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Comparative Evaluation of Immunochromatographic Rapid Diagnostic Tests (Strip and Device) and PCR Methods for Detection of Human Hepatitis B Surface Antigens

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Background and Aims: **Hepatitis B virus (HBV) infection is a leading cause of liver disease worldwide. It is estimated that approximately 350 million people worldwide have chronic HBV infection. In this study, immunochromatographic assays (ICAs) detection methods including rapid tests were compared with serum HBV-DNA detecting by polymerase chain reaction (PCR) system.**

Methods: **240 patients including 120 samples that were positive with quantitative PCR method and 120 that were negative by either PCR or EIA methods were selected. Samples were examined by strip and device from Intec, Blue Cross, Acon, Atlas, DIMA and Cortez companies compare to the quantitative PCR method as gold standard for detecting HBsAg.**

Results: **Strip from Intec and Blue Cross, compare to the Acon, Atlas, DIMA and Cortez devices had higher sensitivity in detecting HBsAg in serum. Also positive and negative predictive values of these two strips were higher compare to the rest. In addition true negative value, specificity and positive predictive value of Acon and DIMA strips were higher for detecting HBsAg compare to the rest of the strips.**

Conclusions: **Rapid diagnostic tests are inexpensive, easy to complete, and impose the minimum discomfort to patients, as well as suitable for case-finding and epidemiological surveillance. But it should be considered that negative results with strips or device dose not exclude the presence of HBV DNA and therefore one can be use rapid tests as a back up to standard testing methods. Immunochromatographic results should be interpreted with caution, when the sample has relatively low reactivity by PCR method.**

Keywords: **Hepatitis B Virus, PCR, Rapid Test**

Introduction

Viral hepatitis is a disease with multiple causes that was first described in the fifth century BC. When Hippocrates described epidemic jaundice, he was undoubtedly referring to persons infected with acute hepatitis B virus (HBV) as well as other agents capable of infecting the liver [(1)]. HBV infection is a global health problem. Two billion people have been infected worldwide; approximately 360 million suffer from chronic HBV infection; over 520000 die each year (50000 from acute hepatitis B and 470000 from cirrhosis or liver cancer) [(2)].

HBV infection management presents us with many challenges. It is expected that more than 8% of the population in sub-Saharan Africa, Australia, and the East Mediterranean are positive to hepatitis B surface antigen (HBsAg) [(3, 4)]. In high prevalence regions, the lifetime risk of HBV infection is greater than 60%, and most infections are acquired at birth or during early childhood when the risk of developing chronic infection is greatest. Because most infections in children are asymptomatic, very little acute disease related to HBV occurs, but rates of chronic liver disease and liver cancer in adults are very high [(4)].

In many developing areas worldwide, field and clinic laboratory capabilities may be insufficient for the detection of infectious agents for definitive clinical diagnostic purposes [(5)].

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Quantitative enzyme immunoassay (EIA) methods are considered to be the most sensitive tests and are widely used at well-equipped reference centers or central blood banks [6-9]. Rapid tests are intended for qualitative detection of HBsAg in human serum, plasma, or whole blood wherever EIA methods are impractical or can not be sustained [10]. Several rapid diagnostic tests were developed for screening purposes, such as, solid-phase assays, flow-through, agglutination, and lateral-flow. The majority of rapid tests are based on immunochromatographic principle [11, 12]. The advantage of immunochromatographic method is that it can be completed in 10-20 minutes and performed by nurses or technicians with a minimum of training. It is practical for use at the provincial or peripheral health care level, since the test strips are stable for one to two years at ambient temperatures, if packaged appropriately [13]. Also nowadays immunochromatographic assays (ICAs) were developed to detect HBsAg from whole blood [14].

The development of sensitive and specific tests to detect HBV infection allowed investigators to define the natural history of HBV infection and develop strategies to prevent transmission. The development of assays to screen blood for HBsAg led to procedures to prevent transfusion-associated hepatitis B [15]. These rapid hepatitis B tests need as a back up to standard EIA testing or DNA-based methods like PCR as gold standard for evaluating their sensitivity and specificity. Comparison of the sensitivity and specificity of ICAs and quantitative immunoassays for detecting HBsAg and anti-HBs were carried out by many researchers [8, 12, 13], but there is not any comparative study using quantitative PCR method as a gold standard. Therefore in this study it was attempt by using these criteria to compare the six widely used diagnostic rapid diagnostic tests sensitivity and specificity with PCR methods.

Materials and Methods

Chronic HBV infection is defined as the presence of HBsAg in serum for at least 6 months. A total of 5-ml blood sample was obtained by trained personnel from 240 patients from the Hospitals in Urmia, Iran during October-December 2006. Serum were separated after 15 minutes at room temperature by bench centrifuge and stored at -40° C upon to be tested. DNA of all the samples were also extracted using pure Art DNA blood Mini-Kit artus (Hamburg, Germany) via spin and vacuum column procedures. Patients' specimens were divided into two groups, including 120 samples that were positive with quantitative PCR method using Roto-GENE 3000 Research (Corbet real time PCR machine) and kit artus (Hamburg, Germany) according to the manufacture instruction and adjusted to 0.5-1 ng/mL of HBsAg by normal saline (0.9g/dL). Negative and positive results were confirmed by running 5ul of HBV-DNA PCR products on 2% agarose gel.

To determine the presence of HBsAg, rapid tests that are based on immunochromatographic method device or strip were used. The device or strip is precoated with anti-HBs antibodies on the test line region of the strip. During experiment the serum reacts with the particle coated with anti-HBs antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBs antibodies to generate a colored line. After 15 minutes, in the positive sample, two distinct red lines appear in the control region (C) and another line in the test region (T), and in negative sample, one red line appear in the control region (C) [16].

Device or strip from six companies: ACON (Acon laboratories Inc., San Diego, USA), Atlas Medical (Williams Jams House, Cambridge, UK), Dima (Geseeschaft fur Diagnostika mbH, Germany), Cortez (Cortez Diagnostics Inc., Calabasas, USA), Blue Cross (Blue Cross Inc., China) and Intec (Intec Products Inc., Xiaman, China) were used.

The testing of sera for HBsAg was carried out using a standard EIA (DiaPlus Inc., USA) method. The rest of the patients (n = 120) were negative by either PCR or EIA methods (DiaPlus Inc USA).

The calculation of accuracy, sensitivity, specificity, positive and negative predictive value, and Pearson correlation value were carried out according to Irwig and collogues methods [17]. Study activities were undertaken only after review and approval by the ethical committee for the protection of human subjects at the Urmia University of Medical Sciences.

Results

True positive and true negative values in 240 patients' samples using strip or device rapid diagnostic methods such as Acon, Atlas, Intec, Blue Cross, DIMA, and Cortez were evaluated for detecting HBsAg, compare to Real Time PCR method which assumed as gold standard method with sensitivity and specificity of 100% and shown in Table 1. According to these findings, strip from Intec and Blue Cross had higher true positive rate in

Table 1. True positive and true Negative values for strip and device compare to the PCR results

Parameters	True positive	True Negative	Total
Acon	118 (98.3%)	119 (99.1%)	240
Atlas	117 (97.5%)	117 (97.5%)	240
Intec	119 (99.1%)	117 (97.5%)	240
Blue Cross	119 (99.1%)	118 (98.3%)	240
DIMA	118 (98.3%)	119 (99.1%)	240
Cortez	118 (98.3%)	118 (98.3%)	240
PCR	120 (100%)	120 (100%)	240

detecting surface HBsAg in patient's serum compare to the Acon, Atlas, DIMA and Cortez. Although true negative results for strips Acon and DIMA were higher compare to the rest of the strips or devices.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Pearson correlation values of the six strips or device used in the experiments in comparison to the PCR methods as gold standard, were calculated and summarized in Table 2. As shown in Table 2, Intec and Blue cross strips had higher sensitivity and NPV for detecting HBsAg in compare to the rest of the strips. Specificity and PPV in DIMA and ACON were higher in comparison to the other strips or device used in the experiment. Typical results of positive HBsAg in serum (two red lines) and negative HBsAg (one red line) for strip or device experiments were shown in Figure 1. Also the result of quantitative PCR which some of its product were amplified by normal PCR were shown in Figure 2.

Table 2. Sensitivity, specificity, PPV, NPV, and Pearson correlation values of the strips or device used in the experiments compare to the PCR methods

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Pearson correlation (%)
Acon	98.3	99.2	99.2	98.3	97.5
Atlas	97.5	97.5	97.5	97.5	95.0
Intec	99.2	97.5	97.5	99.2	96.7
Blue Cross	99.2	98.3	98.3	99.2	97.5
DIMA	98.3	99.2	99.2	98.3	97.5
Cortez	98.3	98.3	98.3	98.3	96.7
PCR	100	100	100	100	100

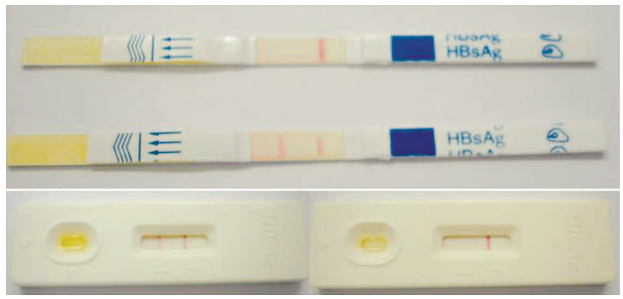

Figure 1. Positive (two red lines) and negative (one red line) for strip or device experiments

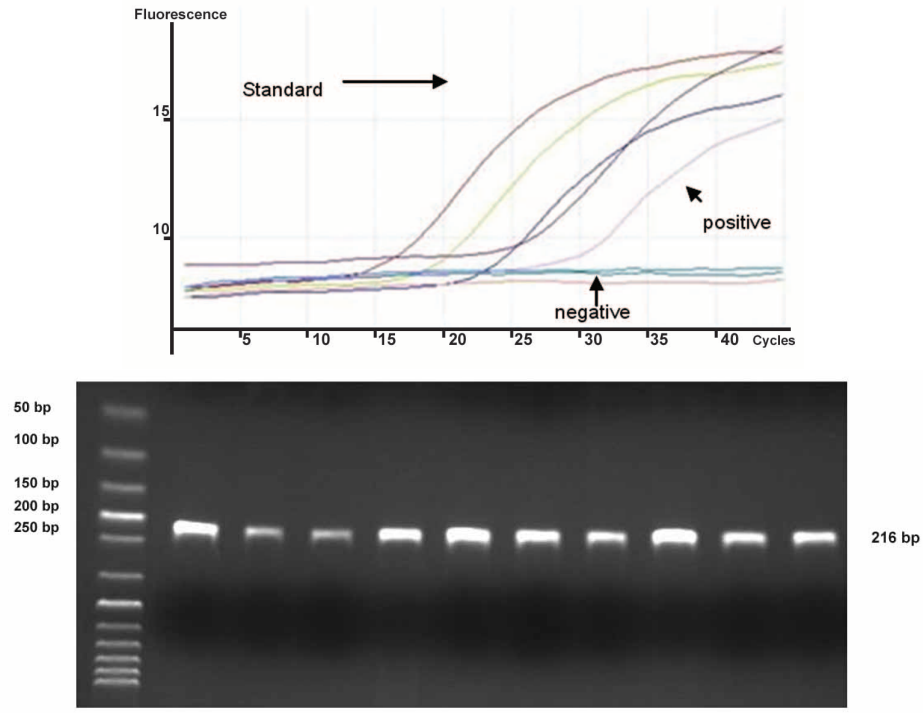

Figure 2. Quantitative PCR (above) and some of its product were amplified by normal PCR (down)

Discussion

Along with increased public education, screening programs can greatly increase public awareness of HBV. This initiates contact with those individuals not normally seeking medical care and allows for the creation of a database so treatment can be managed effectively. However, there are many problems associated with current methods of screening that may account for the lack of target population awareness [4]. Currently, screening of the general population has not been favored. The screening methods available take some time to detect antibodies and antigens, and results are not available immediately. This can cause anxiety during the waiting period for individuals tested, and they will often have to be recalled for the outcome of the screen. This can result in individuals not returning for consultation or proper follow-up. The high laboratory costs associated with screening is another factor that reduces the willingness to screen the general population [4, 18]. Therefore it seems there is a great demand for a reliable screening method, with high efficiency and rapid diagnostic ability. Since there are many trade names that claim they have these abilities, it is necessary to compare them and introduce standards necessary for accepting or rejecting them.

The rapid test is a screening method that is able to discriminate an infected individual from an uninfected subject within minutes is greatly beneficial to the proactive management of HBV. This rapid diagnostic test facilitates screening and helps raise awareness of hepatitis B. The test allows the initiation of treatment or immediate vaccination, reducing the potential loss of follow-up when results are not available straight away [13, 18]. The diagnostic tests evaluated are inexpensive, easy to complete, and impose the minimum discomfort to the patient, since there a very small specimen size is required. The results of any diagnostic test must be valid, accurate, reliable, and reproducible. Because of the potentially severe consequences of acute and chronic HBV infection, it is important to be able monitor the disease progression closely [15]. HBV DNA values persisting longer than 8 weeks may indicate progression to chronic infection which has become increasingly important to monitor low values of HBV DNA.

As compared to quantitative PCR results as a gold standard method, our rapid tests with strips or device showed the sensitivity between 97.5-99.2% and specificity of 97.5-99.2%. For example, among six strip or device rapid diagnostic tests, Intec and Blue Cross showed higher sensitivity than Acon, Atlas, DIMA and Cortez strips. Also Acon and DIMA had higher specificity than others. Pearson correlation of tests was between 96.7% and 97.5%. From our experiments it is possible to conclude that these tests sensitivity were quite acceptable. In addition the specificity of these tests was also quite high. In agreement with our findings a 'rapid' one step immunochromatographic, visually read, antigen capture assay--the "HEPACARD" used for rapid screening of HBsAg was evaluated and showed a sensitivity of 79% and specificity of 98.9%. This study suggested that this particular rapid HBsAg test results have to be confirmed by either an EIA or MEIA where the facility exists. The test may be used only in a small hospital setting where the facilities for EIAs do not exist [18].

Also rapid assays to detect HBsAg in serum were evaluated for their accuracy and suitability at the national bank transfusion service in Zimbabwe and showed that the specificity of tested devices were about 99.5%. They concluded all the rapid/simple tests were easy to perform and interpret, required no (or minimal) laboratory equipment, and could be taught easily to local laboratory personnel. The cost of these tests is equivalent to or less than that of routine EIA methods [15]. In another experiment that carried out in the India using Diagnostic kits (J. Mitra Co. Ltd) for rapid detection of HBsAg; the specificity and sensitivity of the rapid kits were 100% and 93.4%, respectively [19].

In an experiment carried out in Seoul researcher showed immunochromatographic technique has sensitivity of 97% and specificity of 100% for detecting surface HBsAg [17].

In an experiment carried out by Sato and colleagues, they used two ICAs, Dainascreen HBsAg for detecting HBsAg in human serum. The ICA systems are composed of a comb-shaped device that contains nitrocellulose strips on which complex of HBsAg can be visualized. The limit of detection for HBsAg was 3.1 ng/mL. Results of HBsAg detection showed 100% sensitivity and 100% specificity [13]. As we see from these researcher work, the threshold they use (3.1 ng/mL) was very high. The sensitivity the more recently introduced immunochromatographic strips (ICS tests), however, is somewhat better, and sensitivity limit of <1 ng/mL HBsAg have been reported [8, 20, 21].

Since the early 1990s, rapid tests have been available for detection of HIV infection. They were intended for field diagnosis, emergency and home testing. In addition, rapid tests for anti-HIV, HBsAg and anti-HCV have been used for blood screening in many resource-poor areas to save resources and overcome lack of funding, equipment and electrical

supply. The performance of rapid tests varies widely but some have sensitivity and specificity levels that meet standards established by EIA for HBsAg [20, 22]. However, it is known to be less sensitive than EIA [17]. Immunochromatographic results should be interpreted with caution, because the sample containing a relatively low reactivity by the quantitative PCR can show negative result for HBsAg [17].

In summary, the six new rapid diagnostic tests evaluated proved to be accurate testing methods, based on sensitivity and specificity measures, when compared with standard clinical laboratory testing. These six tests are rapid, simple, and provided excellent screening methods, with comparable sensitivity and specificity to the gold standard methods. Application of these serologic tests within the comparative evaluation framework, using the alternative testing strategies of the World Health Organization, proved to be an effective way to determine serostatus related to hepatitis B. The diagnostic test kits can be easily used in small, rural laboratories for serologic screening of high-risk clients. In many instances, false-positive results are preferable to false-negative results when screening large groups of people. Positive serology triggers repeat testing with alternative methods for case confirmation.

References

1. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. *Clin Microbiol Rev* 1999; **12**: 351-66.
2. de Franchis R, Hadengue A, Lau G, Lavanchy D, Lok A, McIntyre N, *et al*. EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version). *J Hepatol* 2003; **39** (Suppl 1): S3-25.
3. Andre F. Hepatitis B: a comprehensive prevention, diagnosis, and treatment program--past, present, and future. *J Gastroenterol Hepatol* 2004; **19** (Suppl): S1-4.
4. Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* 2000; **18** (Suppl 1): S20-2.
5. Aristegui J, Usonis V, Coovadia H, Riedemann S, Win KM, Gatchalian S, *et al*. Facilitating the WHO expanded program of immunization: the clinical profile of a combined diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type b vaccine. *Int J Infect Dis* 2003; **7**: 143-51.
6. Re-evaluation of HBsAg detection kits approved for marketing in Japan. *Japan J Infect Dis* 2001; **54**: 201-7.
7. Clement F, Dewint P, Leroux-Roels G. Evaluation of a new rapid test for the combined detection of hepatitis B virus surface antigen and hepatitis B virus e antigen. *J Clin Microbiol* 2002; **40**: 4603-6.
8. Lau DT, Ma H, Lemon SM, Doo E, Ghany MG, Miskovsky E, *et al*. A rapid immunochromatographic assay for hepatitis B virus screening. *J Viral Hepatitis* 2003; **10**: 331-4.
9. Verstraeten T, Keya A. Evaluation of two rapid hepatitis B surface antigen detection tests for screening in a blood bank. *East African Med J* 1997; **74**: 717-8.
10. Torlesse H, Wurie IM, Hodges M. The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen among pregnant women in West Africa. *Br J Biomed Sci* 1997; **54**: 256-9.
11. Plitt SS, Somily AM, Singh AE. Outcomes from a Canadian public health prenatal screening program for hepatitis B: 1997-2004. *Can J Public Health* 2007; **98**: 194-7.
12. Whang DH, Um T. Comparison of immunochromatography assays and quantitative immunoassays for detecting HBsAg and anti-HBs. *Korean J Lab Med*. 2005; **25**: 186-91.
13. Sato K, Ichiyama S, Iinuma Y, Nada T, Shimokata K, Nakashima N. Evaluation of immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody, Dainascreen HBsAg and Dainascreen Ausab. *J Clin Microbiol* 1996; **34**: 1420-2.
14. Shin HS, Kim CK, Shin KS, Chung HK, Heo TR. Pretreatment of whole blood for use in immunochromatographic assays for hepatitis B virus surface antigen. *Clin Diagn Lab Immunol* 2001; **8**: 9-13.
15. Mvere D, Constantine NT, Katsawde E, Tobaiwa O, Dambire S, Corcoran P. Rapid and simple hepatitis assays: encouraging results from a blood donor population in Zimbabwe. *Bull World Health Organ* 1996; **74**: 19-24.
16. Blumberg BS, Sutnick AI, London WT. Hepatitis and leukemia: their relation to Australia antigen. *Bull N Y Acad Med* 1968; **44**: 1566-86.
17. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates of test accuracy are transferable. *BMJ* 2002; **324**: 669-71.
18. Raj AA, Subramaniam T, Raghuraman S, Abraham P. Evaluation of an indigenously manufactured rapid immunochromatographic test for detection of HBsAg. *Indian J Pathol Microbiol* 2001; **44**: 413-4.
19. Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. *Indian J Med Sci* 2000; **54**: 432-4.
20. Allain JP, Lee H. Rapid tests for detection of viral markers in blood transfusion. *Expert Rev Mol Diagn* 2005; **5**: 31-41.
21. Myint KS, Guan M, Chen HY, Lu Y, Anderson D, Howard T, *et al*. Evaluation of a new rapid immunochromatographic assay for serodiagnosis of acute hepatitis E infection. *Am J Trop Med Hyg* 2005; **73**: 942-6.
22. Lien TX, Tien NT, Chanpong GF, Cuc CT, Yen VT, Soderguist R, *et al*. Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg* 2000; **62**: 301-9.