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

Jong-Han Lee,1 Chae Seung Lim,1 Min-Geol Lee,2 Hyon-Suk Kim3

ABSTRACT

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


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

Outcome measures: HiSens Auto RPR LTIA (HBI, Anyang, Korea) was compared with Macro-Vue RPR Card Tests (Becton Dickinson BD Microbiology Systems, Sparks, Maryland, USA). Treponemal-specific tests were performed by Serodia TPPA assay (Fujirebio, Tokyo, Japan). The percentage agreement, κ value and overall sensitivity and specificity of the two RPR tests were compared. Seroconversion rates after treatment were also compared for each RPR test.

Results: The percentage agreement between the two RPR tests was 78.6% (κ 0.565; 95% CI 0.422 to 0.709). Sensitivity and specificity of the automated RPR test relative to the TPPA test was 52.5% (95% CI 39.1% to 65.7%) and 94.3% (95% CI 84.3% to 98.8%), respectively, while the same values for the conventional RPR card test were 86.4% (95% CI 75% to 93.9%) and 94.3% (95% CI 84.3% to 98.8%), respectively. The conventional RPR card test showed overall higher positivity than the automated RPR test, whereas the automated RPR test showed higher seroconversion (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 than the conventional RPR test based on the treponemal test, but higher seroconversion after treatment. The automated RPR test could be used to monitor treatment response, especially in the reverse screening algorithm in syphilis testing.

INTRODUCTION

There has been a rapid decrease in positive rates for syphilis since the 1970s in Korea, consistent with the global trend. In 2000, ~0.2% of the general Korean population was estimated to be syphilis-positive; since that time, levels appear to have decreased, and the prevalence rate is still very low. 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. Serological analysis of non-treponemal reagin tests, such as the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR) and treponemal tests such as the Treponema pallidum haemagglutination assay (TPHA), the Treponema pallidum particle agglutination (TPPA) test, the fluorescent treponemal antibody absorption test, and the 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

Strengths and limitations of this study

- Automated rapid plasma reagin (RPR) testing has been introduced in clinical laboratories, so we compared the automated test with the conventional RPR card tests.
- The automated RPR test showed overall lower positivity than the conventional RPR test compared with the treponemal test, Treponema pallidum particle agglutination (TPPA).
- The automated RPR test showed higher seroconversion after treatment than the conventional manual RPR test. So, the automated RPR test could be used to monitor treatment response, especially in the reverse screening algorithm for syphilis testing.
- Limitations of this study are small sample size and the patient groups could not be accurately categorised according to the stage of the disease because of the low prevalence of syphilis in Korea.
non-treponemal- or treponemal-specific tests. The Centers for Disease Control and Prevention (CDC) still recommend that a non-treponemal reagin test is used as the first-line diagnostic approach. Two kinds of non-treponemal test have been widely used: VDRL and RPR. RPR is the most common first-line non-treponemal test used to screen for syphilis infection. Recently, automated RPR tests have been introduced, but variable results were reported when the automated test was compared with conventional RPR card tests. 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 with a conventional RPR card test in clinical application.

METHODS

Subjects

All sera testing positive 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 tests after confirmation were included and preserved at −70°C until analysis. Patients were not categorised according to syphilis stage because of the infrequency of syphilis infection. Cases of true syphilis were very rare because of the low prevalence of syphilis in this country. The aim of this study was to evaluate the same RPR tests with ethically protected remnant specimens. This case was exempted by the institutional review board. All study processes complied with the World Medical Association Declaration of Helsinki.

The automated RPR test was compared with the manual RPR card test (Becton Dickinson BD Microbiology Systems, Sparks, Maryland, USA). A confirmatory treponemal-specific test was performed using a TPPA assay according to the manufacturer’s instructions. Seroconversion rates of each non-treponemal RPR test were evaluated on 23 syphilitic patients with a medical history of syphilis treatment.

SEROLOGICAL TESTS

Conventional RPR card test

The Macro-Vue RPR Card Test (Becton Dickinson BD Microbiology Systems) 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 for each test to confirm optimal reactivity of the antigen. The test procedure was followed according to the manufacturer’s instructions.

Automated RPR test

HiSens Auto RPR LTIA (HBI, Anyang, Korea) is a latex turbidimetric immunoassay using latex particles coated with lecithin and cardiolipin. The latex particles react with the reagin in the serum of patients with syphilis. The 15 μL serum samples were allowed to react 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 a CA-400 autoanalyzer (Furuno Electric Co, Nishinomiya, Japan). The CA-400 photometric analyser was used for the automated procedure and analysis. Absorbance at 600 nm was read after 5.3 and 10 s at room temperature, in duplicate. Results of the HiSens auto RPR test equal to or greater than 1.0 RU were considered to indicate reactive RPR. The upper detection limit was 20 RU.

Treponema pallidum particle agglutination

The Serodia TPPA assay (Fujirebio, Tokyo, Japan) is based on agglutination of coloured gelatine particles that have been sensitised (coated) with T. pallidum (Nichols strain) antigen. For each specimen, a 100 μL sample of diluent and 25 μL test specimen were mixed, and then twofold serial dilutions were made with 25 μL sample diluent. The sensitised particles were serially mixed in the neighbouring wells with a plate mixer for 30 s. After 2 h of incubation at room temperature, the result of the agglutination assay was read. The Serodia TPPA assay results were interpreted using the agglutination patterns of positive and negative controls.

STATISTICAL ANALYSIS

The percentage agreement (κ coefficient) of the automated RPR test with the manual RPR card test was calculated. The overall sensitivity and specificity of each test were calculated based on the TPPA results. κ values were used to categorise results as very good (0.81–1.0), good (0.61–0.8), moderate (0.41–0.6), fair (0.21–0.4) or poor (0–0.2). The McNemar test was used to compare seroconversion rates between the automated RPR test and the conventional manual RPR card test and was performed using SPSS Statistics V.20. A p value <0.05 was considered significant.
RESULTS

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

The percentage agreement between the two RPR tests was 78.6% (κ 0.565; 95% CI 0.422 to 0.709; table 1). The strength of agreement between the automated RPR test and the manual RPR card test was considered to be ‘moderate’ according to the κ value scale. Both RPR-positive results (n=32) showed 96.9% (31/32) TPPA-positive results, 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 HBI HiSens Auto RPR LTIA test results that showed positive results on the BD Macro-Vue RPR card test. Of these 22 discrepant results, 20 were TPPA-positive and 2 were TPPA-negative, while 2 cases were positive on the HBI HiSens Auto RPR LTIA test but negative on the BD Macro-Vue RPR card test. These two cases were negative on the TPPA test. There were four results with discrepancies between both the RPR tests and the TPPA assay, which was due to conditions other than syphilis infection (table 2). The strength of agreement between the automated RPR and manual RPR tests was ‘fair’ (κ value 0.296, 59 TPPA-positive results; κ value 0.293, 53 TPPA-negative results) according to the TPPA results (table 3).

The overall sensitivity and specificity of the BD Macro-Vue RPR card test were 86.4% (95% CI 75% to 93.9%) and 94.3% (95% CI 84.3% to 98.8%), respectively (table 4). Automated RPR gave a higher seroconversion rate after syphilis treatment (43.5% (10/23)) than the conventional RPR card test (4.3% (1/23)) (p=0.004) by the McNemar test. A detailed comparison of the treated syphilis cases is given in table 5.

DISCUSSION

The manual RPR test has been used for decades, but recently an automated RPR test was launched and has been used because of its convenience in clinical settings. However, there was a need for thorough inspection and a comparison of results of this new automated test with the conventional manual RPR test in diagnostic approaches. Treponemal test results will not change even after treatment, and the patients live with positive results for the rest 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, the non-treponemal test titre should show a twofold dilution decline after treatment, usually within 6 months.7 Therefore, the non-treponemal test is important for managing syphilitic patients.

We compared an automated RPR test with a conventional RPR card test on sera confirmed by the TPPA test. The TPPA test is known to be less subjective than the

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age/sex</th>
<th>RPR card test</th>
<th>Automated RPR (RPR unit)</th>
<th>TPPA</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28/F</td>
<td>1+</td>
<td>Negative</td>
<td>Negative</td>
<td>Atopic dermatitis, antiphospholipid syndrome</td>
</tr>
<tr>
<td>2</td>
<td>50/F</td>
<td>1+</td>
<td>Negative</td>
<td>Negative</td>
<td>Bronchiectasis, secondary pulmonary hypertension</td>
</tr>
<tr>
<td>3</td>
<td>22/M</td>
<td>Negative</td>
<td>2.2</td>
<td>Negative</td>
<td>Behcet's disease</td>
</tr>
<tr>
<td>4</td>
<td>33/M</td>
<td>Negative</td>
<td>1.1</td>
<td>Negative</td>
<td>Chlamydia, Herpes penis</td>
</tr>
</tbody>
</table>

RPR, rapid plasma reagin; TPPA, Treponema pallidum particle agglutination.

<table>
<thead>
<tr>
<th>TPPA positive (n=59)</th>
<th>HBI HiSens Auto RPR</th>
<th>TPPA negative (n=53)</th>
<th>HBI HiSens Auto RPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Macro-Vue RPR card</td>
<td>Positive 31 20</td>
<td>BD Macro-Vue RPR card</td>
<td>Positive 1 2</td>
</tr>
<tr>
<td>Negative 0 8</td>
<td>Number of observed agreements: 39 (66.1% of the observations)</td>
<td>Number of observed agreements: 49 (92.5% of the observations)</td>
<td></td>
</tr>
<tr>
<td>k=0.296</td>
<td>95% CI 0.118 to 0.474</td>
<td>k=0.293</td>
<td>95% CI −0.212 to 0.798</td>
</tr>
</tbody>
</table>

RPR, rapid plasma reagin; TPPA, Treponema pallidum particle agglutination.
fluorescent treponemal antibody absorption test and easier to read than the microhaemagglutination assay for antibodies to *T. pallidum*. The TPPA test has also been suggested for use on cerebrospinal fluid samples for diagnosing neurosyphilis.

In our study, the conventional BD Macro-Vue RPR card test showed better sensitivity than the HBI HiSens Auto RPR LTIA test in syphilis screening, although the automated RPR test does have some advantages in the clinical setting. For example, the automated RPR test reduced the workload and overall test turnaround time. It can also deal with greater test quantities in a given time than the manual RPR card test and does not require test experts. Furthermore, we observed 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. This reverse algorithm for syphilis testing has been suggested and adopted in many fields because it may be more sensitive and effective than the traditional algorithm in a low-prevalence area and can be automated. However, the CDC still recommend first screening for syphilis with a non-treponemal test such as RPR.

Our study found that the automated RPR test showed earlier seroconversion than the conventional card RPR test after syphilis treatment (p=0.004). If we adopt the reverse algorithm, treponemal tests can be used first to screen sensitively, and then non-treponemal tests can be used to accurately show negative changes in treated cases. In this situation, we could use treponemal tests for first-line screening and non-treponemal tests for monitoring patients allowing us to observe seroconversion more effectively after treatment. Unfortunately, our study had a limited number of syphilitic patients because of the low prevalence of syphilis in our country, so the number of samples was small and could not be classified according to syphilis stage. In fact, in some late or latent syphilis cases, the results of the non-treponemal test were hard to interpret after initial treatment in our study (cases 8 and 9 in table 5). So, further well-designed studies are needed to clarify the serological responses of automated RPR tests after treatment and according to the stage of syphilis infection.

In Korea, automated RPR tests have recently been introduced in clinical laboratories, and evaluations comparing conventional RPR tests and VDRL tests have been reported. However, the results were variable. Onoe 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 titres, because the manual serological testing method for syphilis showed somewhat different results from the automated serological testing methods. In this study, we noticed reasonably consistent results between automated and manual RPR tests.

We found that the automated RPR test has 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 using the treponemal test for initial screening, and then the automated RPR test after treatment because of its rapid seroconversion, although the sensitivity of the automated RPR test is lower than that of the manual RPR test.

In conclusion, the automated RPR test showed an overall lower sensitivity and similar specificity compared with the conventional manual RPR card test. Therefore, we consider that the automated RPR test is not appropriate for use for initial screening for syphilis. However, it produces an earlier seroconversion response in treated cases than the conventional RPR card test. Applying the reverse algorithm, the sensitive treponemal test can be used as the first-line screening test, and then the

<table>
<thead>
<tr>
<th>Non-treponemal tests</th>
<th>TPPA</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Macro-Vue RPR card</td>
<td>Positive</td>
<td>86.4% (95% CI 75% to 93.9%)</td>
<td>94.3% (95% CI 84.3% to 98.8%)</td>
<td>94.4% (95% CI 84.6% to 98.8%)</td>
<td>86.2% (95% CI 74.6% to 93.8%)</td>
</tr>
<tr>
<td>TPPA</td>
<td>Positive</td>
<td>51</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RPR, rapid plasma reagin; TPPA, *Treponema pallidum* particle agglutination.
Table 5  Comparison of manual and automated RPR test after initial syphilis treatment

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age</th>
<th>Gender</th>
<th>Manual RPR</th>
<th>Automated RPR (RU)</th>
<th>TPPA</th>
<th>Pretreatment VDRL test value</th>
<th>Time after initial treatment (days)</th>
<th>Initial treatment</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>Male</td>
<td>2+</td>
<td>0</td>
<td>1:512</td>
<td>1:8 reactive</td>
<td>939</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, latent</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>Male</td>
<td>0.5+</td>
<td>0</td>
<td>1:640</td>
<td>1:1 weakly reactive</td>
<td>903</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Treated syphilis</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>Male</td>
<td>2+</td>
<td>0</td>
<td>1:512</td>
<td>1:4 reactive</td>
<td>222</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, latent</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>Male</td>
<td>2+</td>
<td>0</td>
<td>1:640</td>
<td>1:1 reactive</td>
<td>296</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, other and unspecified</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>Male</td>
<td>1+</td>
<td>0</td>
<td>1:320</td>
<td>1:1 weakly reactive</td>
<td>644</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, latent</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>Male</td>
<td>1+</td>
<td>0</td>
<td>1:640</td>
<td>1:1 weakly reactive</td>
<td>28</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, unspecified</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>Female</td>
<td>0</td>
<td>0</td>
<td>1:1280</td>
<td>N/A</td>
<td>0</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, latent</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>Female</td>
<td>1+</td>
<td>0</td>
<td>1:512</td>
<td>1:1 weakly reactive</td>
<td>7</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, latent</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>Female</td>
<td>2+</td>
<td>0</td>
<td>1:80</td>
<td>1:1 reactive</td>
<td>0</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>Female</td>
<td>1+</td>
<td>0</td>
<td>1:512</td>
<td>1:8 reactive</td>
<td>936</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, other and unspecified</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>Female</td>
<td>2+</td>
<td>1</td>
<td>1:2560</td>
<td>1:1 reactive</td>
<td>1097</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, latent</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>Male</td>
<td>2+</td>
<td>1.1</td>
<td>1:512</td>
<td>1:32 reactive</td>
<td>539</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, congenital, latent</td>
</tr>
<tr>
<td>13</td>
<td>65</td>
<td>Male</td>
<td>3+</td>
<td>1.3</td>
<td>1:640</td>
<td>1:1 reactive</td>
<td>273</td>
<td>Penicillin G Benzathine 1.2×10^6 IU</td>
<td>Treated syphilis</td>
</tr>
<tr>
<td>14</td>
<td>70</td>
<td>Male</td>
<td>3+</td>
<td>2.3</td>
<td>1:1280</td>
<td>1:1 reactive</td>
<td>188</td>
<td>Doxycycline 100 mg</td>
<td>Syphilis, late, latent</td>
</tr>
<tr>
<td>15</td>
<td>48</td>
<td>Female</td>
<td>2+</td>
<td>2.5</td>
<td>1:512</td>
<td>1:1 weakly reactive</td>
<td>665</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Treated syphilis</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>Female</td>
<td>2+</td>
<td>3.8</td>
<td>1:512</td>
<td>1:2 reactive</td>
<td>810</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, latent</td>
</tr>
<tr>
<td>17</td>
<td>74</td>
<td>Female</td>
<td>4+</td>
<td>7.7</td>
<td>1:320</td>
<td>1:4 reactive</td>
<td>669</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, latent</td>
</tr>
<tr>
<td>18</td>
<td>25</td>
<td>Female</td>
<td>4+</td>
<td>8.1</td>
<td>1:512</td>
<td>1:8 reactive</td>
<td>172</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis with pregnancy</td>
</tr>
<tr>
<td>19</td>
<td>64</td>
<td>Female</td>
<td>4+</td>
<td>14.1</td>
<td>1:512</td>
<td>1:8 reactive</td>
<td>0</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Chronic rhinitis</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>Male</td>
<td>4+</td>
<td>20</td>
<td>1:2560</td>
<td>1:16 reactive</td>
<td>7</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, late, unspecified</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>Female</td>
<td>2+</td>
<td>20</td>
<td>1:512</td>
<td>1:16 reactive</td>
<td>3</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis with pregnancy</td>
</tr>
<tr>
<td>22</td>
<td>51</td>
<td>Female</td>
<td>4+</td>
<td>20.4</td>
<td>1:512</td>
<td>1:8 reactive</td>
<td>417</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Syphilis, latent</td>
</tr>
<tr>
<td>23</td>
<td>37</td>
<td>Female</td>
<td>2+</td>
<td>25.6</td>
<td>1:512</td>
<td>1:16 reactive</td>
<td>0</td>
<td>Penicillin G benzathine 1.2×10^6 IU</td>
<td>Treated syphilis</td>
</tr>
</tbody>
</table>

N/A, not applicable; RPR, rapid plasma reagin; RU, RPR unit; TPPA, Treponema pallidum particle agglutination; VDRL, Venereal Disease Research Laboratory.
automated RPR test can be used as an adjunct to detect
earlier seroconversion in treated patients.

Further large-scale studies including patients cate-
gorised by syphilis stage are required to clarify the diag-
nostic efficiency of the automated RPR test.

Contributors H-SK designed and participated in all stages of the study. J-HL
participated in the experiments and statistical analyses and drafted the
manuscript. CSL and M-GL helped in consultations on the study. All authors
read and approved the final manuscript.

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Data sharing statement No additional data are available.

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

Jong-Han Lee, Chae Seung Lim, Min-Geol Lee and Hyon-Suk Kim

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