ORIGINAL ARTICLE

Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa

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Objectives: To assess the rapid plasma reagin (RPR) test performance in the field and to evaluate a new rapid syphilis test (RST) as a primary screen for syphilis.
Methods: 1325 women of reproductive age from rural communities in the Gambia were tested for syphilis seropositivity using a RPR 18 mm circle card and a RST strip. Within 1 week a repeat RPR and a TPHA test were carried out using standard techniques in the laboratory.
Results: Comparing field tests to a diagnosis of "active" syphilis defined as laboratory RPR and TPHA positive, the RPR test was 77.5% sensitive and 94.1% specific; the RST was 75.0% sensitive and 95.2% specific. The RST was easier to use and interpret than the RPR test especially where field conditions were difficult. In this setting with a low prevalence of syphilis in the community (3%), the chance of someone with a positive test being confirmed as having serologically active syphilis was less than 50% for both tests.
Conclusions: The appropriateness of syphilis screening using RPR testing in antenatal clinics and health centres should be questioned if there is a low prevalence in the population, conditions for testing are poor, and resources limited. There is still an urgent need for an appropriate rapid syphilis test for field use.

The rapid plasma reagin (RPR) 18 mm circle card test for syphilis is used as a screening test in many antenatal clinic and health facilities in the developing world. Although it is easy to perform and inexpensive it may be difficult to interpret and requires training of health personnel to ensure testing is carried out and results are read correctly. The test specificity can be limited owing to the non-specific nature of the cardiolipin antigen as biological false positives occur; these can be due to viral infections, malaria, and pregnancy.[1] Additionally, false negatives may occur both in early primary cases[1] and in patients with secondary syphilis, as a result of prozone reactions[2]; this may limit the sensitivity of the test. In many developing country settings where the RPR test would be useful as a screening test, such as antenatal clinics, quality control procedures are suboptimal or lacking entirely and the rate of false positives and false negatives associated with the use of the test (and consequent overtreatment or undertreatment for syphilis) may be higher under operational conditions than that anticipated from research reports. We assessed the RPR test performed under field conditions against RPR/TPHA testing performed in a well appointed laboratory. Testing was carried out in the Gambia, where the national prevalence of serologically active syphilis was recorded as 2.8% in a survey in 1995 (O'Donovan *et al*, unpublished data) but has been reported as high as 7% in 15–34 year old women in some districts.[3] We also evaluated the performance of a rapid syphilis test (RST, Quorum Diagnostics, Vancouver, BC, Canada) as a primary screen. The RST is a one step immunochromatographic strip test, utilising a 47 kDa recombinant antigen of *Treponema pallidum* to detect antibody, developed by Omega Diagnostics in association with the Programme for Appropriate Technology in Health (PATH) and UNAIDS, Sexually Transmitted Diseases Diagnostics Initiative.

MATERIALS AND METHODS

A total of 1325 women aged 15–54 participated in a large community based reproductive heath survey in 20 villages in the Farafenni area of the Gambia, which is described elsewhere.[4] Syphilis testing was included as part of the survey. For this testing a field laboratory was set up in each village. All necessary equipment and consumables were transported daily to the site being surveyed. A portable generator provided electricity for the centrifuge and shaker. All reagents, RPR kits, rapid syphilis test strips, and samples collected were kept in a cool box that was replenished with ice packs daily.

For RPR testing a 10 ml venous blood sample was collected into a plain vacutainer, allowed to clot for about 15 minutes, and centrifuged for 10 minutes at 2000 *g*. A standard RPR 18 mm circle card test (Quorum Diagnostics) was carried out, mixing one drop of serum with one drop of RPR reagent, mixing on a shaker for 8 minutes, and read in the best available light. Positive and negative control sera were included in each day's testing.

For RST testing, 100 µl of serum was aliquoted into a fresh serum tube. A RST strip was removed from the foil pouch and added to the tube. This was left for 15 minutes and the results read, according to the manufacturer's guidelines. Where the sera reacts with the *Treponema pallidum* recombinant antigens in the strip a double pink line results and these were read as positive. Sera were regarded as negative if only the single control line was visible. If no pink line occurred the test was discarded as invalid.

At the end of each day samples were returned to the well equipped laboratory at the MRC Laboratories field station at Farafenni, which has a constant power and water supply. Samples were received, catalogued, and stored frozen. Within 1 week RPR and TPHA testing was carried out using standard techniques. The same laboratory assistant performed the tests in the laboratory as in the field but did not have access to field results.

The TPHA was a standard Fujeriebo test (Mast Laboratories, UK). Sera were diluted to 1/160 and mixed with sensitised and unsensitised red blood cells. This was read after 1 hour at room temperature. Sera were considered positive if agglutination

Table 1 Comparison of RPR testing in the field and the laboratory and the rapid syphilis test in defining cases of "active" syphilis

	Active syphilis (Lab RPR/TPHA positive)				
	Yes	No	Total	Test performance	
Field RPR test					
Positive	31	45	76	Sensitivity	77.5%
Negative	9	1210	1219	Specificity	96.4%
Total	40	1255	1295	PPV/NPV	40.8/99.3%
Rapid syphilis test					
Positive	30	62	92	Sensitivity	75.0%
Negative	10	1223	1233	Specificity	95.2%
Total	40	1285	1325	PPV/NPV	32.6/99.2%

Table 2 Comparison of RPR testing in the field and the laboratory

	Laboratory RPR test		
	Positive	Negative	Total
Field RPR test			
Positive	35	41	76
Negative	12	1207	1219
Total	47	1248	1295

Table 3 Comparison of rapid syphilis test and TPHA results

	Laboratory TPHA test		
	Positive	Negative	Total
Rapid syphilis test			
Positive	51	41	92
Negative	29	1204	1233
Total	80	1245	1325

occurred with sensitised cells only. Samples were considered void if agglutination occurred with unsensitised cells.

RESULTS

From the 1325 serum samples obtained, 1295 samples were RPR tested in the field, and all 1325 samples were tested in the laboratory; the 30 women not screened in the field were revisited and offered treatment if positive results were subsequently found. Field screening in these 30 women was not carried out owing to logistical difficulties, either generator or equipment failure or lack of consumables in the field laboratory. In the field 76 samples were read as RPR positive (5.9%). In the laboratory, 47 samples were read as RPR positive (3.5%), 16 as weakly positive (1.2%), and 40 (3.1%) were RPR/TPHA positive. Using the rapid syphilis strips 92 samples were positive; of these 33 were RPR positive, 51 TPHA positive, and 30 positive by both tests in the laboratory.

The performance of the field RPR and RST tests against serologically active syphilis (defined by laboratory RPR positive and TPHA positive) is shown in table 1. Calculations of sensitivity and specificity against this standard and of positive and negative predictive values for this population (where the prevalence of active syphilis is about 3%) are also presented.

Table 2 directly compares RPR results on samples tested both in the field and in the laboratory. There was agreement in 1207 negative samples and 35 positive samples (95.9%); 41 (3.2%) samples were positive in the field but negative in the laboratory. A further 12 (0.9%) samples were read as negative in the field and positive in the laboratory.

A comparison of the rapid syphilis test with the TPHA test, which is a test also based on *T pallidum* antigens, is shown in table 3. All 29 TPHA positive/RST negative samples remained RST negative on repeat testing.

DISCUSSION

The last guidelines for serological diagnosis for syphilis, produced by the World Health Organization,[5] recommended the use of a cardiolipin test such as the RPR and the TPHA for screening purposes. These guidelines are still in place in many countries but with the development of sensitive and specific treponemal antigen based enzyme immunoassays (EIA) they have been extended in some countries, including the United Kingdom.[6] Results using these assays now suggest a sensitive EIA, as a single screening test would give similar results to RPR and TPHA in combination. In areas where ELISA technology is readily available screening can be automated and more standard reliable results obtained.[7] In addition, the FTA-abs, previously considered as the "gold standard" confirmatory test, has been shown to have a poor specificity and is being superseded by newer, easier antitreponemal IgM ELISAs.[8] In this study we use the traditional standard of laboratory RPR and TPHA positive to indicate active syphilis and we compared this standard both with RPR carried out in field conditions that are typical of many developing country health centres and with the newly developed RST strip.

There is little information available on the performance of syphilis tests under field conditions, although decentralised syphilis prevention programmes in antenatal clinics using RPR testing has been recommended.[9] A study in an antenatal clinic in South Africa[10] using RPR testing showed that clinic testing had a sensitivity of 92.8% and a specificity of 96.3% when compared to reference laboratory results, which led to its recommendation for use. In contrast, Van Dyck *et al*[11] using the RPR teardrop test in field clinics found it to be 69.7% sensitive and 96.5% specific compared to standard RPR/TPHA tests and concluded it was not reliable in these circumstances. The intermediate sensitivity of 77.5% we found here for field RPR testing is closer to that found in the latter study.

The new RST was easier to use and easier to interpret than the RPR test especially where field conditions were difficult. The RPR and RST performed similarly as field screening tests for diagnosing active syphilis, although the field RPR was a little more sensitive and slightly more specific than the RST. Neither test predicted well the presence of active syphilis. Table 1 shows that the chance of someone with a positive test being confirmed as having serological active syphilis in the laboratory was less than 50% for both tests. These predictive values are influenced by the low prevalence of active syphilis (3%) in this population. To extrapolate to settings of differing

Table 4 The calculated predictive values of the field RPR and RST at different active syphilis prevalences

Prevalence of active syphilis	Test:	2%	3%	5%	10%	15%
Probability of syphilis when test is positive	RST	24.2%	32.6%	45.1%	63.4%	73.4%
	Field RPR	30.5%	40.0%	53.2%	70.5%	79.2%
Probability of syphilis when test is negative	RST	0.53%	0.80%	1.36%	2.83%	4.42%
	Field RPR	0.47%	0.71%	1.2%	2.49%	3.90%

prevalence assuming that sensitivity and specificity remain the same we present calculations based on the likelihood ratio for a positive test (LR(+) = sensitivity/(1 − specificity)) and for a negative test (LR(−) = (1 − sensitivity)/specificity). These can be used to estimate the predictive values of the tests in settings of differing prevalence of active syphilis.[12] From table 1 the LR(+) for the RST is 15.6 and that for the field RPR is 21.6. The LR(−) is 0.26 for the RST and 0.23 for the field RPR. Table 4 shows the calculated predictive values of the tests at different active syphilis prevalences. Thus, our data suggest that where syphilis is a major public health problem (10–15% prevalence), and laboratory facilities are limited, a positive test for either field RPR testing or RST would imply a 65–80% probability of active syphilis, and a decision to treat these patients for active syphilis is straightforward. Of more concern is the unreliability of the negative test: 3–4% of subjects with a negative test would escape detection and treatment, reflecting the requirement for screening tests to be as sensitive as possible.

Discrepant samples, for RPR testing, were more likely to be positive in the field and negative in the laboratory (table 2). The field conditions were often poor, the temperature was between 35–42°C, the atmosphere was very dusty, and the light available poor. RPR reagent has been found to be temperature sensitive.[13 14] Reactivity decreases below 23°C and increases above 29°C, and even though the reagent was kept in a cool box between samples the ambient temperature was such that the testing was at temperatures above 29°C. This, we think, explains why more positives were found with the field RPR, though other factors will have contributed to the discordant results. The tests had a tendency to dry within the mixing time and although they were performed under a cover the amount of dust in the atmosphere must also have affected results. Poor light for reading did not help, particularly with samples that were difficult to determine. The laboratory assistants who undertook the tests were well trained and conscientious, used to performing RPR tests, and were provided with controls; this would not always be the case in rural health centres. In contrast, the conditions in the laboratory were good, air conditioned, and had minimal dust and good light. Reproducibility was satisfactory when a subset of samples was retested by RPR in the laboratory for quality control purposes.

A previous evaluation of the RST[15] in a UK laboratory showed a much better performance, with an overall sensitivity of 94%. However, both conditions for testing and the population studied were different from those evaluated in this study. Like the TPHA the RST is based on specific treponemal reactivity, in this case to a recombinant 47 kDa protein antigen. Though this recombinant antigen is specific to *T pallidum*, post-translational processing of the protein in vivo or HLA haplotype restricted responsiveness could lead to lack of reactivity during infection. Conversely, the large number of treponemal antigens exposed during the TPHA test could increase the chance that some cross reaction with other organisms may occur. This may explain some of the discrepancies we observed between the two tests (table 3). Perhaps a mixture of several recombinant test antigens might overcome some of these problems. It is traditional to call for appropriate cheap and better syphilis testing, but it is far from clear how we should decide whether one test for syphilis is better than another, given these kinds of difficulties with defining a sensitive and specific gold standard. One approach may be to compare the utility of different tests by evaluating different testing strategies directly against public heath outcome.[16]

Key messages

- The RPR test does not perform well in hot, dusty field conditions such as are found in many developing country health centres
- The rapid syphilis screening test has the same sensitivity and specificity as RPR but measures specific treponema antibodies
- Use of screening depends upon prevalence in a population to make it worthwhile

Similar questions arise in decision making over screening and testing strategies. If the traditional gold standard, RPR/TPHA positive, means active syphilis in this setting then the field RPR and RST have positive predictive values of only 30–40%. This could be acceptable in higher prevalence settings but some overdiagnosis and overtreatment of patients is bound to occur. There are some hazards to the patients here: suggestions have been made that administering STD treatment and partner notification can cause domestic disruption and even violence in rural societies in similar settings to ours.[17] Social as well as laboratory and treatment issues would need to be taken into account when looking at the benefits of screening policies. If screening tests are used where conditions for testing are poor, some means of communicating the diagnostic doubt is necessary in patient counselling and partner notification policies.

Our data also suggest that negative field testing does not reliably exclude active syphilis in high prevalence situations; hence even in an ideal situation where all mothers are screened adverse birth outcomes could still be anticipated. This situation is unsatisfactory but until better alternatives can be found or a better gold standard for syphilis testing can be achieved these problems must be taken into account when using syphilis screening in public health circumstances.

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CONTRIBUTORS

BW designed the study, coordinated the laboratory testing, and drafted the report; GW conceived and designed a larger study on reproductive health of which this study was a part, coordinated the fieldwork, and edited the report; LM contributed to the study design, was responsible for handling and analysis of data, and edited the report; JB contributed to fieldwork and analysis of data; RB contributed to study design, analysis of data, and edited the report.

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