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# Comparison Between Rapid ICT And ELISA Tests For The Detection Of HBsAg; And Screening Of Hepatitis B Infection In Apparently Healthy Bangladeshi Outbound Staff

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**----------ABSTRACT----------**

*Introduction: Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. This study was performed at the health screening centre of Dhaka Crown Medical Center, Uttar Badda, Dhaka North City Corporation, Bangladesh. It aimed to determine the seroprevalence of this disease by screening the apparently healthy outbound staff who were intending to go abroad for labor work. It also aimed to compare between ICT and ELISA test results;. Materials and Methods: ELISA was used as gold standard for comparative evaluation. 5,500 healthy subjects tested on ELISA and ICT kits using separate panel-sera for each. SPSS version 18.0 was used for statistical analysis. Results: The seroprevalence of HBV was 0.82% (45) by the ICT device and 0.91% (50) by ELISA test. Samples with OD values less than 1.000 in ELISA test could not detect HBsAg by using ICT method. Conclusion: Rapid assays must be used with caution and it is also important to validate these rapid assays by testing them in a given population to assess the effectiveness of these assays in detecting HBsAg in diagnostic laboratories. They should be recommended only in poor settings or remote areas to assess prevalence of HBV infection.*

***KEYWORDS:*** *HBsAg, ICT, ELISA, Prevalence*

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## I. INTRODUCTION

A clinic, health/medical centre, medical institution or hospital in the country of origin, appointed by the Government of employer's country, on a provisional basis, to conduct pre-departure medical examination for foreign workers intending to work in abroad. Accreditation will be given upon recommendation by an institution e.g. Embassy, and/or satisfactory recommendation by a regulatory body e.g. Ministry of Health of respective country without the need for inspection and assessment of the health facility.[1]

Pre-employment health checkup are considered by companies to screen job applicants and generally covers the health history of the candidate. Existing medications or alignments are also reviewed to check if they may pose any threat to the prospective working condition of which both the employee and employer are informed accordingly.[2]

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of causes, including different viruses; such as hepatitis A, B, C, D and E. Hepatitis B and hepatitis C both are serious and common infectious disease of the liver, affecting millions of people throughout the world. The virus is transmitted through contact with the blood or other body fluids of an infected person.[3]

An estimated 240 million people are chronically infected with hepatitis B and 150 million people infected with hepatitis C. Worldwide more than 780,000 people die every year due to complications of hepatitis B and 500,000 people die due to complication of hepatitis C. A significant number of those who are chronically infected will develop liver cirrhosis or liver cancer.[4]

"Hepatitis B is easily preventable by vaccination and safe health practice, yet it still kills more people worldwide than HIV/AIDS. It is also the number one reason for crew failing their pre-employment medical examinations (PEME) and 9.6% of all unfit crew were found to have the disease.[5]

"The disease is caused by a virus that infects the liver and is 50-100 times more infectious that HIV with 10-30 million individuals infected each year. The disease leads to life-long infection, cirrhosis, hepatato-

cellular carcinoma and liver failure with a million people dying each year from hepatitis B and its complications with most people not having symptoms until it is too late. The main symptoms are fatigue, abdominal pain, and jaundice (yellow coloration of the skin).[5]

"Circumstances that put individuals at risk of contracting the disease include unsterile medical equipment, handling clinical waste, intravenous drug abuse and sexual contact. In order to prevent crew from contracting the disease there are highly effective recombinant vaccines available. It is not a mandatory requirement to have received a vaccine to work onboard, although it is highly advisable. Additional preventative measures include the screening of blood donors and safe sex using barrier methods.[5]

Different methods are used for the diagnosis of hepatitis including ICT, ELISA, EIA and PCR. ELISA, EIA and PCR methods are expensive and are used in well equipped labs and major tertiary care hospitals. Rapid diagnostic ICT kits are a good choice as they are less expensive and do not need high tech manpower or infrastructure. Since 1990s, rapid tests are available for detection of HIV infection. They were intended for field survey diagnosis, emergency and home testing. In addition rapid test 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.[6]

For a highly infectious virus like HBV which causes a long term silent infection, accurate detection of the viral marker is essential for controlling the transmission of the virus. For this reason, it is necessary to validate detection methods prior to allowing their use in diagnostic laboratories. In many developing countries, ICT based rapid diagnostic tests are widely used to detect HBsAg antibody for both diagnosis and screening of acute and chronic infections, although ideally, screening should be done using more advanced and accurate methods such as EIA, PCR or ELISA. Negative samples from patients referred for screening assays (rapid assays) are seldom re-tested, considering the costs of retesting in resource poor settings. Hence, choosing a test with high sensitivity and negative predictive values (NPV) is more important than choosing a test with high specificity and positive predictive values (PPV) for routine use.[7]

The aim of this study was to estimate the prevalence of hepatitis B infection in apparently healthy subjects those were intending to go abroad for employment in foreign countries those came in Dhaka, Bangladesh for pro-employment medical screening. Since there is a possibility of them being asymptomatic carriers of hepatitis, they need to be screened out. Dhaka is the capital city of Bangladesh with tremendous diversity of population associated with various fields of life and social status. This retrospective study will give us the idea about the burden of disease in healthy looking general people living in different areas of Bangladesh. This data can be used to make important plans and policies for assuring safe blood. The two diagnostic techniques; a one step diagnostic kit and ELISA technique were used and the sensitivity comparison of both techniques was also studied.

## II. MATERIALS AND METHODS

**Materials**

**Study Location:**

Dhaka Crown Medical Center, Uttar Badda, Dhaka North City Corporation, Bangladesh.

**Study Population:**

A total of 5,500 healthy blood donors visiting Dhaka Crown Medical Center, Uttar Badda, Dhaka North City Corporation, Bangladesh were considered in the present study. They attended over the period of two years from June 2016 to June 2018. Out of these 5,500 donors 80% (4,400) were males and 20% (1100) were females.

**Study Time:**

June 2016 to June 2018.

**Study Samples:**

Blood collected from outbound staff on the strict basis of the standard operating procedures of Bangladesh. Whole blood from participants is drawn by venipuncture into dipotassium ethylenediamine-tetraacetate (K2-EDTA)-containing evacuated Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) after informed consent was obtained. Samples were packaged according to CDC guidelines and transported by courier in insulated containers to this CLIA-certified laboratory. Most samples are delivered within 4 hours of collection. Samples held overnight before delivery are kept at room temperature (65-76°F).[8]

**Methods**

Outbound staffs were screened for Hepatitis B using two diagnostic methods, i.e., i). ELISA technique and ii). One step Immune-Chromatographic Stripe Test Method.

**i. Enzyme Linked Immuno Sorbant Assay (ELISA)**

Monolisa™ HBs Ag ULTRA assay is a one step enzyme immunoassay based on the principle of the "sandwich" type using monoclonal antibodies and polyclonal antibodies selected for their ability to bind

themselves to the various subtypes of HBs Ag now recognized by the WHO and the most part of variant HBV strains.

**Preparation of the Reagents**
**NB:** Before use, allow reagents to reach room temperature (18-30°C).
**1) Ready for use reagents**
**Reagent 1 (R1):** Microplate
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at +2-8°C.
**Reagent 3 (R3):** Negative control
**Reagent 4 (R4**): Positive control
**Reagent 10 (R10):** Stopping solution
**2) Reagents to reconstitute**
**Concentrated washing solution (20X): Reagent 2 (R2)**
Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips.
**Conjugate working solution (R6 + R7)**

- Gently tap the vial of the lyophilized conjugate (R7) on the work-bench to remove any substance from the rubber cap.
- Carefully remove the cap and pour the content of a conjugate diluent vial (R6) into the lyophilized conjugate vial (R7).
- Put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution.

**Enzyme development solution: Reagent 8 (R8) + Reagent 9 (R9)**
Dilute 1:11 the chromogen (R9) in the Substrate Buffer (R8) (ex: 1ml reagent R9+10ml reagent R8). Stability is for 6 hours in the dark once prepared.

**Assay Procedure**
Strictly follow the proposed procedure.
Use the negative (R3) and (R4) positive controls for each series of determinations to validate the test results.
Follow the following Good Laboratory Practice:
1. Carefully establish the sample distribution and identification plan.
2. Prepare the diluted washing solution.
3. Prepare the conjugate R6+R7 working solution.
4. Take out from the protective packing the support frame and the necessary number of strips (R1). Put the unused strips back in their packing and reclose it.
5. Distribute in the wells in the following order (advisable plate distribution):
• Wells A1, B1, C1 and D1: 100µl of negative control (R3)
• Well E1: 100µl of positive control (R4)
• Well F1: 100µl of the first unknown sample if this well is not used as control well for the validation of the sample and conjugate deposition (optional)
• Wells G1, H1,...etc : 100µl of unknown sample.
Depending on the used system, it is possible to modify the position of controls or the order of distribution.
**NB:** The sample distribution can be visually controlled at this step of the manipulation: there is a difference of coloration between empty well and well with sample (Refer to section 14 for automatic verification).
6. Quickly dispense 50µl of conjugate solution (R6+R7) into all wells, the conjugate solution must be shaken before use. Homogenize the reaction mixture.
**NB:** The sample distribution can also be visually controlled at this step of the manipulation, as well as the conjugate distribution: The conjugate solution (R6+R7), which is colored red, can be visually controlled at this step of the manipulation.
7. When possible, cover the plate with new adhesive film and incubate for 1hour and 30 ± 5 minutes at 37±1°C.
8. Remove the adhesive film, empty all wells by aspiration and wash a minimum of 5 times. The residual volume must be lower than 10µl (if necessary, dry the strips by turning them upside down on absorbent paper).
9. Quickly dispense into each well 100µl of prepared development solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30±5 minutes at room temperature (18-30°C). Do not use adhesive film during this incubation.
**N.B.:** The distribution of the development solution, which is colored pink, can be visually controlled at this step of the manipulation: There is a clear difference of coloration between empty well and well containing the pink

substrate solution. (refer to section 14 for automatic verification: spectrophotometric verification of sample and reagent pipeting)
10. Add 100µl stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture.
**N.B.:** The distribution of the stopping solution, which is not colored, can be visually controlled at this step of the manipulation. After the addition of the stopping solution the pink coloration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples).
11. Carefully wipe the plate bottom. Wait at least 4 minutes after stopping solution addition before reading and within 30minutes of stopping the reaction, read the optical density at 450/620-700 nm using a plate reader.
12. Check for agreement between the spectrophotometric and visual readings and against the plate and sample distribution and identification plans.

**ii. Immune-Chromatographic Test Method (ICT)**

ASPEN's (catalog no. 1156071101) the HBsAg One Step Hepatitis B Surface Antigen Test Strip (Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in serum or plasma.

**Reagents:**

The test strip contains anti-HBsAg particles and anti-HBsAg coated on the membrane.

**Specimen Collection and Preparation**

• The HBsAg One Step Hepatitis B Surface Antigen Test Strip (Serum/Plasma) can be performed using either serum or plasma.
• Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
• Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
• Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
• If specimens are to be shipped, they should be packed in compliance with federal, state or local regulations for the transportation of etiologic agents.

**Procedure**

Allow test strip, serum or plasma specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. With arrows pointing toward the serum or plasma specimen, immerse the test strip vertically in the serum or plasma for at least 10-15 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip.
3. Place the test strip on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 15 minutes.
**Note:** A low HBsAg concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 30 minutes.

**Statistical analysis**

SPSS version 18.0 was used for statistical analysis of this study.

## III. RESULTS

Among the 5,500 Bangladeshi outbound staff 5,450 individuals were found healthy regarding hepatitis B virus infection, and 50 individuals were identified as HBsAg seropositive by ELISA method and obtained OD values range was 0.1130-3.5530. Of the HBsAg seropositive 50 samples 45 were identified as HBsAg seropositive by ICT method and obtained OD values range in ELISA method was 1.0150-3.5530. Samples OD values less than 1.000 obtained in ELISA method HBsAg could not identified by ICT method. In respect to the total outbound staff ELISA method identified 0.91% and ICT method identified 0.82% subjects were HBsAg seropositive (Table-1).

**Table-1:** ELISA and ICT test results

| Sl No. | Test Name | E L I S A Method | | | ICT Method |
|---|---|---|---|---|---|
| | | OD Value | Cut off Value | Result | Result |
| 1. | HBsAg | 3.5230 | 0.0830 | Positive | Positive |
| 2. | HBsAg | 3.5110 | 0.0920 | Positive | Positive |
| 3. | HBsAg | 3.5530 | 0.0810 | Positive | Positive |
| 4. | HBsAg | 3.5510 | 0.0720 | Positive | Positive |
| 5. | HBsAg | 3.5320 | 0.0760 | Positive | Positive |
| 6. | HBsAg | 3.5111 | 0.0830 | Positive | Positive |
| 7. | HBsAg | 3.5320 | 0.0880 | Positive | Positive |
| 8. | HBsAg | 3.5220 | 0.0920 | Positive | Positive |
| 9. | HBsAg | 3.5100 | 0.0820 | Positive | Positive |
| 10. | HBsAg | 3.5100 | 0.0750 | Positive | Positive |
| 11. | HBsAg | 3.5300 | 0.0840 | Positive | Positive |
| 12. | HBsAg | 3.5210 | 0.0760 | Positive | Positive |
| 13. | HBsAg | 3.5250 | 0.0810 | Positive | Positive |
| 14. | HBsAg | 3.5150 | 0.0830 | Positive | Positive |
| 15. | HBsAg | 3.5200 | 0.0780 | Positive | Positive |
| 16. | HBsAg | 3.5140 | 0.0720 | Positive | Positive |
| 17. | HBsAg | 3.5230 | 0.0820 | Positive | Positive |
| 18. | HBsAg | 2.5250 | 0.0920 | Positive | Positive |
| 19. | HBsAg | 2.5320 | 0.0720 | Positive | Positive |
| 20. | HBsAg | 2.5150 | 0.0830 | Positive | Positive |
| 21. | HBsAg | 2.5250 | 0.0760 | Positive | Positive |
| 22. | HBsAg | 2.5320 | 0.0930 | Positive | Positive |
| 23. | HBsAg | 2.5020 | 0.0790 | Positive | Positive |
| 24. | HBsAg | 2.5030 | 0.0860 | Positive | Positive |
| 25. | HBsAg | 2.5600 | 0.0740 | Positive | Positive |
| 26. | HBsAg | 2.5010 | 0.0820 | Positive | Positive |
| 27. | HBsAg | 2.5200 | 0.0930 | Positive | Positive |
| 28. | HBsAg | 2.5210 | 0.0810 | Positive | Positive |
| 29. | HBsAg | 2.4200 | 0.0750 | Positive | Positive |
| 30. | HBsAg | 2.4020 | 0.0760 | Positive | Positive |
| 31. | HBsAg | 2.4620 | 0.0930 | Positive | Positive |
| 32. | HBsAg | 2.4250 | 0.0830 | Positive | Positive |
| 33. | HBsAg | 2.4240 | 0.0720 | Positive | Positive |
| 34. | HBsAg | 2.4100 | 0.0820 | Positive | Positive |
| 35. | HBsAg | 2.4200 | 0.0860 | Positive | Positive |
| 36. | HBsAg | 2.1015 | 0.0840 | Positive | Positive |
| 37. | HBsAg | 2.1530 | 0.0830 | Positive | Positive |
| 38. | HBsAg | 2.1080 | 0.0740 | Positive | Positive |
| 39. | HBsAg | 2.1600 | 0.0920 | Positive | Positive |
| 40. | HBsAg | 2.1050 | 0.0720 | Positive | Positive |
| 41. | HBsAg | 1.5230 | 0.0930 | Positive | Positive |
| 42. | HBsAg | 1.5500 | 0.0940 | Positive | Positive |
| 43. | HBsAg | 1.0250 | 0.0820 | Positive | Positive |
| 44. | HBsAg | 1.0800 | 0.0730 | Positive | Positive |
| 45. | HBsAg | 1.0150 | 0.0840 | Positive | Positive |
| 46. | HBsAg | 0.1430 | 0.0880 | Positive | Negative |
| 47. | HBsAg | 0.1130 | 0.0920 | Positive | Negative |
| 48. | HBsAg | 0.1320 | 0.0720 | Positive | Negative |
| 49. | HBsAg | 0.1530 | 0.0830 | Positive | Negative |
| 50. | HBsAg | 0.1230 | 0.0920 | Positive | Negative |

## IV. DISCUSSION

Hepatitis B surface antigen (HBs Ag) seropositivity of 0.91% recorded among healthy outbound staff implies an incidence of hepatitis B virus infection in a health check center of Bangladesh.

Bangladesh is a country with intermediate endemic HBV and a chronic HBV carriage rate of 2–6%. The prevalence of chronic HBV among the general population and various high-risks groups, including intravenous drug users, ranges from 0.8% to 6.2%.[9, 10]

A study from India has observed that immunochromatographic assays (ICAs) has a specificity of 100% but the sensitivity was 93.4%.[11] Study from Seoul showed 97% sensitivity and 100% specificity for detecting HBsAg.[12] In another study in healthy individuals from Karachi showed comparable sensitivity and specificity of ICT kits with ELISA technique.[13]

Different ICA based rapid assays used for HBsAg detection in the serum may not have the same accuracy index in every region since there can be differences in the prevalence of HBV infection in a given

population. Most of these rapid assay use recombinant proteins from the prototype virus alone, specifically for HCV. Eleven type of genotype of HCV and eight type of genotype of HBV prevalent in different region of world. Moreover, the circulating subtype/s and genotypes of HBV and HCV show varied geographical and epidemiological distribution.[4,14] In such cases ICA that does not cover this particular subtype/s will not detect this type when testing. This may be the reason why one serum sample that was non-reactive for one step test was reactive using the ELISA.[15] In our study 5 serum samples were not identified by ICT but identified by ELISA among HBsAg seropositive 50 samples from the 5,500 healthy outbound staff.

Failure of rapid test kit to detect HBV and HCV reactive samples may be due to inadequate coating of the antigen, different nature of antigen used and genetic heterogeneity of the virus prevalent in that area.[16] Rapid assays must be used with caution and it is also important to validate these rapid assays by testing them in a given population to assess the effectiveness of these assays in detecting the genotypes and subtypes of HBV and HCV circulating in the region before using these tests routinely in diagnostic laboratories. There are no approved rapid assays by the food and drug administration (FDA) and CE mark for European Union for HBsAg and HCV detection although several rapid tests for screening for HIV have been approved.[17]

In conclusion we reported rapid test are less efficient than ELISA. They should be recommended only in poor settings, remote areas and peripheral health facilities. HBV is highly dangerous infection for community; false negative results leave a threat of silent transmission and spreading of diseases among people and also create an urge for more sensitive assays like ELISA.

## V. CONCLUSION

ICT test must be used with caution and validation is required for these rapid assays by testing them in a given population to assess the effectiveness of these assays in detecting HBsAg in diagnostic laboratories. It should be carried out in poor settings or remote areas to assess prevalence of HBV infection and can help to prevent vertical transmission in some extent.

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