EVALUATION OF A LOW COST, MACROSCOPIC NONTREPONEMAL TEST FOR SYPHILIS USING REUSEABLE SUPPLIES

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SYNOPSIS

The rapid plasma reagin (RPR) 18-mm circle card test for syphilis serology has been used widely by many laboratories because of the stability of the antigen and the test results are read macroscopically. It is, however, a costly test for many routine laboratories. Our laboratory has modified the test using the same antigen but other items are reuseable. As a result the cost of the test is reduced by over 90%. The test compares favourably with the original RPR (97.5% agreement) and other macroscopic non-treponemal tests which are commercially available.

INTRODUCTION

The original rapid plasma reagin (RPR) (1) test produced by Hynson, Westcott and Dunning Inc. has been used by many laboratories as the standard nontreponemal test for syphilis for many years. The advantages of the test over the Venereal Disease Research Laboratory (VDRL) slide test (2) are that the antigen is stable, and the test results are read macroscopically with the naked eyes.

Because of its success, several other versions have appeared in recent years (3, 4, 5, 6, 7, 8). They all utilise stabilised antigen to which is added either charcoal particle or an appropriate toning agent. The tests, however, are either costly or the antigen preparation requires skill and special facilities available only in the larger laboratories. Consequently, most medium size laboratories especially in developing countries, are still using the VDRL slide tests as their standard method.

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The cost of the RPR test is due mainly to the disposable items and not the antigen. We have recently evaluated a modificiation of the RPR test using reuseable items instead of disposable items. The antigen used is the commercial RPR antigen. This paper presents the findings of the study in which other macroscopic nontreponemal tests, which are commercially available in Singapore were also evaluated.

MATERIALS AND METHODS

The sera used were collected from specimens which were sent to the laboratory for routine syphilis serology. They came from the veneral disease clinic in Middle Road (75), hospitalised patients with various disorders (100), blood bank (50) and antenatal clinics (16).

The following tests were performed using kits supplied by various companies: RPR (HWD) by Hynson, Wescott & Dunning; RPR (Beck) by Beckman; Syphilis Reagin Card (SRC) by Cambridge Biomedical Ltd; RPR Syfacard-R (Syfa) by Wellcome Diagnostics; RPR biotrolame (biot) by Laboratories Biotrol; Reagin Screen Test (RST) by Fisher Diagnostics and TRUST (Preco) by Preco Inc. The RPR (CSL) carbon antigen manufacturered by Commonwealth Serum Laboratories was also used to test the sera utilising the dispenstirs, Brewer diagnostic cards and methodology of the RPR (HWD) test.

The RPR (R) reuseable test which was developed in the laboratory consisted of replacing the Brewer diagnostic card with a white perspex plastic (polymethylmethacrylate) measuring 12.8 × 7.5 × 0.3 cm. Two rows of five 18-mm diameter circles were drawn on one surface using a template and a black permanent pen marker with a fine tip. A fixed volume pipettor with disposable tips was used to deliver 0.05 ml sera. Toothpicks were used spread the sera within the circles. The antigen used was the one produced by Hynson, Westcott and Dunning for the Automated Reagen test. The antigen dropper and the rest of the methodology were similar to the RPR (HWD) test. A solution of disinfectant-detergent containing 4% chlorhexidine, followed by tap water was used to wash the reuseable plastic items.

The FTA-ABS test was used to test the sera according to the method as described in the Manual of Test for Syphilis (2).

RESULTS

Although the various tests were similar in principle, differences were noticeable between them. The main differences were in the fineness of the particles, and the ability of the antigens to coalesce to form centrral aggregrates with non-reactive sera. Such centralised aggregations of antigen particles were seen consistently with the RPR (HWD), RPR (R), RPR (Beck), RPR (CSL) and RPR (Camb) tests. Their presence was helpful in distinguishing minimal reactivity from non-reactivity.

The antigens of the RPR (HWD) and RPR (R) tests were very smooth. The reactivity of RPR (R), however, was marginally weaker. After incubation it was necessary to delay reading for a mintue, with a few manual rotations in between, in order to bring out the reactivity of weak sera. The results were then read under an illuminating lamp. The RPR (Beck) antigen was less smooth, and we preferred to read the results without the illuminating lamp, which tended to enhance the coarseness of the particles. The RPR (CSL) antigen was slightly smoother, but the RPR (Camb) antigen was coarser than the Beckman antigen. Like the RPR (Beck) test, the results of the RPR (Camb) test were not read under enhanced illumination.

The reactivity of the RST was lower than the RPR (HWD), and it was necessary to view the results under good illumination to detect the fine aggregates given by weakly reactive sera. The RPR (Syfa) and RPR (biot) antigens were coarser than the RPR (HWD) antigen. The RPR (biot) not infrequently gave aggregates, especially at the rim, with non-reactive sera. As a result, there were occasional problems in distinguishing the non-reactive from weakly reactive sera. The TRUST (Preco) gave problem with a number of non-reactiv sera, as specks of aggregates were detected in the serum-antigen mixture. Most of them were at the rim, but some were in the centre.

Table I shows the sensitivity and specificity of the various tests. The sensitivity is the ability of the test to give reactive results for FTA-ABS reactive sera, while the specificity is the ability to give non-reactive results for FTA-ABS non-reactive sera. The sensitivity and specificity were over 80% for the RPR (HWD), RPR (R), RPR (Beck), RPR (CSL) and RPR (Camb) tests. On the other hand, the results were below 80% for RPR (Syfa), RPR (biot) and TRUST (Preco). Table 2 shows the qualitative agreement of the tests with the RPR (HWD) test. The best agreements were shown by RPR (R), RPR (Beck), RPR (CSL) and RPR (Camb), all of which achieved over 95% agreement. However, both RPR (biot) and TRUST (Preco) gave low levels of agreement at 86.6% and 77.9% respectively.

TABLE 1
COMPARATIVE SENSITIVITY AND SPECIFICITY OF NONTREPONEMAL TESTS

	Sensitivity (%)	Specificity (%)
RPR (HWD)	93/108 (86.1)	107/133 (80.5)
RPR (R)	91/108 (84.3)	109/133 (82.0)
RPR (Beck)	89/108 (82.4)	112/133 (84.2)
RPR (CSL)	75/91 (82.4)	90/108 (83.3)
RPR (Camb)	63/75 (84.0)	87/100 (87.0)
RST	82/108 (75.9)	109/133 (82.0)
RPR (Syfa)	77/102 (75.5)	91/117 (77.8)
RPR (biot)	62/84 (73.8)	87/110 (79.1)
TRUST (Preco)	56/82 (68.3)	75/99 (75.8)

DISCUSSION

The results of the study indicate that there are major differences in the performance of the various macroscopic nontreponemal tests for syphilis. The best results were obtained with the RPR (HWD), RPR (R), DPR (Beck), RPR (CSL) and RPR (Camb). With these tests there was little problem in identifying sera giving minimally reactive results. However, even for them there were minor differences in the fineness of the particles, which had to be taken into consideration in reading the test results. Experience with each of them will enhance the confidence of the staff reading them. In view of this, it is preferable for laboratory staff to

TABLE 2
QUALITATIVE AGREEMENT OF NONTREPONEMAL TESTS

		RPR (HWD)		
		Reactive	Non reactive	% Agreement
RPR (R)	R	114	1	
	N	5	121	97.5
RPR (Beck)	R	109	1	
	N	10	121	95.4
RPR (CSL)	R	90	3	
	N	5	101	96.0
RPR (Camb)	R	76	0	
	N	5	94	97.1
RST	R	102	4	
	N	17	118	91.3
RPR (Syfa)	R	94	9	
	N	6	110	93.2
RPR (biot)	R	76	9	
	N	17	92	86.6
TRUST (Preco)	R	64	16	
	N	24	77	77.9

R — reactive
N — nonreactive

stick to one test rather than to change them at frequent intervals.

It is difficult to compare reliably the levels of sensitivity and specificity obtained from this study with the results obtained from other studies without identifying more definitively the nature of the patients' illnesses. This is because the sensitivity will be influenced by the number of patients with primary, latent or treated syphilis, while specificity is influenced by the number of patients giving biologically false positive results. However, it is better to compare the levels of agreement with a standard test, such as the RPR (HWD) test.

The levels of agreement for RPR (Beck), RST and RPR (Syfa) (Table 2) are only slightly lower than the 96.7%, 94.1% and 94.4% (quantitative) which were found by others respectively (3, 5, 7). The level for TRUST (Preco), however, is somewhat lower than the 86.5% previously reported (8). This is probably due to the high proportion of patients (19.1%) giving biologically false positive results. Thus if those sera which gave false positive results were excluded, the agreements for RPR (Beck), RST and RPR (Syfa) would have been 98.6%, 96.4% and 96.1% respectively.

The RPR (R) gave 97.5% agreement. Though it was necessary to use a lamp to read minimally reactive test results, it has the advantage of being cheap. All the supplies needed, except the antigen, are reuseable. The perspex is obtainable from signboard makers. Both the plastic plates and disposable tips can be recycled after washing with a disinfectant-detergent and tap water. The cost of the RPR antigen is less than 10% of the standard RPR (HWD) test which comes in a kit form. The antigen can be purchased by itself, since it is also used in the Automated Reagin Test. This obviates the need for laboratories to prepare and standardise their own antigen preparation. The test appears suitable for use by laboratories in developing countries. We have not evaluated the RPR (R) with other brands of antigen. If these are to be used, they should first be evaluated.

Unsatisfactory results were obtained with the RPR (biot) and TRUST (Preco) tests. Both tests gave rise to difficulty in distinguishing minimally reactive from nonreactive sera. The agreements of both tests with the RPR (HWD) were low. It should be stated that the TRUST (PRECO) is not the same at the TRUST test as originally described (6). In this test the firm has dried the antigen on the concave surface of the card where the test is done. The test serum itself is used to reconstitute the antigen.

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