronchiectasis, Secondary pulmonary hypertension
3	22/M	Negative	2.2	Negative	Behcet's disease
4	33/M	Negative	1.1	Negative	Chlamydia, Herpes penis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 3 Comparison of non-treponemal RPR tests according to *Treponema pallidum* particle agglutination (TPPA) test results

TPPA positive (n=59)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	31	20
	Negative	0	8
Number of observed agreements: 39 (66.1% of the observations)			
Kappa= 0.296			
95% confidence interval: 0.118 to 0.474			

TPPA negative (n=53)		HBI HiSens Auto RPR	
		Positive	Negative
BD Macro-Vue RPR card	Positive	1	2
	Negative	2	48
Number of observed agreements: 49 (92.5% of the observations)			
Kappa= 0.293			
95% confidence interval: -0.212 to 0.798			

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 4 Performance characteristics of RPR tests for diagnosis syphilis

Non-treponemal tests		TPPA	
		Positive	Negative
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	Negative	28	50
Sensitivity		52.5%	(95% CI: 39.1 % to 65.7 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		91.2%	(95% CI: 76.3 % to 98 %)
Negative predictive value		64.1%	(95% CI: 52.4 % to 74.7 %)
		TPPA	
		Positive	Negative
BD Macro-Vue RPR card	Positive	51	3
	Negative	8	50
Sensitivity		86.4%	(95% CI: 75 % to 93.9 %)
Specificity		94.3%	(95% CI: 84.3 % to 98.8 %)
Positive predictive value		94.4 %	(95% CI: 84.6 % to 98.8 %)
Negative predictive value		86.2 %	(95% CI: 74.6 % to 93.8 %)

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination.

Table 5 Comparisons between manual and automated RPR test after initial syphilis treatment

Case No.	Age	Gender	Manual RPR	Automated RPR (R.U.)	TPPA	Pretreatment VDRL test value	Day after initial treatment	Initial treatment	Diagnosis
1	54	Male	2+	0	1:5120	1:8 reactive	939	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
2	66	Male	0.5+	0	1:640	1:1 weakly reactive	903	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
3	17	Male	2+	0	1:5120	1:4 reactive	222	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
4	62	Male	2+	0	1:640	1:1 reactive	296	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
5	68	Male	1+	0	1:320	1:1 weakly reactive	644	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
6	72	Male	1+	0	1:640	1:1 weakly reactive	28	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
7	55	Female	0	0	1:1280	N/A	0	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
8	56	Female	1+	0	1:5120	1:1 weakly reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
9	65	Female	2+	0	1:80	1:1 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Late syphilis
10	33	Female	1+	0	1:5120	1:8 reactive	936	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, other and unspecified
11	28	Female	2+	1	1:2560	1:1 reactive	1097	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
12	2	Male	2+	1.1	1:5120	1:32 reactive	539	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, congenital, latent
13	65	Male	3+	1.3	1:640	1:1 reactive	273	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
14	70	Male	3+	2.3	1:1280	1:1 reactive	188	Doxycycline 100 mg	Syphilis, late, latent
15	48	Female	2+	2.5	1:5120	1:1 weakly reactive	665	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis
16	36	Female	2+	3.8	1:5120	1:2 reactive	810	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
17	74	Female	4+	7.7	1:320	1:4 reactive	669	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, latent
18	25	Female	4+	8.1	1:5120	1:8 reactive	172	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
19	64	Female	4+	14.1	1:5120	1:8 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Chronic rhinitis
20	30	Male	4+	20	1:2560	1:16 reactive	7	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, late, unspecified
21	31	Female	2+	20	1:5120	1:16 reactive	3	Penicillin G Benzathine 1.2×10^6 IU	Syphilis with pregnancy
22	51	Female	4+	20.4	1:5120	1:8 reactive	417	Penicillin G Benzathine 1.2×10^6 IU	Syphilis, latent
23	37	Female	2+	25.6	1:5120	1:16 reactive	0	Penicillin G Benzathine 1.2×10^6 IU	Treated syphilis

Abbreviations: RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination; N/A, not applicable.

STARD checklist for reporting of studies of diagnostic accuracy
(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5-6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5-6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	5-6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	N/A
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	N/A
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	7
	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	7
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	8-10