RESEARCH NOTE

RAPID DETECTION OF HEPATITIS B SURFACE ANTIGEN BY RED CELL AGGLUTINATION ASSAY

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Hepatitis B virus is an important worldwide public health problem especially in areas where the prevalence are high, such as China, Southeast Asia, tropical Africa. In Thailand, the carrier rate is about 5-10% of the total population (Kamolratanakul *et al,* 1985; Poovorawan *et al,* 1989). The detection of hepatitis B surface antigen (HBsAg) is one of the indicators of the hepatitis B infectious stage. The rapid detection of HBsAg in whole blood within minutes is very useful in management of postexposure of vertical transmission prophylaxis. Proper prophylactic intervention can be immediately instituted.

In the present report we described the rapid detection of HBsAg by autologous cell immunoassay technology (AIT) to evaluate the sensitivity and specificity of the test. Radioimmunoassay (RIA) was used as the gold standard. We also modified the test by using blood group O positive cells instead of O negative as recommended by the manufacturer for the assay of serum.

Whole blood was drawn from 28 hepatitis B surface antigen positive mothers and 38 children with high titer antiHBs from vaccination. We also tested one hundred sera of adolescents, age range from 11-18 years. The sera were kept at -20°C since May 1991 with code numbers. AIT utilizes a monoclonal antibody which was selected to react with all major HBs antigen subtypes (SimpliRED® HBsAg test, AGEN Biomedical Limited, Brisbane, Australia). The test was performed on whole blood samples by the adding of a single reagent to initiate an agglutination reaction of the patients' own blood cells as an indicator of the presence of antigen. For the stored sera we added whole blood sample from a donor who was HBsAg negative, blood group O, Rhesus positive. Citrate was used as anticoagulant.

The active agent is a chemical conjugate of two monoclonal antibodies, one which binds to the red blood cell surface (but itself does not cause agglutination) and the other which is an anti-HBsAg antibody. The conjugate coated the red cells but did not cause agglutination in HBsAg negative samples. HBsAg present in a blood sample binds to the conjugate on the red blood cell causing crosslinking between cells resulting in visible agglutination (Rylatt *et al,* 1990). The red cell agglutination assays were performed in duplicate.

The commercial radioimmunoassay kit (AUSRIA II, Abbott Laboratories, North Chicago, Ill, USA,) was used. The principle of this test is sandwich radioimmunoassay, using a bead coated with guinea pig antibody to HBsAg. HBsAg was bound to the solid phase antibody, then human ^{125}I anti-HBs reacted with the antibody-antigen complex on the bead. The radioactivity remaining on the beads was counted in a gamma counter. The cut-off positive value was over 2.1 times the negative control (S/N ratio).

All of 28 women who have been known to be positive for HBsAg by RIA method had positive results by the rapid AIT. Thirty-eight children with high titer of antiHBs had also negative HBsAg by AIT. The comparative results of serum AIT with RIA in 100 adolescent sera are shown in Table 1. Two specimens with negative results by RIA method were positive by serum AIT. The S/N ratio data are shown in Table 2.

The sensitivity and specificity of AIT were 100% and 96.9% respectively. The positive predictive value was 94.59% and the negative predictive value was 100%. The accuracy was 98%.

Table 1

Comparative results of HBsAg by RIA and AIT methods.

		Radioimmunoassay		Total
		Positive	Negative	
AIT	positive	35	2	37
	negative	0	63	63
Total		35	65	100

Table 2

False positive specimens by AIT as compared to gold standard RIA and S/N ratio.

Number	RIA*	S/N ratio	AIT
1	negative	1.43	positive
2	negative	1.86	positive

* The cut off value of RIA for positive HBsAg was over 2.1 S/N ratio.

Among the methods used for HBsAg detection such as RPHA, CIE, ELISA, RIA, the latter two are considered the most sensitive with specificity tests, but they require special equipment, technical skill and are time consuming. RPHA is less expensive and the result is obtainable in 2-24 hours but the specificity and sensitivity is lower than ELISA and RIA (Skulramrung, 1985). AIT is a rapid test. Within 2 minutes the reading can be completed. Whole blood or serum can be used. When using whole blood, time required to separate serum is saved. By the kit's instruction, if serum is used, blood group O, Rh negative can be added for hemagglutination reading. In this study, we used a group O, Rh positive donor due to the prevalence of this group in Thailand. Neither the group O Rh positive blood nor the high HBsAb interfered with the test results. There were 2 false positive tests and no false negatives in 166 specimens. These findings indicated that AIT is a reliable screening test. Due to the procedure simplicity, it can be used in the field or at the bed side and applied as a guideline for the prophylaxic approach. The possibility of false positive reaction should be kept in mind and confirmed by RIA method when indicated.

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