International Journal of STD & AIDS 2001; **12**: 299–301

ORIGINAL ARTICLE

Effect of adverse storage conditions of antigen reagent on performance of the rapid plasma reagin test

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Summary: The aim of this study was to evaluate the performance of rapid plasma reagin (RPR) testing using expired and adversely stored antigen reagent. The sensitivity of RPR using antigen stored at 36°C was compared at 3-monthly intervals with RPR using fresh antigen on 116 sera reactive by RPR and by *Treponema pallidum* particle agglutination (TPPA). After multiple phases of freezing and thawing, 8.3% of initial RPR reactive sera seroreverted. After storage at 36°C for one year and 24 weeks after expiration the overall sensitivity of the adversely stored antigen was 93.8% compared with fresh antigen; the sensitivity was 100% for sera with RPR titres $\geqslant$1:4 and 85.4% for sera with RPR titres of 1:1 and 1:2. The high stability of the reagent may increase the feasibility of the RPR test for use in poorly-equipped healthcare centres in developing countries.

Keywords: Rapid plasma reagin, performance, adverse storage

INTRODUCTION

The currently used syphilis non-treponemal flocculation tests are modifications of the microscopic Venereal Disease Research Laboratory (VDRL) test, in that they all use a phospholipid antigenic component, cardiolipin. The RPR test was developed in the early sixties and designed as a teardrop card test and rapidly modified to a circle card test for batch processing[1]. The RPR test has remained unchanged for almost 40 years and is still the most frequently used non-treponemal test worldwide.

Many copies of the original RPR test have become commercially available. Unfortunately, several brands are of poor quality and may result in high false positive and/or false negative rates. The accuracy and reliability of non-treponemal tests, derived from the VDRL, could probably be increased by replacing the natural cardiolipin, extracted from beef hearts, with a purified synthetic cardiolipin[2]. RPR is a cheap and rapid test and is considered to be a simple assay; its reliability, however, greatly depends on the experience of the user. Without 'test' and 'user' validation and without internal quality control, the performance of an RPR can be very poor. Another problem of the cardiolipin test is the biologic false positive reaction due to small amounts of anti-phospholipid antibodies that may be present in sera from healthy individuals or higher amounts of such antibodies associated with some non-treponemal infections and systemic diseases[3]. Laboratories are recommended to confirm positive cardiolipin test results with a specific treponemal test like *Treponema pallidum* haemagglutination (TPHA) or TPPA. TPPA is a recently developed particle agglutination assay, and has been proven to be a very accurate specific syphilis test[4]. For screening of high-risk populations and for case finding of syphilis in endemic areas, however, confirmation is not always a major priority because the prevalence of biologic false positive cardiolipin test seems to be 1–2% in healthy populations[5]. A more important obstacle to the field use of most diagnostics, including RPR, is that they have to be stored between 2°C and 8°C; and many healthcare centres in developing countries are not equipped with a refrigerator. Another drawback with using diagnostic products in developing countries is that the products often reach their expiry date shortly after arrival or even before arrival, due to the prior sale of these products and to irregular procurement practices in the public healthcare sector.

We are often confronted with these problems and therefore decided to test the robustness of the RPR assay by evaluating expired reagents that have been exposed for a long time to high temperatures.

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Table 1. Performance of qualitative rapid plasma reagin (RPR) test using adversely stored reagents compared with results obtained using fresh antigen

Tested sera		No. of reactive sera							
		At 3 months		At 6 months		At 9 months		At 12 months	
RPR titre at start	No.	4°C	36°C	4°C	36°C	4°C	36°C	4°C	36°C
1:2	59	59	58	57	55	48	44	41	35
1:4	45	45	45	45	45	45	44	44	44
1:8	7	7	7	7	7	7	7	7	7
1:16	5	5	5	5	5	5	5	5	5
All sera	116	116	115	114	112	105	100	97	91

MATERIALS AND METHODS

A panel of 116 African human sera, reactive in RPR (Becton Dickinson, Cockeysville, MD, USA) and with TPPA (Fujirebio, Tokyo, Japan) were re-tested using a quantitative RPR test at the start of the study and were stored frozen at −20°C for later use. Four ampoules of RPR antigen, expiring after 28 weeks, were stored in an incubator at 36°C. Sera were tested by qualitative RPR assays after 3, 6 and 9 months and by quantitative RPR assays after 12 months using fresh antigen and antigen stored at 36°C.

For analysis of the sensitivity of RPR using adversely stored antigen, 95% confidence intervals (CI) were calculated based on binomial distribution of observed results.

RESULTS

Qualitative RPR test results of initial RPR reactive sera obtained with fresh antigen and with reagents stored at 36°C for 3, 6, 9, and 12 months after the initial testing are shown in Table 1. Sera were frozen at −20°C and thawed 4 times during the study period: 18 sera with an initial RPR titre of 1:2 and one serum with an initial RPR titre of 1:4 became negative after one year when tested with fresh RPR antigen. Besides these 19, 6 more sera with an initial RPR titre of 1:2 became negative with antigen stored for one year at 36°C and expired for 5 months.

Overall, 12 months after the start of the experiment, 97 sera were still RPR-reactive with fresh antigen, 91 (93.8%) of these were reactive with antigen stored at 36°C. Compared with fresh antigen, the sensitivity of the adversely stored reagent was 99.1% (95% CI: 95.3–100) and 98.2% (95% CI: 93.8–99.8) after 3 and 6 months, respectively; the 2 sera missed after 6 months had a RPR titre of 1:2. After expiration of reagents in the 7th month of the study, the sensitivity of the antigen stored at 36°C was 95.2% (95% CI: 89.2–98.4) and 93.8% (CI: 87.0–97.7) after 9 and 12 months, respectively. For sera with an initial RPR titre of $\geqslant$1:4 and for sera with an initial RPR titre of 1:2, an RPR performed with antigen stored at 36°C for one year and expired for more than 5 months, showed sensitivities of 100% (95% CI: 93.6–100) and 85.4% (95% CI: 70.8–94.4), respectively, when compared with a normal RPR at the end of the study. For the 91 sera RPR reactive with the adversely stored antigen at the end of the study, the titres of 75 samples were similar to the titres obtained with fresh antigen, and only one dilution lower for the other 16 samples.

DISCUSSION

Venereal syphilis remains a serious health problem in less developed countries. In 1995, the World Health Organization estimated that 4.2 million syphilis infections occurred in South and Southeast Asia and in sub-Saharan Africa, which is 76% of the global incidence[6]. A severe epidemic of syphilis occurred in the nineties in eastern European, and in the central Asian states which were previously a part of the Soviet Union, and the rates of congenital syphilis still seem to be increasing[7–9]. 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[9–11]. To prevent congenital syphilis, screening of all pregnant women using a non-treponemal test is recommended early in pregnancy and during the third trimester, as well as during delivery, in regions and populations with a high incidence[12,13]. Despite these recommendations, and despite the serious consequences of syphilis in pregnancy, screening and treatment in antenatal clinics in developing countries is seldom practised effectively. The main reasons are a lack of interest among health providers, a lack of expertise, poor procurement of diagnostics in the public health sector, and a lack of refrigerators for storing diagnostic reagents.

In a previous study, we described the stability of the RPR antigen after storage at 30°C for 3 months[14]. Our current study shows that the performance of qualitative and quantitative RPR assays is altered very slightly using expired antigen and after long-term storage of antigen at 36°C. All sera with RPR titres of $\geqslant$1:4, defined with fresh antigen at the end of the study, were also positive with antigen stored at 36°C for a full year and used

24 weeks after expiration; and for sera with RPR titres of 1:1 and 1:2, only 14.6% (95% CI: 5.6–29.2) tested negative. The false negative results obtained with low titre RPR reactive sera in this study, using adversely stored antigen, very probably underestimate the sensitivity of such RPR testing for screening or case finding of syphilis in asymptomatic populations, because a substantial number of sera with 1:1 and 1:2 RPR titres will be detected in individuals with inactive or recently treated syphilis.

Other observations made here were seroreversion and decreasing RPR titres for sera exposed to multiple phases of freezing and thawing. The phenomenon of unstable low level anti-cardiolipin antibodies in sera that have been repeatedly frozen and thawed has been seen in our laboratory since we introduced cardiolipin testing some decades ago.

The high robustness of the RPR test shown in this study may remove certain obstacles of conservation and expiration, and may make this test more feasible for poorly-equipped and remote health centres in developing countries. Despite the high stability, the use of adversely stored and expired RPR reagents may only be recommended if RPR test performance is assured by internal quality control measures. As for the quality differences associated with diverse commercial RPR tests, our findings should not be taken as a stability guarantee for all available non-treponemal cardiolipin tests.

Acknowledgements: We are grateful to K Janssens and T James for secretarial work and to C Tilborghs for data entry.

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(Accepted 12 December 2000)