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CLINICAL EVALUATION OF ANTIBODY SENSITIZED LATEX PARTICLTES METHOD FOR AUSTRALIA ANTIGEN IN SERUM

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ABSTRACT

There are several testing methods for the detection of Australia antigen. Among them an antibody sensitized latex particle agglutination assay (LAT) for the detection is technologically simple, sensitive, and rapid. So the detection of the antigen in the 896 specimens from hepatic diseases was carried out by LAT, IEOP, and SRID and the detection of rheumatoid factor in the LAT positives was also made by the modified method of Heller *et al.* The results of testing the 896 specimens from blood donors are positive in 0.8%, negative in 4.1%, false positive in 5.1%, and false negative in 0.1%. And also, it is suggested that false positive to LAT is partially due to rheumatoid factor, since 7 specimens of 9 positives for rheumatoid factor were to LAT (a rate of 67.8%), and partially due to the freshness of blood, since of 59 storage specimens at 4°C for a week there were different reactions in 21 specimens between storage specimens and fresh specimens. Two false negatives in the 61 specimens from hepatic diseases showed positive reactions in diluted specimens for LAT. The cause of false negative reactions to LAT is unclear but it may partly be due to the rheumatoid factor and other factors. These results led us to the conclusions that the LAT requires further development and that the LAT appears to be useful as a preliminary screening for detection of the antigen.

INTRODUCTION

It is well known that the detection of Australia antigen is important for the diagnosis of viral hepatitis[1]. There are several testing methods for detection of Australia antigen (Au-Ag) such as single radial immunodiffusion (SRID), immunoelectroosmophoresis (IEOP)[2], complement fixation (CF), passive haemaagglutination inhibition (HI), immune adherance (IA) and radioimmunoassay (RIA) but these techniques are too time consuming for a screening test. Ideally, such a test should be sensitive, highly specific, rapid, and easily performed at low cost and readily adaptable to a screening test[1]. This paper describes an antibody sensitized latex particle agglutination assay for Australia antigen.

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MATERIALS AND METHODS

Blood samples the studied were obtained from 61 patients with hepatic diseases in Nagoya University Hospital and 896 blood donors of the Aichi Red Cross Blood Bank. The sensitized latex particles were purchased from Behringwerke (Latex-HAA reagent)[3].

The detection of Australia antigen in sera was carried out by the latex agglutination test (LAT), immunelectroosmophoresis (IEOP), and single radial immunodiffusion (SRID). In addition, the detection of rheumatoid factor in the latex test positives was made by the modified method of Heller *et al.*[4] The LAT was carried out on plastic agglutination slides by mixing well one drop of serum with one drop of sensitized latex particles and examined for agglutination at room temperature after five minutes.

RESULTS AND DISCUSSIONS

The results of testing 957 specimens by the methods of LAT, and SRID are shown in the Table 1.

Of the 896 specimens collected from blood donors, 53 were positive to LAT, 7 to IEOP and 7 to SRID. Of the latex positives, 46 were negative by two other methods. One specimen positive by two other methods was negative to LAT, so this reaction must have been false negative to LAT. That is to say, the false positive rate was 5.1% and the false negative rate 0.1%. Other

TABLE 1. Distribution of Australia Antigen Positive and Negative Reactions by three Different Methods

Groups of specimens	LAT	IEOP	SRID	No. of R.F. positive	No. of patients in each combination
Blood donors	+	−	−	2	53 (5.1%)
	+	+	+	0	6 (0.8%)
	−	+	+	0	1 (0.1%)
	−	−	−	0	836 (94.0%)
Total	59	8	8	2	896
Hepatic diseases	+	−	−	2	9 (14.7%)
	+	+	−	2	13 (21.3%)
	−	+	+	1	2 (3.4%)
	−	−	−	2	37 (60.6%)
Total	24	15	15	7	61

(1) Hepatic diseases: infectious hepatitis 25, posttransfusion hepatitis 3, chronic hepatitis 20, liver cirrhosis 10, lupoid hepatitis 2, and S.L.E. 1 specimen.

(2) Rheumatoid factor: (R.F.)

(3) Of 61 spcimens, two specimens showed positive reaction to L.E. test.

investigators have reported false positive reactions to LAT in about 3% and false negative reactions in about 0.3% of blood donors and patients[5)6)].

In addition, of specimens positive to LAT, two specimens were positive for rheumatoid factor.

Of the 61 specimens from hepatic diseases, 24 were positive to LAT and 15 to IEOP and SRID. False positive specimens were 9 in LAT, whereas two specimens of latex positives were positive for rheumatoid factor. It is suggested that false positive to LAT is partially due to rheumatoid factor, since 7 specimens of 9 positives for rheumatoid factor were positive to LAT (a rate of 67.8%).

In the experiments described above, sera were subjected to LAT on the day of blood collection. For examination of the influence of the sample storage to LAT, specimens positives to LAT were kept in the refrigerator at 4°C for a week and the samples were again subjected to LAT. The results are shown in Table 2. Of 59 storage specimens at 4°C for a week, there were different reactions in 21 serum specimens between storage specimens and fresh specimens.

These 21 storage specimens showed negative reactions to LAT, although 21 fresh specimens showed positive reactions (a rate of 35.6%).

These reactions were in agreement with the reactions to IEOP and SRID.

In LAT, no differences were found between storage specimens and fresh specimens of 38 specimens. Of these specimens, 32 were negative by other two methods, that is to say, a false positive rate of 3.5%, and 6 specimens

TABLE 2. Detection of Australia Antigen Positive and Negative Reactions by three Different Methods in 59 Specimens of Latex Positive Reactions after Storage at 4°C for a Week

	LAT	IEOP	SRID	No. of specimens in each combination
	+	+	+	6 (10.3%)
	+	−	−	32 (54.0%)
	−	+	+	0 (0 %)
	−	−	−	21 (35.6%)
Total	38	6	6	59

TABLE 3. Detection of Australia antigen in LAT and IEOP in Diluted and Undiluted Specimens

Method	1 X	2 X	4 X	8 X	16 X	32 X	64 X	128 X ···
IEOP	+	+	+	+	−	−	−	−
LAT	++	++	+	+	−	−	−	−
IEOP	+	+	+	+	+	+	+	+
LAT	−	+	++	++	+	±	−	−

were positive by other two methods. Fresh specimens resulted in an increase in false positive reaction rate for LAT. These different results of LAT between storage specimens and fresh specimens seem to be partially due to inactivation of complement. Some investigators reported that false positive reaction rate decreased by adding thrombin reagent to fresh blood specimens[7].

2 specimens positive by two other methods were negative to LAT so these were false negative reaction by LAT. The failure to defect two Australia antigen positive specimens may result in serious disadvantage. But these two false negative specimens showed positive reactions in only diluted specimens for LAT. The cause of false negative reactions to LAT is unclear, but it may partly be due to the rheumatoid factor and other factors and also to the formation of a prozone of nonreaction in specimens containing a high titer of the antigen[3]. Therefore, it is necessary to test both diluted and undiluted specimens as shown in the accompanying Table 3.

These experimental results led us to the conclusions that the latex agglutination test for Australia antigen requires further development before it can be used in screening positive specimens, and that despite such false positive reactions, the LAT appears to be useful as a preliminary screening for detection of the antigen.

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