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Evaluation of Immunochromatographic Assay Systems for Rapid Detection of Hepatitis B Surface Antigen and Antibody, Dainascreen HBsAg and Dainascreen Ausab

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We evaluated two immunochromatographic assays (ICAs), Dainascreen HBsAg for detecting human hepatitis B surface antigen (HBsAg) and Dainascreen Ausab for detecting human hepatitis B surface antibody (anti-HBs) in human serum. The ICA systems are composed of a comb-shaped device that contains nitrocellulose strips on which complexes of HBsAg and anti-HBs can be visualized. The results can be read within 15 min of incubation. The limit of detection for HBsAg was 3.1 ng/ml, and that for anti-HBs was 42 mIU/ml. Results of HBsAg detection agreed completely with those of conventional enzyme immunoassays (EIAs) and showed a 100% sensitivity (158 of 158 samples) and a 100% specificity (304 of 304 samples). The Dainascreen Ausab detected 184 of the 199 EIA-positive samples (sensitivity, 92.5%) and yielded 6 positive results among the 281 EIA-negative samples (specificity, 97.9%). The ICA systems are rapid and sensitive methods for detecting HBsAg and anti-HBs. They are low-cost systems that need no complex instrumentation for analysis and can be recommended for routine use in clinical microbiology laboratories.

Hepatitis B virus (HBV) is a major cause of hepatitis associated with transfusion or acquired in the community (6, 8, 10, 11). Worldwide, more than 300 million people chronically carry the hepatitis B surface antigen (HBsAg); in Asian countries 220 million people (73%) carry HBsAg and in North American and European countries fewer than 2 million carry HBsAg. The number of deaths from acute or chronic HBV infection is estimated to be approximately 1 million per year worldwide (12).

HBsAg and hepatitis B antibody (anti-HBs) are measured by enzyme immunoassays (EIAs), radioimmunoassay, reversed passive hemagglutination assays (RPHAs), or passive hemagglutination assays (PHAs) (2, 4, 7, 9). Although EIAs and radioimmunoassays are widely used because of their excellent sensitivities, they are expensive and require complex instrumentation or radioisotopes, and the methods are too complex for emergency use (1, 2, 4). The RPHA and PHA methods are rapid and simple to perform, but they lack sensitivity and the results are difficult to interpret (1, 9). Considering the limitations mentioned above, a rapid, simple, and highly sensitive method for detecting HBsAg and anti-HBs is desired.

Methods for detecting HBsAg and anti-HBs based on immunochromatographic assays (ICAs), specifically, Dainascreen HBsAg and Dainascreen Ausab (Dainabot Co., Ltd., Tokyo, Japan), have been developed. We evaluated the utilities of these new systems for use in the clinical microbiology laboratory.

MATERIALS AND METHODS

Clinical samples. A total of 462 serum samples were analyzed for HBsAg, and 480 serum samples were analyzed for anti-HBs. All samples were obtained from Nagoya University Hospital between October 1994 and March 1995. Sera were stored at −80°C until they were assayed.

Dainascreen (ICA) systems. The apparatus for the ICA systems is composed of a comb-shaped device containing nitrocellulose strips to which anti-HBs or HBsAg is attached (Fig. 1). The assays are based on the sandwich immunoassay. To detect HBsAg, samples are incubated with mouse monoclonal anti-HBs (first antibody) conjugated to a selenium colloid in a microtiter plate, thus forming the first step of the sandwich (antigen-antibody complex) if HBsAg is present in the sample. The comb-shaped device is then lowered into the microtiter plate, and the sandwich complex migrates through the nitrocellulose strip to be captured at the site of mouse monoclonal anti-HBs (second antibody). Accumulation of the colloid at the capture site results in the formation of a red line, indicating a positive result (Fig. 2).

The procedure was first carried out according to the manufacturer's instructions, which are intended for qualitative testing. In other experiments, modifications of the procedure were made for quantitative tests. The lyophilized anti-HBs monoclonal conjugate was reconstituted with 1.5 ml of the conjugate diluent, and the mixture was allowed to stand for at least 5 min at room temperature. A volume of 25 μl of the sample and 25 μl of the diluted conjugate were dispensed into the U-shaped wells of the microtiter plate at room temperature for another 2 min. The test strips of the comb-shaped device were placed in the wells and were allowed to react for 15 min. A positive reaction was indicated by a red line on the strip. The method for detecting anti-HBs was the same as that for detecting HBsAg except that a selenium colloid conjugated to HBsAg was used in the first step.

EIA. Solid-phase EIAs for HBsAg and anti-HBs were performed with the Auszyme Monoclonal and Ausab EIA (Abbott Laboratories, North Chicago, Ill.) (2) according to the manufacturer's instructions. For detecting HBsAg, sera were incubated with beads coated with mouse monoclonal anti-HBs and mouse monoclonal anti-HBs conjugated with horseradish peroxidase for 3 h at 40°C. *o*-Phenylenediamine solution containing hydrogen peroxide was added to the beads, and the color was allowed to develop for 30 min at room temperature in the dark before the reaction was stopped with 1 N H_2SO_4. The A_{492} was measured with a beam spectrophotometer. Specimens giving absorbances equal to or greater than the absorbance of the mean for the negative control plus a factor indicated by the manufacturer were considered to be positive. The method for detecting anti-HBs was principally the same as that for detecting HBsAg.

RPHAs and PHAs. HBsAg and anti-HBs were also detected by RPHA (Serodia-HBs; Fujirebio. Inc., Tokyo, Japan) and PHA (Serodia-antiHBs; Fujirebio. Inc.), respectively (9). Semiquantitative RPHA was performed with sensitized erythrocytes consisting of fixed chicken erythrocytes (0.6%; wt/wt) with highly purified anti-HBs guinea pig-specific antibody (immunoglobulin G) adsorbed onto them and unsensitized erythrocytes (as a negative control) consisting of fixed chicken erythrocytes (0.6%; wt/wt) adsorbed with normal guinea pig immunoglobulin G. They were added in 25-μl aliquots to serial twofold dilutions of the serum in phosphate-buffered saline up to a 1:40 dilution, and the mixtures

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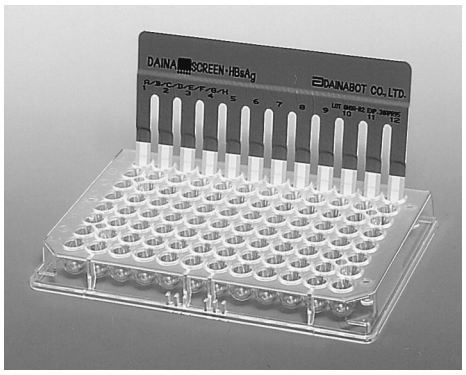

FIG. 1. Dainascreen ICA system. The apparatus consists of a comb-shaped device, a lyophilized conjugate (mouse monoclonal anti-HBs conjugated to a selenium colloid), a conjugate diluent, a positive control, and a U-shaped microtiter plate. The comb includes 12 test strips.

were incubated at room temperature for 1 h; this was followed by reading for hemagglutination. The methods for PHA were principally the same as those for RPHA.

Evaluation of the results. The sensitivity of HBsAg detection by ICA was examined by using the refined HBsAg subtyping panels (HBs/ad and HBs/ay; Dainabot panel; Dainabot Co.). Each panel consisted of six concentrations (1.6, 3.1, 6.3, 12.5, 25.0, and 50.0 ng/ml). The sensitivity of anti-HBs detection by ICA was examined by using a positive clinical sample quantitatively detected by IMx-Ausab (Abbott Laboratories). The sensitivities of HBsAg and anti-HBs detection by ICAs were also evaluated semiquantitatively with clinical samples by the twofold dilution method. The results of HBsAg detection by ICA were compared with those by EIA (Auszyme Monoclonal) and RPHA (Serodia-HBs). The results of ICA for anti-HBs detection were compared with those of EIA (Ausab EIA) and PHA (Serodia-antiHBs). Qualitative evaluations of the ICAs for HBsAg and anti-HBs detection were also done by comparing the results of the EIAs and the RPHA (or PHA) by using clinical samples. Samples with discrepant results were reevaluated by a hepatitis B core antibody (anti-HBc) assay (IMx CORE; Abbott Laboratories) to evaluate the history of HBV infection and another EIA (IMx-HBsAg and IMx-Ausab; Abbott Laboratories) (3, 5). These kits were used according to the manufacturer's instructions.

RESULTS

HBsAg ICA. The detection limits of the ICA system with the HBs/ad and HBs/ay panel were 3.1 and 6.3 ng/ml, respectively, when the assay systems were incubated for 15 min. The endpoint titer of the semiquantitative assay by the twofold dilution method was 2^8 for the ICA, 2^{11} for the EIA, and 2^5 for the RPHA methods. The sensitivity of HBsAg detection by ICA was improved by extending the incubation time; values of 2^9 and 2^{10} were obtained after incubation for 60 and 120 min, respectively. Overnight incubation did not increase the sensitivity further.

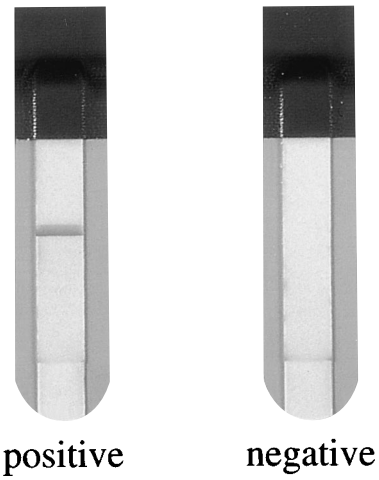

FIG. 2. Positive result with the ICA system. A positive result is indicated by the development of a positive immunoreaction, shown by a red line on the comb-shaped device, on which the mouse monoclonal anti-HBs or the HBsAg has been coated.

TABLE 1. Analysis of samples with discrepant results for HBsAg detection after reevaluating anti-HBc and another EIA (IMx-HBsAg) method

Results of[a]:			No. of specimens	No. of samples with the indicated results by:			
				Anti-HBc assay		IMx-HBsAg method	
ICA[b]	EIA[c]	RPHA[d]		Positive	Negative	Positive	Negative
+	+	−	7	7	0	7	0
−	−	+	1	0	1	0	1

[a] +, positive detection; −, negative detection.
[b] Dainascreen HBsAg.
[c] Auszyme Monoclonal.
[d] Serodia-HBs.

Qualitative evaluation of the ICA and EIA for HBsAg detection in the 462 samples is as follows. The results of ICA for HBsAg completely agreed with those of the EIA, with a sensitivity of 100% (158 of 158 samples) and a specificity of 100% (304 of 304 samples). Table 1 provides the results for samples with discrepant results by the ICA, EIA, and RPHA methods. The RPHA method yielded negative results for seven samples that were positive by both the ICA and the EIA methods, as well as a positive result for one sample that was negative by each of the other two methods. These samples were reevaluated by the anti-HBc assay and another EIA method (IMx). All seven samples that were ICA positive, EIA positive, and RPHA negative were anti-HBc positive and IMx positive. On the other hand, the sample that was ICA negative, EIA negative, and RPHA positive was anti-HBc negative and IMx negative. Therefore, the RPHA-positive result was considered to be false positive.

Anti-HBs ICA. The ICA system detected anti-HBs at a level of 42 mIU/ml after incubation for 15 min and at a level of 24 mIU/ml after overnight incubation. The endpoint titers of the semiquantitative assay were 2^5 for the ICA, 2^7 for the EIA, and 2^3 for the PHA methods. The sensitivity of the ICA was improved by extending the incubation time; values of 2^6 and 2^7 were obtained after incubation for 60 and 120 min, respectively. Overnight incubation did not further increase the sensitivity.

Qualitative evaluation of anti-HBs detection by the ICA and EIA methods showed that of the 199 EIA-positive samples, 184 were positive by ICA (sensitivity, 92.5%) and that of the 281 EIA-negative samples, 6 were positive by ICA (specificity, 97.5%). This shows a 95.6% agreement between the two methods.

Table 2 shows the discrepant results obtained by the ICA, EIA, and PHA methods. All 15 samples that yielded ICA-negative and EIA-positive results were positive by the second EIA method (IMx-Ausab), and the 6 samples with ICA-positive and EIA-negative results were also IMx-Ausab positive. Extending the incubation time to 60 min did not improve the ICA-negative and EIA-positive results. The PHA method gave negative results for the 40 samples that were positive by both the ICA and the EIA methods. The PHA method gave two positive results that were negative by both the ICA and the EIA methods.

TABLE 2. Analysis of the samples with discrepant results for anti-HBs detection after reevaluating anti-HBc and another EIA (IMx-Ausab) method

Results of[a]:			No. of specimens	No. of samples with the indicated results by:			
				Anti-HBc		IMx-Ausab method	
ICA[b]	EIA[c]	PHA[d]		Positive	Negative	Positive	Negative
−	+	−	15	3	12	15	0
+	−	−	6	2	4	6	0
+	+	−	40	NT[e]	NT	40	0
−	−	+	2	0	2	0	2

[a] +, positive detection; −, negative detection.
[b] Dainascreen Ausab.
[c] Ausab EIA.
[d] Serodia-antiHBs.
[e] NT, not tested.

DISCUSSION

We evaluated the detection sensitivity and specificity and the clinical utility of new HBsAg and anti-HBs detection systems that use ICAs. Reactions are completed in as little as 15 min, and a single sample can be simply examined by cutting the chromatographic strip as required. The cost of this assay system is four times less than that of EIA. The system does not require any specific instrumentation. Furthermore, this test requires only a small amount of sample (25 μl) and can be readily performed.

Although the sensitivity of ICA was slightly lower than that of EIA at an incubation time of 15 min, it was improved to a level comparable to that of EIA by extending the incubation time to 60 min in the analysis of the sensitivity with respect to the endpoint titer. With clinical samples, the sensitivities and specificities with 15 and 60 min of incubation were not different for either HBsAg or anti-HBs detection. For HBsAg and anti-HBs detection, however, we observed 20 samples which were positive but whose results were difficult to interpret. These samples gave clear positive results by extending the incubation time to 60 min. We think that there may be a possibility that the sensitivity of the assay for clinical samples can be increased by extending the incubation time to 60 min. We therefore considered 60 min of incubation to be optimal. The sensitivity of the ICA was 8 to 16 times higher than that of the conventional RPHA (or PHA) method.

In a qualitative evaluation, results of the ICA for HBsAg detection agreed completely with those of the conventional EIAs, showing a 100% sensitivity (158 of 158 samples) and a 100% specificity (304 of 304 samples). The ICA system for anti-HBs detection also showed excellent results, with a 92.5% sensitivity and a 97.9% specificity when compared with EIA.

In detecting anti-HBs, of the 15 and 6 samples with discrepant results by the ICA and EIA methods, respectively, 16 samples were anti-HBc negative. All 16 samples were from HBV vaccinees. This finding does not indicate that the EIA-positive or ICA-positive results from the vaccinees are false positive, since HBV vaccination does not result in a positive conversion to anti-HBc. Actually, the mean EIA index obtained for the 15 discrepant samples with EIA-positive and ICA-negative results was 1.2 ± 0.2 (mean ± standard error) which is near the detection limit of the EIA method. Therefore, these discrepancies were considered to be due to the low anti-HBs titer in the serum. The reason for the six discrepant results for the samples that were ICA positive and EIA negative is unclear. These variations may have arisen from differences in the types and proportions of anti-HBs detected in the serum, as well as differences in the HBsAg types used in the test kits.

The Dainascreen HBsAg and Dainascreen Ausab ICAs are sensitive and reliable ways of detecting HBsAg and anti-HBs with speed and simplicity. The costs of the systems are low, and the tests do not require complex instrumentation. These tests can be recommended for use in routine screening, especially for emergency use, in the clinical microbiology laboratory.

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REFERENCES

1. **Barr, A., B. C. Dow, and I. Macvarish.** 1979. HBsAg detection—results of comparative large scale testing of blood donations. Med. Lab. Sci. **36:**109–114.
2. **Hendry, R. M., L. W. Herzog, and J. E. Herrman.** 1983. Comparison of enzyme immunoassay with radioimmunoassay for the detection of hepatitis B surface antigen. J. Med. Microbiol. **16:**111–116.
3. **Kroes, A. C., W. G. Quint, and R. A. Heijtink.** 1991. Significance of isolated hepatitis B core antibodies detected by enzyme immunoassay in a high risk population. J. Med. Virol. **35:**96–100.
4. **McCready, J. A., D. Morens, and H. A. Fields.** 1991. Evaluation of enzyme immunoassay (EIA) as a screening method for hepatitis B markers in an open population. Epidemiol. Infect. **107:**673–684.
5. **McMahon, B. J., A. J. Parkinson, and C. Helminiak.** 1992. Response to hepatitis B vaccine of persons positive for antibody to hepatitis B core antigen. Gastroenterology **103:**590–594.
6. **Par, A., S. Luminita, and I. Erdosy.** 1992. Hepatitis virus (HBV, HCV, HDV) markers in chronic liver diseases. Comparative studies in two East-Central European countries. Orv. Hetil. **133**(Suppl. 1)**:**48–50.
7. **Ranga, S., J. Pandey, and V. H. Talib.** 1994. Evaluation of two techniques (RPHA and latex agglutination) for use as a screening tool for HBsAg. Indian J. Pathol. Microbiol. **37:**375–380.
8. **Shiels, M. T., H. F. Taswell, and A. J. Czaja.** 1987. Frequency and significance of concurrent hepatitis B surface antigen and antibody in acute and chronic hepatitis B. Gastroenterology **93:**675–680.
9. **Takeo, J., and Y. Taeko.** 1969. Hemagglutination technique with erythrocyte coated with specific antibody for detection of Australia antigen. J. Exp. Med. **39:**615–620.
10. **Treichel, U., K. H. Meyer zum Buschenfelde, and R. J. Stockert.** 1994. The asialoglycoprotein receptor mediates hepatic binding and uptake of natural hepatitis B virus particles derived from viraemic carriers. J. Gen. Virol. **75:**3021–3029.
11. **Van Damme, P., M. Cramm, and J.-C. Van der Auwera.** 1995. Horizontal transmission of hepatitis B virus. Lancet **345:**27–29.
12. **World Health Organization.** 1994. Epidemiology of infection, p. 54–59. IARC monographs on the evaluation of carcinogenic risks to humans, hepatitis viruses, vol. 59. World Health Organization Geneva.