

## Effect of Using Heat-Inactivated Serum with the Abbott Human T-Cell Lymphotropic Virus Type III Antibody Test

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**The Abbott enzyme immunoassay (Abbott Laboratories, North Chicago, Ill.) for human T-cell lymphotropic virus type III (HTLV-III) antibody was evaluated to determine the effect of using heat-inactivated (56°C for 30 min) serum as the sample. Each of 58 nonreactive serum samples gave a higher  $A_{492}$  value when tested after heat inactivation. Ten of the samples became reactive after heating. Heat-inactivated serum should not be used in the current Abbott HTLV-III antibody test, because this can cause false-positive results.**

The Abbott enzyme immunoassay (EIA) (Abbott Laboratories, North Chicago, Ill.) for antibody to human T-cell lymphotropic virus type III (HTLV-III) is commonly used by blood banks to screen donors so that the chance of transmission of the virus through transfusions is minimized (1, 2, 11). When used in conjunction with other tests and clinical findings, the test can have some usefulness in evaluating certain patients, but it is not a test for acquired immunodeficiency syndrome (AIDS) (4, 9, 10).

We had two reasons for investigating the effect of using heat-inactivated serum in this test. The blood from known AIDS patients can be infectious (2, 4). Infection of laboratory personnel by working with blood specimens has not been demonstrated and is very unlikely even after a direct puncture of the skin (5). Despite the evidence so far that laboratory workers are not at high risk of developing AIDS by handling serum and performing laboratory tests, the personnel doing the work should use precautions when possible until more long-term information is available concerning the transmission of the virus, the spectrum of the disease, and the natural history of antibody development to HTLV-III (3, 5). In any EIA procedure of this type, there is the potential for a laboratory accident that could result in exposure to HTLV-III present in the patient sample. In the Abbott test, no specific measure is taken to inactivate virus present in the sample itself.

HTLV-III has been reported to be easily killed by heat, and titers expected in a patient serum specimen should be inactivated to a significant degree by heating at 56°C for 30 min (8). The time required at 56°C to reduce the titer by 1 log is approximately 2 min. Heating serum specimens at 56°C for 30 min to inactivate complement is a standard practice before the measurement of complement-fixing antibodies (6). Therefore, virus inactivation based on the heating of the serum sample could make subsequent antibody testing procedures safer in the event of an accidental exposure.

The second reason for reporting on the effect of heat inactivation is that in large clinical immunology laboratories multiple tests on a single blood specimen are often ordered. Because some procedures, such as the complement fixation test, require heat inactivation of the sample before testing, both types of sample could be present in the laboratory serum storage area.

The Abbott test for HTLV-III antibody was performed as directed on the package insert. An Abbott Quantum II

spectrophotometer with the Abbott Memory Module A was used for reading the optical density in  $A_{492}$  units and for the calculation of results. Serum samples were split, and one aliquot was heated in a water bath at 56°C for 30 min and then cooled to room temperature before being tested. The remainder was kept at 4°C and then warmed to room temperature before testing. The means and standard deviations of the absorbance measurements were calculated, and Student's two-tailed  $t$  test of paired samples was used to determine the significance of any difference in the means (7).

Fifty-eight blood samples were collected from healthy donors who denied belonging to any of the known high-risk groups associated with AIDS. These sera were refrigerated before being tested. In addition, sera from 11 patients known to have AIDS were tested. These sera had been stored at -20°C. The mean optical density  $A_{492}$  values of the HTLV-III antibody tests on the unheated and heat-inactivated aliquots are given in Table 1. Student's  $t$  test gave a  $P$  value of <0.0001, indicating that the results of the tests done on the heated and unheated specimens were significantly different in the normal population. For the sera from the AIDS patients, Student's  $t$  test gave a  $P$  value of 0.29.

These results indicated that heating nonreactive serum samples at 56°C for 30 min followed by testing for HTLV-III antibody in the usual way results in an increase in the absorbance values of the tests. Of 58 healthy blood donors who had nonreactive test values with unheated serum, 10 had reactive readings after heat inactivation. All would have been considered to be weak-positives. The  $A_{492}$  values for these false-positives ranged from 0.164 to 0.363 and averaged 33% (range, 0 to 121%) higher than the minimum cutoff absorbance value for a reactive test. The positive cutoff  $A_{492}$  values for the different runs ranged from 0.150 to 0.201. The exact mechanism of the false-positive phenomenon is unknown.

The serum samples from the AIDS patients all gave much higher absorbance values and were strongly reactive. This was in keeping with the EIA results for most AIDS patients in a previous study (11). For 7 of the 11 true-positive sera, the heated sample gave higher absorbance values than the unheated sample. However, we were not able to prove any significant difference in absorbance values for the two types of samples from these patients. This could have been due to the fact that three of the samples gave values that were  $\geq 2.000$  and were at the upper limit of the absorbance scale. The high absorbance resulting from the presence of HTLV-III antibody may have obscured a weak reaction owing to

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TABLE 1. Effect of using heat-inactivated serum<sup>a</sup> in the Abbott HTLV-III antibody EIA

Patient group	No. of sera tested	$A_{492}$ [mean (range)]	
		Unheated serum	Heated serum
Normal donors	58	0.056 (0.032–0.156)	0.138 (0.059–0.363)
AIDS patients	11	1.727 (1.122–2.000)	1.819 (1.527–2.000)

<sup>a</sup> Heated at 56°C for 30 min before testing.

heat inactivation. Regardless of whether there was a slight increase in absorbance, when high-titer sera were tested the interpretation of the test was not altered by the use of heat-inactivated specimens.

These studies indicated that heating at 56°C for 30 min as a means of inactivating HTLV-III in the serum sample is not an acceptable modification of the current Abbott HTLV-III antibody test. Heat inactivation might still be considered if the test analysis program were changed to allow for a higher absorbance cutoff point for categorizing reactive and nonreactive specimens. It may be necessary to use other methods for inactivation of the HTLV-III potentially present in the sample. One method currently being tried is included in the Bio-EnzaBead test for HTLV-III antibody (Bionetics Lab Products, Charleston, S.C.). That kit uses a specimen-inactivator medium which contains salts and surfactant. The serum specimen is diluted in the specimen-inactivator medium as step 1 of the testing procedure. However, studies to determine whether that procedure inactivates HTLV-III in test specimens have not been released or published.

In conclusion, when the Abbott EIA for HTLV-III antibody is performed by the current method, laboratories should not inadvertently or purposely use serum which has been heat inactivated, because this can lead to false-positive results.

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