Heated Versus Unheated Sera in a Microhemagglutination Assay for Antibodies to *Treponema pallidum*

SANDRA A. LARSEN,* DONNA D. CRUCE, CAROL E. FARSHY, AND JOHN C. FEELEY

Bureau of Laboratories, Center for Disease Control, Public Health Service, United States Department of Health, Education and Welfare, Atlanta, Georgia 30333

Received for publication 24 July 1978

The effect of prior heating of sera on the results of the microhemagglutination assay for antibodies to *Treponema pallidum* was evaluated. Findings indicate that heating of sera had no significant effect on the results.

Since the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP) (1a-3) has gained popularity as a treponemal or confirmatory test for syphilis, a number of questions have arisen. One concerns the suitability of heated sera for the test. Although the MHA-TP kit currently available (Sera-Tek; Ames Co., Division of Miles Laboratories, Inc., Elkhart, Ind.) specifies the use of unheated sera, many reference laboratories, such as ours, are often faced with the problem of testing sera without knowledge of their prior treatment.

In 1976 our laboratory, in cooperation with Harold Jaffe, Venereal Disease Control Division, Bureau of State Services, Center for Disease Control, and Peter Dans, Department of Medicine, University of Colorado Medical Center, Denver, evaluated the MHA-TP test for its sensitivity and specificity (1). In addition, we studied the effect of prior heating of the specimens on the results of the MHA-TP test. These specimens were submitted refrigerated, but not frozen. Intervals between collection and shipment of the specimen to the Center for Disease Control varied. A small portion of each of the 1,015 specimens received was heated at 56°C for 30 min. They were then cooled to room temperature and coded to prevent bias on the part of the technologist. Both the unheated and heated samples were tested on the same day with the Sera-Tek kit according to the directions of the manufacturer by the same technologist and with the same antigen working dilution, thereby eliminating these major variables.

The results are shown in Table 1. Agreement between the heated and unheated sera was 99.2% (1,007/1,015). Of the eight sera that did not

TABLE 1. Effect of heating sera at 56°C for 30 min on outcome of the MHA-TP test

Unheated sera reactions	Heated sera reactions	
	R ^a	N ^b
R	138	4
N	4	869

R, Reactive 1+ - 4+.
N, Nonreactive.

agree, four were from patients with syphilis; two of these four were reactive only when not heated, and two were reactive only when heated. The other four sera were from patients with no history or clinical symptoms of syphilis. Seven of the eight discrepant readings were in the critical R 1+ reading range, but one was R 2+.

As shown in the prior study of Jaffe et al. (1), reproducibility was 100% for paired specimens tested on the same day. Therefore, from our findings we can conclude that heating had no significant effect on the results of the MHA-TP (Sera-Tek) test ($P < 0.0001$ by the chi square test).

LITERATURE CITED

1. Jaffe, H. W., S. A. Larsen, O. G. Jones, and P. E. Dans. 1978. Hemagglutination tests for syphilis antibody. *Am. J. Clin. Pathol.* **70**:230-233.
- 1a. Logan, L. C., and P. M. Cox. 1970. Evaluation of a quantitative automated microhemagglutination assay for antibodies to *Treponema pallidum*. *Am. J. Clin. Pathol.* **53**:163-166.
2. Rathlev, T. 1965. Haemagglutination tests utilizing antigens from pathogenic and apathogenic *Treponema pallidum*. World Health Organization Document VDT/RES/77.65. World Health Organization, Geneva.
3. Tomizawa, T. 1968. On the hemagglutination reaction using *Treponema pallidum* as an antigen (TPHA). *Jpn. J. Clin. Pathol.* **26**:360-364.