Effect of Temperature of the Sensitization Process on the Passive Hemagglutination Test for Hepatitis B Antibody

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The temperature at which the coupling of antigen to erythrocytes takes place is an important factor in the passive hemagglutination test for hepatitis B antibody. Erythrocytes sensitized at 16 C are much less sensitive for the detection of antibody than are those sensitized at 22 to 41 C.

As a detector of hepatitis B antibody (HB Ab) and, to a lesser degree, hepatitis B antigen (HB Ag), the passive hemagglutination technique of Vyas and Shulman (4) ranks next in order of sensitivity to the much more expensive and time-consuming radioisotope methods (5). Because of the simplicity of equipment needed and ease of performance, the test is suitable for smaller laboratories unable to afford or operate radioisotope equipment. However, one factor inhibiting widespread use of the technique has been the difficulty experienced by some laboratories in obtaining reproducible results (5).

In our hands a high degree of consistency had been obtained by the practice of preparing large batches of reagents and storing them in the frozen state. (Erythrocyte diluent, serum diluent, negative and positive control antigens, and antisera are stored at –20 C, sensitizing antigen is stored at –70 C, and erythrocytes are preserved at –196 C in a glycerol-mannitol solution [3]).

After several months of consistent behavior, aberrant results occurred with no evident cause. These coincided with the sudden onset of winter and a failure of the air-conditioning system to adjust, with the result that the ambient temperature was noticeably lower than normal. Consequently, the effects of temperature on the test were investigated.

The method used followed that described by Vyas and Shulman, modifications being that 0.25% bovine albumin fraction V in Veronal-buffered saline, pH 7.4, (erythrocyte diluent) was used to wash and resuspend the human group O erythrocytes after sensitization, the final concentration of erythrocytes was 0.35%, and incubation of the microtiter plates after the addition of sensitized erythrocytes was carried out for 1 h at 37 C followed by 1 h at 4 C before reading.

The sensitization process itself (i.e., mixing of erythrocytes, antigen, and chromic chloride) had always been carried out at room temperature, as specified by Vyas and Shulman (4).

Water baths were set up at temperatures ranging from 16 C upwards to a maximum at 41 C, thus embracing the range of minimum and maximum ambient temperatures which could normally be expected in Sydney. Graduated centrifuge tubes containing 0.1 ml of sensitizing antigen were allowed to equilibrate to the temperature of each water bath, as were samples of 0.03% chromic chloride solution. To each tube containing antigen were added 0.03 ml of a 40% suspension of washed human group O erythrocytes and 0.03 ml of the temperature-equilibrated chromic chloride solution. The mixtures were allowed to stand in the water baths, with occasional gentle agitation, for 10 min, washed twice in 10 ml of erythrocyte diluent, and resuspended to a concentration of 0.35%. To avoid variability due to serum dilution procedures, all dilutions of antisera were made in tubes, and replicates were dispensed from these into the microtiter plates.

Several combinations of erythrocytes (two donors, frozen and fresh), sensitizing antigen (subtypes ad and ay), and antisera were used. In each case the cells sensitized at 16 C gave significantly lower antibody titers than those obtained with cells sensitized at 22 C and above, although the magnitude of the difference between the titers given by the 16 C cells...
compared with those sensitized at 22°C and above varied with the combination of cells, sensitizing antigen, and antibody used. There was no appreciable effect (less than twofold difference in titer) when the temperature of sensitization was varied between 22 and 41°C. Representative experiments are presented in Table 1.

To investigate the effect of washing the sensitized cells in erythrocyte diluents at different temperatures, cells sensitized at 16 and 22°C were washed and finally resuspended in diluent at both 6 to 10°C and 26 to 30°C. The temperature of the washing fluid had no effect on the antibody titer given by cells sensitized at either temperature. Thus, the temperature-dependent phase appeared to be that of the coupling of the antigen to the erythrocytes by chromic chloride.

To achieve satisfactory results with the passive hemagglutination test for HB Ag and HB Ab, it is necessary to standardize as many variables as possible. Mention has been made of batch of erythrocytes, glassware preparation, and general performance of the sensitizing procedure in this respect (5). A hitherto unsuspected variable is the ambient temperature at the time of cell sensitization. In their original description of the method for HB Ab, Vyas and Shulman suggested room temperature as being suitable for the sensitization procedure. This was reasonable on the basis of previous reports stating that the sensitization phase of the passive hemagglutination test with other antigens was independent of temperature (1, 2). Unfortunately, sensitization of erythrocytes with HB Ag is temperature dependent at temperature ranges approximating to ambient in many parts of the world. To ensure uniformity of results, it is suggested that attention be paid to this factor, and that, if necessary, sensitization be carried out in a water bath at or above 22°C.

| Expt | Temp (°C) | Antibody titer |
|------|-----------|----------------|
| 1    | 16        | <20            |
|      | 24        | 1,280          |
|      | 37        | 2,560          |
| 2    | 16        | 320            |
|      | 21        | 2,560          |
|      | 26        | 2,560          |
|      | 31        | 2,560          |
|      | 36        | 1,280          |
|      | 41        | 2,560          |
| 3    | 16        | 640            |
|      | 22        | 2,560          |

* Erythrocyte donor 1, frozen cells, sensitizing antigen subtype ay, antiserum A.
* Erythrocyte donor 2, unfrozen cells, sensitizing antigen subtype ay, antiserum A.
* Erythrocyte donor 2, frozen cells, sensitizing antigen subtype ad, antiserum B.

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