Heated Versus Unheated Sera in the Hemagglutination Treponemal Test for Syphilis


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Sera (920) were tested to evaluate the use of heated versus unheated sera in the hemagglutination treponemal test for syphilis. The heated and unheated samples were tested on the same day with the hemagglutination treponemal test for syphilis kit according to the manufacturer's protocol. Agreement of results between the heated and unheated sera was 99.2%. The reading pattern of agglutination was clearer and more distinct with heated sera; therefore, based solely on our preference for the reading patterns, we suggest that heated sera be used in the hemagglutination treponemal test for syphilis.

In 1978, a new confirmatory test for syphilis, the hemagglutination treponemal test for syphilis (HATTS), was introduced in the United States. When the HATTS was still in the investigational stage and before it was introduced as a commercial reagent, all evaluations of the test used sera heated at 56°C for 30 min (2, 3). Since many laboratories use nontreponemal screening tests which do not require heated sera for testing, it would be more convenient to test unheated sera for confirmation of the reactivity of nontreponemal tests when the diagnosis of syphilis is unclear. Also, many reference laboratories receive sera for testing without information as to previous heat treatment of the sera. In a previous study involving the microhemagglutination assay for antibodies to Treponema pallidum findings indicated that whether heated or unheated sera were used, there was no significant effect on the test results (1). Therefore, in the present study, our laboratory was concerned with the effect of using heated versus unheated sera in the HATTS.

We used 920 human sera (328 syphilitic and 592 nonsyphilitic) to evaluate the effect of using heated versus unheated sera in the HATTS. Fresh sera (481) were obtained from the DeKalb County, Ga., Sexually Transmitted Disease Clinic, and frozen sera (439) were obtained from the Venereal Disease Serology Serum Bank. The HATTS was performed according to the manufacturer's protocol (Difco Laboratories, Detroit, Mich.), with heated and unheated serum samples that were tested on the same day by the same technologist. Briefly, 0.1 ml of Treponema pallidum-sensitized turkey erythrocytes was added to microtitration U-plate wells containing 0.025-ml volumes of 1:16 dilutions of heated and unheated sera in HATTS test diluent. Into a second row of wells, 0.1-ml volumes of control unsensitized erythrocytes were added to 0.025-ml amounts of serum diluted 1:16. After the cells were added, the final serum dilution was 1:80. Plates were gently tapped, incubated for 1 h at room temperature, and then read visually with a microtitration reading mirror. Reactive and negative control sera were included on each day of testing. The HATTS reagents were tested with reference sera before being used in this study. A single lot of HATTS reagent was used throughout the tests.

The results of using unheated sera in the HATTS are shown in Table 1. Agreement between the heated and unheated sera was 99.2% (913/920). Of the seven sera that gave discrepant results, one, from a patient with untreated latent syphilis, was reactive only when heated, two nonsyphilitic sera were reactive only when heated, and four nonsyphilitic sera were reactive only when unheated. The seven discrepant readings were read as N or R1+. These seven sera were retested, and results were repeatable.

Our findings would seem to indicate that the HATTS may be used as a confirmation test to detect antibodies to T. pallidum in the diagnosis

<table>
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<th>TABLE 1. Effect of testing unheated sera in the HATTS</th>
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<td>Heated serum reaction</td>
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<sup>a</sup> R, Reactive 1+ to 4+.
<sup>b</sup> N, Nonreactive.
<sup>c</sup> Number of sera.
of syphilis with either heated or unheated sera, since there was no significant difference in the sensitivity of the test. However, some of the reading patterns were slightly altered when the unheated sera were used. Although the report of reactive or nonreactive remained constant whether heated or unheated sera were used, the pattern of agglutination was more distinct and easier to read with heated sera. Therefore, as recommended by the manufacturer and based solely on our preference for the reading patterns obtained with the heated sera, we suggest that heated sera be used in the HATTS.

LITERATURE CITED

