STREPTOMYCIN, SODIUM AZIDE, AND MERTHIOLATE AS BACTERIOSTATIC AGENTS FOR USE WITH THE *TREPONEMA PALLIDUM* IMMOBILIZATION TEST*

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The problems of bacterial contamination associated with the shipment or preservation of serum specimens to be tested with the conventional serologic tests for syphilis are increased when such specimens are to be tested with the *Treponema pallidum* Immobilization (TPI) test. Bacterial contamination interferes with the ease and accuracy of reading the test and the metabolic or somatic products of certain contaminants produce non-specific immobilization. This report confirms the value of streptomycin as a bacteriostat, as reported by Ledbetter and Cumming (1954), and also shows that sodium azide and merthiolate are satisfactory bacteriostats for use with the TPI test.

Ledbetter and Cumming employed streptomycin at a concentration of 100 $\mu\text{g./ml.}$ to prevent further growth in bacterially contaminated specimens submitted for testing. They concluded that a concentration of 100 $\mu\text{g./ml.}$ did not have a harmful effect on the viability of *Treponema pallidum* or the residual complement reaction. They did not report on the effect of 100 $\mu\text{g./ml.}$ concentration on the sensitivity of the test. Studies in this laboratory indicate that 100 $\mu\text{g./ml.}$ does not lower test sensitivity significantly. Eleven quantitative tests with sera containing 100 $\mu\text{g./ml.}$ gave a geometric mean 50 per cent. immobilization titre of 1:53, while without streptomycin the geometric mean titre was 1:74. This small series of comparative tests indicates that streptomycin, in the concentration employed by Ledbetter and Cumming, does not reduce significantly the sensitivity of the test.

In addition to the report by Ledbetter and Cumming, it has been reported from the Royal Free Hospital, London (WHO, 1957), that a final test concentration of 200 $\mu\text{g./ml.}$ is also satisfactory. Streptomycin is added to the complement and treponeme suspension so that when the mixture is

added to the test proper a final concentration of about 200 $\mu\text{g./ml.}$ is obtained. This permits the use of unsterile serum specimens without further treatment.

The use of sodium azide as a satisfactory bacteriostatic agent for the preservation of serum for flocculation and complement fixation tests for syphilis has been reported by Stansell (1954), who stated that serum preserved with sodium azide at 1:1000 concentration remained clear and exhibited fewer anticomplementary reactions over a longer period than serum treated with merthiolate 1:3000. He also found sodium azide to be anticomplementary at 1:100 but not at 1:1000 or 1:3000. Stansell concluded that sodium azide is superior to merthiolate for preserving sera in serologic surveys. These experiences and observations of Stansell with the usual serologic tests for syphilis and the results reported here with the TPI test indicate that sodium azide may be employed in concentration of 1:1000 as a bacteriostat with serum specimens for TPI testing.

Positive and negative sera without sodium azide added and with concentrations of 1:1000, 1:2000, 1:4000, and 1:8000 sodium azide were placed at room temperature for one day and for one week. These concentrations were diluted tenfold in the final test mixture. There was no essential difference in the 50 per cent. immobilization titres of the specimens containing various concentrations of sodium azide and of serum without sodium azide added.

Ten quantitative tests gave a geometric mean 50 per cent. immobilization titre of 1:146 without sodium azide added, and with 1:2500, 1:5000, and 1:10,000 sodium azide concentrations the geometric mean titres were 1:83, 1:98, and 1:167 respectively. It is evident from these results that there was no essential difference in the titres of the serum without sodium azide added and the titres in which the final tube

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concentration of sodium azide was 1:10,000 (1:1000 concentration in the serum specimen).

Merthiolate (sodium ethylmercurithiosalicylate) has been repeatedly reported to be of value in the preservation of serum and cerebrospinal fluid specimens to be tested with serologic tests employing tissue extract or cardiolipin antigens (Harris and Mahoney, 1944; Powell and Jamieson, 1938; Rein and Kelcec, 1954). Nelson, Zheutlin, Diesendruck, and Austin (1950), reporting the unpublished results of Barron, stated that merthiolate, in the usual dilutions used in serum, exerts a marked toxic action on *Treponema pallidum in vitro*. It has since become a serologic axiom that specimens preserved with merthiolate are unsuitable for TPI testing. Employing the technique described below, it has been found that merthiolated specimens are suitable for TPI testing.

Serum specimens containing 1:1000 to 1:2000 merthiolate have been tested with the TPI test as given by Nelson and Diesendruck (1951), with added sodium thioglycollate and complement as recommended by Portnoy, Harris, and Olansky (1953). Furthermore, the sodium thioglycollate must have a minimum assay of 95 per cent. sulphhydryl content or it is not used for TPI testing. It has been found that sodium thioglycollate will retain its sulphhydryl level for three years or more if it is sealed in all-glass ampoules.

Quantitative tests have shown that similar titres are obtainable, with and without merthiolate, when the sulphhydryl level is adequate. For example, a given serum resulted in a titre of 1:205 without added merthiolate and of 1:215 when the serum contained 1:1000 merthiolate. It appears to make no difference whether the thioglycollate is contained in the medium or is placed with merthiolate-containing serum

before mixing with the treponeme suspension. A titre of 1:210 was obtained when sodium thioglycollate was added to the serum before adding the treponeme suspension, and a titre of 1:215 was obtained when all the sodium thioglycollate was present in the medium.

Qualitative testing of 250 serum specimens with concentrations of merthiolate ranging from 1:1000 to 1:2000 was found to yield satisfactory tests. The average motility of the controls of the 250 specimens was 84 per cent.; the complement and other control tubes had an average motility of 88 per cent. after 16 to 18 hours' incubation.

It thus appears that, by the use of an adequate concentration of sulphhydryl (sodium thioglycollate), merthiolated serum specimens may be tested and valid results obtained with the TPI test.

Conclusions

The observations presented in this paper confirm the use of streptomycin as a bacteriostat for use with the TPI test as reported by Ledbetter and Cumming (1954). Original data are presented which show that sodium azide and merthiolate are satisfactory bacteriostats for use with the TPI test.

REFERENCES

- Harris, A., and Mahoney, J. F. (1944). *Vener. Dis. Inform.*, 25, 46.
 Ledbetter, R. K., Jr., and Cumming R. J. (1954). *Brit. J. vener. Dis.*, 30, 214.
 Nelson, R. A., Jr., and Diesendruck, J. A. (1951). *J. Immunol.*, 66, 667.
 —, Zheutlin, H. E. C., Diesendruck, J. A., and Austin, P. G. M., Jr. (1950). *Amer. J. Syph.*, 34, 101.
 Portnoy, J., Harris, A., and Olansky, S. (1953). *Ibid.*, 37, 101.
 Powell, H. M., and Jamieson, W. A. (1938). *Proc. Indiana Acad. Sci.*, 47, 65.
 Rein, C. R., and Kelcec, L. C. (1954). *Amer. J. Syph.*, 38, 308.
 Stansell, G. B. (Quoted by Rein and Kelcec, 1954).
 World Health Organization Co-operative Study on the TPI Test (WHO/TPI/3, January 15, 1957).