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Comparison between the automated reagin test and reagin screen test methods of VDRL screening tests for syphilis in use in a routine laboratory

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SUMMARY A comparison is made between the automated reagin test (ART) using Technicon AutoAnalyzer equipment and the reagin screen test (RST) introduced by Lederle using a new antigen formulation. Treponemal haemagglutination tests (TPHA) were done simultaneously as second screen tests. The absorbed fluorescent treponemal antibody test (FTA-ABS) was used in all seropositive cases. Altogether 1154 sera were tested; 1028 were negative with all tests, 66 were positive with all tests, 42 were positive with TPHA and negative with ART and RST, and 16 were positive with TPHA and RST and negative with ART. It was concluded that the use of the RST and TPHA together would be the more sensitive screen test.

Criteria for tests to be used in a routine laboratory screen for the detection of serum antibodies against *Treponema pallidum* include: sensitivity, reproducibility, moderate cost, and sufficiently good design to minimise operating error without producing boredom in the technical staff carrying out the test.

In the laboratory attached to the James Pringle House Clinic of The Middlesex Hospital, two screening tests are currently used to examine approximately 500 sera each week for the presence of syphilitic antibodies. These tests are the *Treponema pallidum* haemagglutination (TPHA) test (Rathlev, 1967) and the automated reagin test (ART) using Technicon AutoAnalyzer equipment (McGrew *et al.*, 1968) with VDRL carbon antigen. Fluorescent treponemal antibody absorbed (FTA-ABS) tests are done on all sera from new positive cases of syphilis, contacts, and other possible cases, and if necessary these sera are also sent to the Venereal Diseases Reference Laboratory at The London Hospital for further tests including the *Treponema pallidum* immobilisation (TPI) test.

All positive ART screen tests are titred out to give a quantitative result. This is used both as a check on the correctness of the original result and to follow the response to treatment of the patient on subsequent visits to the clinic.

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This paper presents the results of a trial of methods and materials run as a preliminary to possible changes in the routine screen tests for syphilis used in the clinic laboratory in view of new antigens and techniques becoming commercially available.

Material and methods

SERA

All clotted-blood samples from the clinic were received in 100 × 12 mm screw-capped glass vials which can be put directly into the AutoAnalyzer trays as no inactivation is needed for any of the three tests. The specimens were centrifuged after clot retraction had taken place and if necessary glass beads were added to raise the serum level in tubes where it was too low for the AutoAnalyzer probe to reach. Serum from specimens sent from outside the clinic had usually to be transferred into standard AutoAnalyzer cups because of non-standard tubes. All three tests were done on the same tube of blood. Control sera from a batch of known strength were included in each day's run of the three tests.

Treponema pallidum HAEMAGGLUTINATION TEST

A microhaemagglutination technique was used modified, but using the materials provided in the kits available from Fujizoke Pharmaceutical Co Ltd, with 'U' shaped WHO microtitre trays and a Compu-Pet automatic diluter (General Diagnostics

Department, William R. Warner and Co Ltd). The trays were sealed with Sellotape and the results were read after three hours' incubation at room temperature.

AUTOMATED REAGIN TEST

The Technicon AutoAnalyzer System was used following the method laid down in the manual. VDRL carbon antigen, as supplied by Searle Diagnostic (National Communicable Disease Center, Venereal Disease Programme, 1969) was used in qualitative and quantitative tests; twofold dilutions for the latter were made by hand and put through the machine in standard AutoAnalyzer cups at the end of the daily run.

REAGIN SCREEN TEST

This macroscopic non-treponemal flocculation test was done using Lederle Diagnostics kit and following the method given in the kit. The same samples were tested which had already been used in the two previous tests. The antigen supplied in these kits, the instructions state, has been '... dyed with a lipid-soluble dye imparting a light blue coloration to the antigen (March *et al.*, 1974)' and an intense blue flocculation forms with reactive serum, which is readily visible macroscopically.

FLUORESCENT TREPONEMAL ANTIBODY ABSORBED TEST

FTA-ABS tests were done using commercially available antigen, sorbent, and conjugate and a method based on that described by the Venereal Disease Research Laboratory (1968) on sera collected at the time the screen tests were done and stored at −20°C until required.

Table 1 *Distribution of positive reactions with ART, VDRL, RST, and TPHA tests of 126 reactive sera out of the 1154 screened*

Derivation of serum	TPHA + ART + RST + FTA +	TPHA + ART − RST − FTA +	†TPHA + ART − RST + FTA +	TPHA − ART + RST + FTA −	TPHA − ART − RST + FTA ? TPI −
Patients with previously positive test	51	38	12	1*	—
Patients with previously negative test	15	4	4	—	1
Total	66	42	16	1	1
% of positive sera	52·4	33·3	12·7	0·8	0·8

*Known BFP.

†The sera mentioned in this column occurred early in the investigation and in common with the other sera reported here were not titrated owing to a shortage of antigen. The reactions were graded on a scheme from + to +++ inclusive. As soon as antigen became freely available all positive sera were titrated. Subsequent experience has shown that + approximates to a serum dilution of Neat −½, ++ to a dilution of ½-¼, and 3+ to a dilution of ⌟¼.

On this scheme, 13 of the 16 sera were graded 1+ and the remaining three were ++.

Table 2 *Sequential results from three patients in different stages of syphilis*

Patient	*Date*	*Treatment*	*RST*	*ART*	*TPHA*	*FTA*	*WR*	*RPCFT*
A3 (1960)								
USA	19 February 71		.	−	.	+	−	±
	9 September 71		.	−	.	.	−	−
A1 Italy	8 September 73	Penicillin, 600 000 units × 10 days	.	1:4	3+	.	.	.
	2 November 73		.	1:8	3+	.	.	.
	10 December 73		.	1:2	3+	.	.	.
	11 April 74		.	−	3+	.	.	.
	7 April 75		.	−	3+	.	.	.
	12 April 76		2+	−	3+	.	.	.
A3	18 February 76	Penicillin, 600 000 units × 10 days	.	1:2	2+	1+	.	.
	24 March 76		.	N	3+	.	.	.
	7 April 76		.	N	3+	.	.	.
	5 May 76		2+	−	3+	.	.	.
A2	11 August 75	Penicillin, 600 000 units × 10 days	.	−	−	.	.	.
	9 January 76		.	1:16	2+	1+	.	.
	30 January 76		.	N	2+	.	.	.
	12 April 76		2+	−	2+	.	.	.

Results

Negative results with all three screen tests were obtained in 1028 (89%) of the sera tested and as none of the patients had any history of contact with syphilis at the time no FTA or TPI tests were done on these negative sera. One other serum was also in this group but as the patient was a contact of latent syphilis an FTA was done, giving a doubtful positive result; a subsequent TPI was negative. One other serum with positive RST and ART was a known biological false positive reactor.

Table 1 shows that over half (66) of the remaining 126 sera were positive with all three screening tests; the majority of these tests were a follow-up of treatment and were expected to be positive. The next highest total (42) is again chiefly of follow-up cases, in which the TPHA test is the only positive screen test, although four of these patients had not previously had a positive test and might have been missed if the TPHA test had not been done. Column 3, Table 1, shows 16 sera in which the ART was negative but the RST positive. These 16 results were all from patients attending the clinic. Two of the new patients had had previous courses of antibiotics for various reasons, and the other two new ones had treponemes present on dark-ground examination of lesions and were presumably of such low titre antibody that the less sensitive ART was not yet showing up as positive.

Table 2 gives details of three of the previously positive ART cases, in which follow-up titrations had given a steadily falling ART titre which had become negative before the RST was done; comparative titrations undertaken when more antigen was obtained later (the results of which are shown in Table 3) make the RST two- to four-fold more sensitive than the ART. These results were obtained from the same titration done simultaneously to eliminate diluting errors or variation in media, etc.

Table 3 *Comparison of titres with ART and RST antigen tests*

No. of sera	*Titre with:*	
	ART	*RST*
2	N	1:2
3	N	1:4
3	1:2	1:4
3	1:4	1:8
1	1:4	1:16
1	1:8	1:32
1	1:16	1:32
1	1:64	1:64

Discussion

Correlation between VDRL tests done by the ART or RST methods is to be expected, and our results confirm this. The bias in favour of the RST is partly due to the variation in titre as the finely divided weak positive results of the ART tend to be invisible in the paper-strip. (This has been evident for some years when we have compared the results of urgently requested tests, done by hand on glass slides while the patient waited, with the repeat titration done the next day by ART.) The RST was positive in 16 patients in whom the ART was negative, as shown in Table 1, column 3, and if the RST had not been used 16 more samples would have been (wrongly) added to the total of 1028 already found to be negative.

The finely divided RST antigen undoubtedly makes the results of weak positive tests easier to read than the ART tests using some commercially available carbon type VDRL antigens, and there is also a saving in time. Against this must be set the greater risk of technical error involved in individual testing of sera where large numbers are concerned although many of these mistakes can be eliminated by titrating all positive reacting sera.

It must also be pointed out that if the TPHA had not been in use 42 more samples would have been (wrongly) added to the total of 1028 found negative with ART and RST as screen tests, or the total of 1044 found negative if the RST had not been used instead of the ART.

Conclusions

It appears that the use of the TPHA together with the RST as screen tests would be a good sensitive combination to use at this time, taking convenience and expense into consideration.

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References

McGrew, B. E., Du Cros, M. J. F., Stout, G. W., and Falcone, V. H. (1968). Automation of a flocculation test for syphilis. *American Journal of Clinical Pathology*, **50**, 52-59.

March, R. W., Stiles, G. E., and Forgione, P. S. (1974). A new reagin card test for syphilis. *Abstracts of Annual Meeting of American Society of Microbiologists*, 93-98.

National Communicable Disease Center. Venereal

Disease Program. (1969.) *Manual of Tests for Syphilis* (Public Health Service Publication, 411). U.S. Government Printing Office, Washington D.C.

Rathlev, T. (1967). Haemagglutination test utilizing pathogenic *Treponema pallidum* for the sero-diagnosis of syphilis. *British Journal of Venereal Diseases*, **43**, 181-185.

Venereal Disease Research Laboratory (1968). Technic for fluorescent treponemal antibody-absorption test (FTA-ABS) test. *Health Laboratory Science*, **5**, 23-37.