The Reiter protein complement-fixation test using the AutoAnalyzer

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SYNOPSIS A method for the Reiter protein complement-fixation test using the Technicon AutoAnalyzer is described. Two hundred and fifty sera were tested, when six false-positive and nine false-negative results were obtained when compared with results obtained by the manual method. Deep freezing the sera before testing on the AutoAnalyzer increased the sensitivity so that in a further 250 sera tested no false-negative and two false-positive results were obtained when compared with the results obtained at the Venereal Diseases Reference Laboratory, London.

Following the successful development of the routine automated technique for the Wassermann reaction (Pugh and Gaze, 1965 and 1966), it was thought necessary to develop some other serological test for syphilis, which could be carried out routinely on the AutoAnalyzer in parallel with the Wassermann reaction.

Perusal of the literature led to the conclusion that the complement-fixation test using Reiter protein antigen would be the most useful. Sequeira (1959) found that the specificity and the sensitivity of the Reiter test were higher than those of the standard tests for syphilis and suggested that the ideal combination of tests for the diagnosis of the treponematoses is the Reiter test and two tests for reagin, one at normal and one at high sensitivity, and any discrepancies should be submitted to the treponemal immobilization test. Wilkinson and Johnston (1959) pointed out that in tests on sera which presented diagnostic problems, the Reiter protein complement-fixation test gave results which were in close agreement with the treponemal immobilization test. Foster, Nicol, and Stone (1959) suggested that the Reiter test is a good routine test for treponemal disease, because it is sensitive and reproducible, it appears to be specific, and it depends upon an antibody different from reagin.

MATERIALS

- 1 Complement freeze dried from Burroughs Wellcome.
- 2 Haemolysin (Stayne Laboratories).
- 3 Sheep cells (Burroughs Wellcome).
- 4 Buffered saline as complement-fixation test dilution tablets (Oxoid Division, Oxo Ltd.) (Wilkinson 1962).

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5 Reiter protein antigen (Organon Ltd.).

6 The manifold is illustrated in Figure 1 (Pugh and Gaze, 1966).

METHOD

Complement-haemolysin titration as described for the automated Wassermann reaction is carried out, and the weakest dilution of complement with the weakest dilution of haemolysin which gives complete haemolysis is used to calculate the strength of the haemolytic system for the Reiter reaction. The complement is used at 1.5 M.H.D. (instead of 2 M.H.D. as in the Wassermann reaction) and the haemolysin is used at the 1½ M.H.D., the same strength as for the Wassermann reaction (Pugh and Gaze, 1965).

The automated Reiter protein complement-fixation test is also carried out exactly as described for the automated Wassermann reaction (Pugh and Gaze, 1965 and 1966), using the buffered saline given above and the Reiter antigen in place of the Maltaner antigen. Since the Reiter protein complement-fixation test was not carried out as one of the routine serological tests in this laboratory, Dr. P. J. L. Sequeira at the Central Serological Laboratory, Manchester, kindly agreed to examine 250 routine sera using the manual Reiter test so that the results of the automated method could be assessed.

INTERPRETATION

A pool of negative sera and a positive serum are included with each batch and any serum giving a peak of one transmission line above the negative peak is regarded as positive and the serum is 'run' again at the end, in the absence of antigen, as the anticomplementary check. Figure 2 is a short run of Reiter tests together with the anticomplementary check. It shows that sera nos. 4 and 15 are positive, serum no. 14 is a weak positive, and serum no. 10 is anticomplementary.

FIG. 1. *Manifold and flow diagram.*

FIG. 2

RESULTS

Of the 250 sera examined, 206 were found to be negative and 29 positive by both automated and manual methods and there were 15 discrepancies. In six instances the automated method gave positive results which were not confirmed, two were negative and four anticomplementary, by Dr. Sequeira. Nine sera which gave negative results by the automated method gave positive reactions by the manual method (Fig. 3).

This failure of the automated method to detect a positive reaction on nine occasions (especially since three of the four sera tested for treponemal immobilization were positive) caused much concern. It was thought that this failure in sensitivity was due to the fact that the automatic method does not allow for the 18 hours' refrigeration of the serum-antigen-complement mixture.

In a critical evaluation of the Reiter protein complement-fixation test, Taylor Peterson, De Haven, Wright, and Miller (1960) found that deep freezing the sera enhanced the reactivity and that the results of the Reiter tests were then closely related to the results for treponemal immobilization and to the

clinical conditions of the patients. These findings were confirmed by Neblett and Merriam (1965), who found that 82.6% of negative Reiter tests became weakly reactive or reactive after deep freezing the sera. It was decided that if this practice of deep freezing the sera were followed, the results of the automated Reiter tests would agree more closely with the results of the manual method. The sera that had been tested by Dr. Sequeira had been kept deep frozen before dispatch.

The manual Reiter protein complement-fixation test was started in this laboratory and the tests were carried out in parallel with the automated method. Dr. A. E. Wilkinson, at the Venereal Diseases Reference Laboratory, London, kindly agreed to test any sera in which discrepant results were obtained.

Two hundred and fifty sera, mainly from the special treatment centre of the Royal Infirmary, Leicester, were examined in this way. The sera were tested by the manual method and by the automated method after deep freezing for three days. One hundred and ninety-four sera were negative and 40 were positive by both automated and manual methods. Five were anticomplementary. There were

FIG. 3. Analysis of discrepant results between AutoAnalyzer and manual (Dr. P. J. L. Sequeira) methods for Reiter complement-fixation tests.

TABLE I
DISCREPANCIES BETWEEN AUTOMATED (AFTER DEEP FREEZING) AND MANUAL (LEICESTER ROYAL INFIRMARY) METHODS
Serial No. Reiter Protein Complement-fixation Test of Leicester Royal Infirmary Venereal Diseases Reference Laboratory, London

	Machine	Manual	Reiter Test	V.D.R.L. Slide Flocculation Test	Cardiolipin Wassermann Reaction	Fluorescent Antibody Tests
64231	+	-	+	+	+	+
651092	+	-	+	+	+	+
65393	+	-	-	+	+	+
65944	+W	-	-	-	-	+W
11638	-	+W	-	-	-	-
65895	-	+W	-	-	-	-
66131	-	+	-	-	-	-
20774	-	+W	-	+W	-	-
65856	-	+	-	+½	+	+(TPI+)
65820	-	+W	-	-	-	-
17683	-	+W	-	+	+	+

11 discrepancies which were tested by Dr. Wilkinson (Table I). Four sera which gave positive results by the automated method were negative by the manual (Leicester Royal Infirmary) method; two were confirmed as positive by Dr. Wilkinson; the other two were found to be negative by him, but gave positive

fluorescent antibody tests; one of these sera was also positive for the cardiolipin Wassermann reaction and by the Venereal Diseases Reference Laboratory slide flocculation test. There were seven sera which were negative by the automated technique and positive by our manual method. Seven were confirm-

FIG. 4. Analysis of discrepant results between AutoAnalyzer (sera frozen) and manual (Leicester Royal Infirmary and Dr. A. E. Wilkinson) methods for Reiter complement-fixation tests.

ed as negative by Dr. Wilkinson, two of which gave positive reactions for the cardiolipin Wassermann reaction the Venereal Diseases Reference Laboratory slide flocculation test, and the fluorescent treponemal antibody test (Fig. 4).

CONCLUSIONS

The adaptation of the Reiter protein complement-fixation test for use on the AutoAnalyzer is satisfactory and the results obtained show excellent agreement with the manual method when the sera to be tested are deep frozen for three days before doing the automated method. When the results are compared with the results of the manual method as carried out by the Venereal Diseases Reference Laboratory, London, no false negatives were ob-

tained; two false positives occurred, although both of these gave positive fluorescent antibody tests.

Our thanks are due to Dr. P. J. L. Sequeira and to Dr. A. E. Wilkinson for carrying out the many serological tests and for making their results available to us.

REFERENCES

- Foster, W. D., Nicol, C. S., and Stone, A. H. (1959). *Brit. J. vener. Dis.*, 35, 181.
- Neblett, T. R., and Merriam, L. R. (1965). *Amer. J. clin. Path.*, 43, 281.
- Pugh, V. W., and Gaze, R. W. T. (1965). *Brit. J. vener. Dis.*, 41, 221.
- , — (1966). *J. Med. lab. Technol.*, in the press.
- Sequeira, P. J. L. (1959). *Brit. J. vener. Dis.*, 35, 139.
- Taylor Peterson, E., De Haven, R. C., Wright, E. T., and Miller, J. N. (1960). *Ibid.*, 34, 231.
- Wilkinson, A. E. (1962). Serological Tests for Syphilis. Association of Clinical Pathologists. Broadsheet No. 41 (New Series). October 1962.
- , and Johnston, N. A. (1959). *Brit. J. vener. Dis.*, 35, 175.

FIG. 1. Flow diagram.

sheep serum as for the manual test with a final concentration of 1%. The cells are kept in suspension by means of a magnetic stirrer. Serum samples are diluted with 0.85% sodium chloride, mixed in a single mixing coil and sensitized sheep erythrocytes added, mixing again in a single coil, then into a 40-foot glass coil at 37°C.

From the delay coil, the agglutinates settle in a straight horizontal piece of glass tubing. Removal of agglutinates is accomplished by a standard T piece. The remaining cell suspensions are re-mixed in a single glass mixing coil and passed into a 15 mm. flow cell in an N colorimeter using 550 mμ filters.

Initially a base line of 98% transmission is set with all lines pumping saline. Sheep cells are pumped through the appropriate line until a 5% transmission is obtained. Any sample giving a rise of more than 2% transmission

from this base line is regarded as a potential positive and titrated manually. To facilitate the identification of samples a known positive is placed at the beginning of the batch and at every tenth cup as a marker.

Some 500 tests have so far been performed and the results compared with those from the manual technique. No titre below 1/16 as shown by the manual method has been recorded by the automated method nor has any automated titre of less than 1/16 been subsequently found to be greater after manual titration.

This screening technique represents a saving in manual titration of approximately 60% of samples.

REFERENCES

- Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O. (1948). *Proc. Soc. exp. Biol. (N.Y.)*, 85, 4.

CORRECTION

This is figure 2 of the paper by V. P. Pugh and R. W. T. Gaze, entitled 'The Reiter protein complement-fixation test using the AutoAnalyzer' (*J. Clin. Path.*, 19, 595), which was wrongly printed.