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Elimination of Non-Specific Reaction in Latex Agglutination Test for the Detection of Hepatitis-Associated Antigen

Brief Report

By

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In evaluating the applicability of the HAA latex agglutination test[1] for routine diagnostic use, it was found that it compares favourably in sensitivity with counterimmunoelectrophoresis (CIE) for the detection of hepatitis-associated antigen (HAA) and has the added advantage of being more rapid and simpler to perform. It has, however, a serious disadvantage of giving a disturbingly high percentage (5—8% in our hands) of false-positive reactions.

Table 1. *Rheumatoid Factor (Rf) Positive Sera in the HAA Latex Test*

Rf titre[a]	No. reacted/No. tested	% reacted
1:5120	10/10	100
2560	16/16	100
1280	31/34	91
640	16/22	73
320	21/43	49
160	16/50	31
80	10/55	18
40	2/29	7

[a] Rheumatoid factor titre.

In investigating various factors and possibilities which may be responsible for the non-specific reactions, evidence was obtained to indicate that the factor implicated in these false-positive reactions is associated with the IgM fraction of immunoglobulins. This was clearly demonstrated when 287 sera, negative for HAA by CIE but positive for rheumatoid factor in titres ranging from 1:40 to 1:5120 were tested. The results of this test are summarized in Table 1.

[1] Preparation used was supplied by the Behringwerke AG, Marburg (Lahn), Federal Republic Germany.

From this Table it can be seen that the percentage of non-specific reactions depends on the concentration of the rheumatoid factor and that all sera with rheumatoid factor in titres 1:2560 and higher agglutinated latex particles.

These results, of course, do not necessarily prove that the rheumatoid factor alone is responsible for these false-positive reactions; they do, however, indicate that the rheumatoid factor or a component associated with it, plays a major role in bringing about non-specific agglutination of the sensitized latex particles. It has been established that the rheumatoid factor is associated with the IgM class of immunoglobulins and that it is capable of binding not only human, but also rabbit IgG globulins (1). Since the latex particles used in the HAA test were coated with rabbit IgG globulins, the agglutination, therefore, may well be due to the reaction between the IgM in test serum and the IgG molecules coating the latex particles.

In an attempt to remove this factor, the following procedure, which gives completely satisfactory results, has been developed:

The sera found to agglutinate sensitized latex particles on screen test are heated at 62° C for 10 minutes with constant agitation to prevent coagulation and then treated with antihuman IgM globulin (rabbit or goat origin) in the proportion of one part of anti-IgM globulin (0.025 ml) to two parts of test serum (0.05 ml) and tested immediately. Heating the sera at a lower temperature was found to be not as effective as can be seen from the results given in Table 2.

Table 2. *Effect of Heat and Anti-IgM Pre-Treatment of Sera on the False-Positive Reactions*

	Sera pre-treated at			
	56° C for 1 hour		62° C for 10 minutes	
Rf titre[a]	− anti-IgM	+ anti-IgM	− anti-IgM	+ anti-IgM
5120	6/6	4/6[b]	6/6	0/6
2560	4/4	3/4	4/4	0/4
1280	12/12	6/12	6/12	0/12
640	5/5	3/5	3/5	0/5
320	16/19	0/19	not done	not done
160	8/16	0/16	not done	not done

[a] Rheumatoid factor titre.
[b] Number reacted/number tested.

From this Table, it is apparent that with heat and anti-IgM pretreatment, all the false-positive reactions were eliminated. Heating sera at 62° C and pretreatment with the anti-IgM globulin, on the other hand, did not in any way affect the HAA specific latex agglutination nor interfree with the CIE. All the sera found positive for HAA by the CIE before treatment remained positive after the treatment. The only effect this pretreatment has is slowing down the reaction time for latex agglutination without affecting its specificity.

It has recently been reported by FRITZ and RIVERS (2) that non-specific latex agglutination (2.1%) also occurs when latex particles are sensitized with guinea pig anti-human-HAA globulins. These authors have also found that the sensitivity of HAA latex agglutination test is comparable with that of complement-fixation and hemagglutination inhibition and, because of the rapidity and simplicity of the test, suggest its use for large scale blood donor screening.

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References

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