Rapid plasma reagin card test: evaluation of a hand-rotation procedure and stability of the RPR antigen

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Reported are the results of a comparative study of two procedures for the rapid plasma reagin (RPR) test for syphilis serology: in one approach a mechanical rotator was used and in the other, hand rotation was employed. Both procedures were performed on 327 sera. The agreement between both was 98.8%. Three sera that exhibited minimal reactivity (titre = 1) in the mechanical-rotation RPR were non-reactive in the hand-rotation RPR.

RPR antigen was stored for 3 months at room temperature (mean, 21 °C) and 30 °C. There was no difference in the reactivity of 62 sera (50 positive, 12 negative) that were tested using either adversely stored antigens or antigen stored at 4 °C.

In poorly equipped settings at the primary health care level, the hand-rotation RPR is a practical alternative to mechanical rotation. Also, the stability of the antigen under adverse storage conditions is an additional advantage of RPR for use in tropical areas.

Introduction

Syphilis is a common disease in many developing countries. In Africa, the prevalence of syphilis seroreactivity among pregnant women attending antenatal clinics ranges from 4% to 15% (1). Adverse pregnancy outcomes associated with the disease include spontaneous abortion, stillbirth, neonatal death, and infants with congenital syphilis. A total of 50–80% of pregnancies involving syphilis-seroreactive women result in an adverse outcome (2–6). Syphilis, as well as other causes of genital ulcer disease (GUD), is also associated with human immunodeficiency virus (HIV) infection, suggesting that it increases the risk of acquisition and transmission of HIV (7–10). The prevention and treatment of syphilis in adults and the prevention of congenital syphilis are among the most cost-effective health interventions in developing countries (11).

The identification of syphilis cases depends on the detection of serum antibody, with the rapid plasma reagin (RPR) card test probably being the most widely available method of screening for recently acquired infection. The RPR procedure requires the use of a mechanical rotator and the antigen needed for the test must be stored at 2–8 °C. In most developing countries the majority of pregnant women attend antenatal clinics or health centres, where cold storage conditions for diagnostic reagents are often not feasible and where mechanical rotation devices are not available or practicable. Laboratory procedures that do not require equipment and reagents that do not require refrigeration could therefore provide more appropriate diagnostic tests for modestly equipped and low-resource settings.

In this article we compare the results of an RPR hand-rotation procedure (RPR–HR) for syphilis with those obtained with an RPR procedure that used mechanical rotation (RPR–MR), using sera from patients with sexually transmitted diseases (STD). Described also is the effect on the RPR antigen of suboptimal storage conditions.

Materials and methods

A total of 327 serum specimens obtained in Kigali, Rwanda, from consecutive STD patients with GUD were tested using an RPR procedurea and a Treponema pallidum haemagglutination assay (TPHA).b Reactive RPR/non-reactive TPHA sera were also tested with a fluorescent treponemal antibody absorption (FTA-Abs) assay.c All specimens were first

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examined using a qualitative RPR–MR test, and reactive samples were retested using a quantitative RPR–MR procedure to determine antibody titres. All sera were also tested blind by the same technician using an RPR–HR procedure; the test cards were tilted manually back and forth and rotated for 8 minutes consistently at 80–85 rotations per minute.

To test the stability of the RPR antigen, we stored different ampoules from the same manufacturer’s lot at 4°C, room temperature (mean, 21°C; range, 20–24°C), and 30°C for 3 months and then, within the product expiry date, used them to test in parallel a panel of 12 non-reactive and 50 reactive RPR sera. Qualitative and quantitative RPR tests were performed by one technician and the results were read and recorded blind by a second technician.

Results

The sera of 72 (22%) of the 327 GUD patients were reactive in the RPR–MR test, with antibody titres in the range 1–512. Seven reactive sera (10%) had an antibody titre of only 1. Two sera (titres 1 and 32) were non-reactive when tested with TPHA and FTA-Abs, and were considered to be false-positives. The remaining 70 sera were TPHA-reactive, resulting in a specificity of 99.2% (255/257) for the RPR–MR. All 255 non-reactive RPR–MR sera were also non-reactive when tested with the RPR–HR. As shown in Table 1, 68 of the 72 RPR–MR-reactive sera were reactive also in the RPR–HR, including one of the false-positive sera (titre, 32). The second false-positive RPR–MR serum (titre, 1) was non-reactive in the RPR–HR test, and three of the six 1:1 true-positive RPR–MR sera were non-reactive in the hand-rotation procedure. Discrepant results were not due to technical error since the reproducibility of both RPR procedures, determined on a random subsample, including the specimens with discordant results, was 100%. If the two false-positive RPR–MR sera are excluded, the sensitivity of the RPR–HR was 95.7% (67/70) relative to the classic test procedure with mechanical rotation. All of the 65 sera with RPR–MR antibody titres ≥2 were reactive in the RPR–HR test. The agreement between both RPR procedures was 98.8% (323/327).

The stability of the RPR antigen samples that were stored for 3 months at room temperature and at 30°C was determined by testing a panel of 62 sera; the results were compared with those obtained using an RPR–MR method performed with antigen from the same manufacturer’s lot stored at 4°C. Of the 62 sera, 12 were non-reactive, and 50 exhibited various degrees of reactivity as determined by the RPR test using the antigen stored at 4°C. For all specimens tested, the reactivity with the antigen stored at room temperature was similar to that with the antigen stored at 4°C. With the antigen stored at 30°C a non-significant twofold dilution increase was observed in 7/50 (14%) of the reactive sera. All non-reactive sera were also non-reactive with antigen stored under suboptimal conditions.

Discussion

The requirement for special equipment makes many laboratory tests less practicable at the primary health care level in developing countries. Cold storage requirements, short expiry periods, and the low stability of diagnostic reagents may preclude the use of even simple diagnostic tests in poorly equipped settings. Previously we evaluated the RPR "teardrop" test on fingerstick blood samples for the screening of syphilis under field conditions and found that the classic RPR card test using serum from venepuncture and mechanical rotation was a significantly more reliable procedure (12).

The objectives of the present study were to determine the practicability of a hand-rotation RPR card test performed on venepuncture serum specimens, and the stability of the RPR antigen under storage under suboptimal conditions. In most primary health care centres and antenatal clinics, the daily number of new pregnancies seen is small, and a mechanical rotator is not needed because only a few specimens have to be tested. Also, in many health centres, the power supply required to maintain the cold storage of reagents is not available or its continuity cannot be guaranteed.

The agreement between the results of RPR–HR and the RPR–MR was 98.8%. Among the patients with serum antibody for syphilis, false-negative RPR–HR results were observed in 3/6 sera from cases with minimal RPR–MR reactivity (titre, 1).

Data on the stability of the RPR antigen after storage under adverse conditions are not available and this information could also not be obtained from the manufacturer. For the toluidine red unheated

Table 1: Comparison of the reactivity of the rapid plasma reagin (RPR) card test, by mechanical (MR) and hand rotation (HR), on 372 sera

<table>
<thead>
<tr>
<th>RPR–HR</th>
<th>No. reactive</th>
<th>No. non-reactive</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>RPR–MR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. reactive</td>
<td>68</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>No. non-reactive</td>
<td>4</td>
<td>255</td>
<td>259</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>255</td>
<td>327</td>
</tr>
</tbody>
</table>
serum test (TRUST), which is a modification of the RPR test, the stability of the antigen after suboptimal storage has been evaluated for 11 reactive and 7 non-reactive sera (13). Of the negative sera, one serum specimen gave a false-positive reaction with antigens that had been stored for 2 weeks at 20 °C and 37 °C. For the other serum specimens no differences were observed using the TRUST antigens that had been stored adversely for 12 weeks. The antigen, however, rapidly changed its physical properties and it became more difficult to distinguish between minimal reactivity and non-reactivity (13). This inconvenience was not observed in our study with RPR antigen stored for 3 months at room temperature or at 30 °C. There was no difference in the reactivity pattern between adversely stored antigen and that stored at 4 °C, and the qualitative and quantitative agreement between the results with 50 reactive and 12 non-reactive sera was 100%.

Our findings show that the RPR hand-rotation procedure is feasible and practicable, and that the results obtained are very accurate. This manual procedure may be appropriate for poorly equipped settings if few serum specimens have to be tested. Adverse storage of RPR antigen at temperatures up to 30 °C for 3 months does not affect its stability, thereby removing another obstacle for the promotion of RPR testing of sera, which is essential for the more efficient control of syphilis in developing countries.

Résumé

Epreuve rapide de mise en évidence de la réagène plasmatique sur carte: évaluation d'une méthode avec agitation manuelle et stabilité de l'antigène RPR

On a rapporté ici les résultats d'une étude comparative de deux méthodes de mise en évidence rapide de la réagène plasmatique utilisées dans la sérologie de la syphilis: on a utilisé dans l'une un agitateur mécanique et pratiqué dans l'autre l'agitation manuelle. On a appliqué ces deux méthodes à 327 sérums. La concordance entre elles a été de 98,8%. Trois sérums ayant montré une réactivité minimale (titre = 1) avec l'agitation mécanique n'ont pas réagi du tout avec l'agitation manuelle.

L'antigène RPR a été conservé pendant 3 mois à température ambiannte (± 21 °C) et à 30 °C. On n'a observé aucune différence de réactivité dans les 62 sérums (50 positifs, 12 négatifs) testés en présence d'antigènes conservés dans de mauvaises conditions ou conservés à 4 °C.

Dans les endroits mal équipés au niveau des soins de santé primaires, le test rapide avec agitation manuelle est une alternative pratique à l'agitation mécanique. De plus, la stabilité de l'antigène dans de mauvaises conditions de conservation constitue un avantage supplémentaire de ce test dans les régions tropicales.

References