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Evaluation of Rapid Immunochromatographic Assay Kit for HBsAg-Screening Using Whole Blood

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Abstract A rapid immunochromatographic assay kit using whole blood to screen hepatitis B surface antigen was developed and evaluated by using sera from 240 patients. The reference diagnosis was based on the results obtained with GENEDIA Anti-HBs Rapid kit which is very similar to the above kit except for the use of serum. The test demonstrated a good correlation with the reference immunochromatographic assay kit, that is, the sensitivity and the specificity of the kit was 100%, respectively. The rapid test kit using whole blood should be more convenient and useful for the diagnosis of hepatitis B virus because the kit does not need machines and time to prepare serum. In addition, this kit is safe from inadvertent infection during sample treatment because the blood is sterilized with hydrogen peroxide, eliminates the procedure required to prepare serum and reduces the possibility of exposure to infectious agents.

Keywords: HBsAg, immunochromatographic assay, whole blood, sensitivity, specificity.

INTRODUCTION

Immunochromatographic assay (ICA) is also referred to as rapid test kit due to its rapidity and simplicity [1] and the ICA kits are being widely used for the detection of various analytes such as hormones [2], antigens [3], antibodies [4] and drugs [5]. In such an assay, tracer antibody molecules conjugated with gold particles bind to a particular antigen contained in a serum sample, after which the formed complexes pass through microspores of nitrocellulose (NC) membrane in due to capillary forces. The complexes finally bind to capture antibodies immobilized on the inner surface of micropores of the NC membrane and develop color of a positive line, whereby determining easily the existence of a particular antigen in the serum sample with the naked eyes.

There are two major constituents to an ICA kit. One is a NC membrane which has two invisible lines on the surface and the other is a glass fiber filter containing antibody-gold particle conjugates in a dry state on the surface. Two kinds of antibodies, that is, the monoclonal anti-HBs being specific to the antigen to be detected and Goat anti-mouse IgG, are immobilized on the lower line and the upper line of the NC membrane, respectively. A sample is added to the sample well of the ICA kit and then the antibody-gold particle conjugates on the surface of the NC membrane in a dry state are rehydrated and then bound to antigens in the serum sample, after which the formed complexes pass through micropores of the NC membrane due to capillary forces. Thereinafter, the antigens of the complexes reacts with the monoclonal anti-HBs immobilized on the lower line, resulting in color development. In addition, the upper line develops a color because the goat anti-mouse IgG immobilized on the upper line may react with the antibody-gold particle conjugates although no antigen is present, thus the upper line always develops a color in each run of the test and may serve as a control line. That is to say, when antigens exist in a serum sample, both the positive line and the control line of the ICA kit become visible but only the control line becomes visible, when no antigen is present (Fig. 1).

Meanwhile, whole blood can not be used in the ICA kits due to the visual hindrance of the color of red blood cells (RBCs). Hence, current ICA kits employ clear serum as a sample to be tested, which has to be previously subjected to coagulation and centrifugation to separate the blood cells. This pretreatment process reduces the rapidity and simplicity of the ICA kit because additional time and machines are required for coagulation and separation to prepare serum after collecting whole blood. In order to solve this problem, a blood separation filter for blocking blood cells, which prohibits blood cells in the whole blood from moving across and ensures only filtered serum to be developed, has been adapted to the kit (Pall Corporation, WO 960314; and Boehringer Mannheim, EP 586789). However, the filter retards the development of serum, and some of samples sometimes do not run because of blocking the sample well by clotting of intact whole blood in the well. Furthermore, the drier the applied sample serum becomes after developing through the NC membrane, the higher the concentration of salts of whole blood in the sample well becomes and finally inducing rupture

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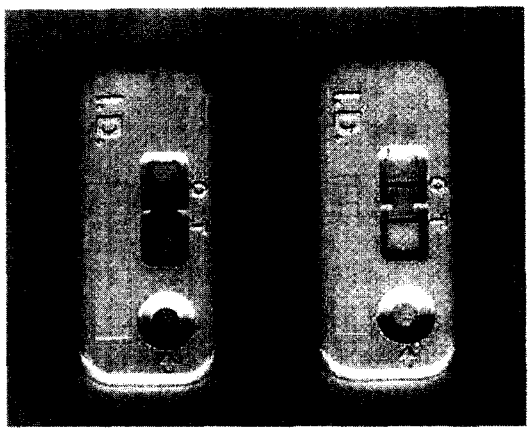

Fig. 1. ICA kits showing a negative reaction (left) and a positive (right) against HBsAg. Serum samples were loaded into the sample well.

of the red blood cells. The intracellular materials including the red pigment may move across the filter and cover the NC membrane to prevent the correct reading. The current authors already developed an ICA kit for HBsAg using serum [6], however, this kit has to be used with serum like most of the others. Thus, in order to perform ICA with whole blood, we developed the ICA kit for HBsAg using whole blood and compared this to reference ICA kit by using mimic blood.

MATERIALS AND METHODS

Serum Samples

Serum samples were collected from patients and determinded to be either HBsAg-positive (120 sera) or HBsAg-negative (120 sera) at Korea University Hospital according to the test result from an enzyme immunoassay (EIA) kit (Enzygnost HBsAg, Boehringer Mannheim, Germany).

Comparison Test with a Reference ICA Kit

The ICA kit for HBsAg using whole blood was made by adding a small container containing pretreating solution to decolor RBCs to a conventional ICA kit for HBsAg using serum. The pretreating solution was composed of 1.5% hydrogen peroxide (Sigma, St. Louis, MO, USA) [7], 0.016% Sag 471 (OSi, USA), 0.003% sodium azide (Sigma, USA) [8] and 5 mM ethylenediaminetetraacetic acid (EDTA, Sigma, USA) in phosphate buffered saline (PBS) and each component plays a role of decoloring agent, antifoam, enzyme inhibitor, and chelating agent, respectively.

The serum samples were tested with GENEDIA HBsAg Rapid kit (GCC, Korea) using serum as the reference kit and retested with the ICA kit for HBsAg using whole blood (Fig. 1). The blood samples were reconstituted by adding 40 μL of packed cells from O-typed normal human blood (HBsAg negative, anti-HBs negative) which was collected with heparinated syringe (Sigma, USA) to 60 μL of the serum samples to mimic whole blood. The reconstituted samples (40 μL) were added to 160 μL of pretreating solution and decolored for a while, and then the mixtures were applied into the sample well of the individual ICA kit.

Sensitivity

Sensitivity is a value to evaluate a certain assay system to be able to show true positive results among total positive samples (with reference system) [9], which may include false negative samples, and is obtained from the equation below.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

Specificity

Specificity is a value to evaluate a certain assay system to be able to show true negative results among total negative samples (with reference system), which may include false positive samples, and is obtained from the equation below.

$$\text{Sensitivity} = \frac{\text{True negative}}{\text{True positive} + \text{False negative}} \times 100$$

RESULTS

Comparison Test with a Reference ICA Kit

Assay results were appeared on the window of an individual kit within 10 min and 120 HBsAg-positive samples were positive (Table 1) and 120 negatives were negative (Table 2) without any false negative and false positive readings with the ICA kits for HBsAg using whole blood and the same results were obtained with the reference kits.

The color intensity of the test line on the ICA kits for HBsAg using whole blood was similar to or stronger (9 cases) than that on the reference kits.

Sensitivity

The HBsAg-positive serum samples collected at Korea University Hospital showed the same results between the ICA kits for HBsAg using whole blood and the reference kits without any false negative results. Therefore, the sensitivity of the ICA kit for HBsAg using whole blood was 100% when GENEDIA HBsAg Rapid kit was employed as the reference kit.

Specificity

The HBsAg-negative serum samples collected at Korea

Table 1. Clinical data of immunochromatographic assay with 120 positive samples

HBsAg-screening ICA kit using whole blood				
		+	−	Total
GENEDIA HBsAg Rapid	+	120	0	120
	-	0	0	0
	Total	120	0	120

Table 2. Clinical data of immunochromatographic assay with 120 negative samples

HBsAg-screening ICA kit using whole blood				
		+	−	Total
GENEDIA HBsAg Rapid	+	0	120	120
	−	0	0	0
	Total	0	120	120

university hospital showed the same results between the ICA kits for HBsAg using whole blood and the reference kits without any false positive results. Therefore, the specificity of an ICA kit for HBsAg using whole blood was 100% when GENEDIA HBsAg Rapid kit was employed as the reference kit.

DISCUSSION

A rapid and accurate method for the diagnosis of infectious diseases like hepatitis B is important for both clinicians and patients. An ICA kit for HBsAg using whole blood was developed on the basis of this demand. A blood sample is diluted by five times; compared of the volume of pretreating solution, 20 vol.% of the sample is added. Furthermore, the amount of serum which contains HBsAg would be relatively reduced when using blood, since only serum is employed in GENEDIA HBsAg Rapid kit but serum plus blood cells are employed in the ICA kit for HBsAg using whole blood. The dilution factor would be 2.5 in this case because the portion of cells in whole blood is about 40% [10]. Thus, the total dilution factor when adding pretreated sample to an ICA kit using whole blood would be 12.5. Despite this large dilution factor, the color intensity of the test lines of the ICA kits for HBsAg using whole blood was not weaker than that of the kits using serum. Nine of the positive samples showed stronger color when the ICA kits for HBsAg using whole blood were used compared to when the kits using serum were used, contrary to our expectation. There are two possible reasons for this phenomenon; one reason is the characteristics and the amounts of the capture antibody immobilized in the NC membrane and the tracer antibody deposited in the glass fiber filter, and the other is the color and the amount of gold particles, because the test result is basically dependent upon the binding of antigens (HBsAg) and antibodies (monoclonal anti-HBs, polyclonal anti-HBs). It is estimated that the antibodies and gold particles employed in ICA kits for HBsAg using whole blood should be able to compensate for dilution factors and to show the similar intensity of color as conventional ICA kits using serum. Especially, it is assumed that ICA kits for HBsAg using whole blood should overcome the prozone effect [11] for the dilution of blood with pretreating solution and show more intense color than the kits using serum.

There are some other values such as detection limit and reproducibility [9] besides sensitivity and specificity to evaluate the performance of a diagnostic kit. However, in this study, we did not include the former two values because the detection limit of this ICA kit was reported to be 4 μg/L [6] and the reproducibility was not adequate for ICA. The reproducibility is able to be gained from standard deviation of numeric results as in EIA and radioimmuno assay.

The ICA kit for HBsAg using whole blood demonstrated a good correlation with the reference ICA kit and was so convenient that no additional time and machines were needed to prepare serum. In addition, this kit was safe from inadvertent infection during sample treatment because the blood was sterilized with hydrogen peroxide [12], eliminates the procedure required to prepare serum, and reduces the possibility of exposure to infectious agents. Therefore, this new HBsAg-screening kit using whole blood would be a good substitute for the conventional ICA kits due to its rapidity, simplicity and safety.

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