Detection of hepatitis B virus infection: A systematic review

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AIM: To review published methods for detection of hepatitis B virus (HBV) infection.

METHODS: A thorough search on Medline database was conducted to find original articles describing different methods or techniques of detection of HBV, which are published in English in last 10 years. Articles outlining methods of detection of mutants or drug resistance were excluded. Full texts and abstracts (if full text not available) were reviewed thoroughly. Manual search of references of retrieved articles were also done. We extracted data on different samples and techniques of detection of HBV, their sensitivity (Sn), specificity (Sp) and applicability.

RESULTS: A total of 72 studies were reviewed. HBV was detected from dried blood/plasma spots, hepatocytes, ovarian tissue, cerumen, saliva, parotid tissue, renal tissue, oocytes and embryos, cholangiocarcinoma tissue, etc. Sensitivity of dried blood spot for detecting HBV was > 90% in all the studies. In case of seronegative patients, HBV DNA or serological markers have been detected from hepatocytes or renal tissue in many instances. Enzyme linked immunosorbent assay and Chemiluminescent immunoassay (CLIA) are most commonly used serological tests for detection. CLIA systems are also used for quantitation. Molecular techniques are used qualitatively as well as for quantitative detection. Among the molecular techniques version 2.0 of the CobasAmpliprep/CobasTaqMan assay and Abbott’s real time polymerase chain reaction kit were found to be most sensitive with a lower detection limit of only 6.25 IU/mL and 1.48 IU/mL respectively.

CONCLUSION: Serological and molecular assays are predominant and reliable methods for HBV detection. Automated systems are highly sensitive and quantify HBV DNA and serological markers for monitoring.

Key words: Chemiluminescent immunoassay; Serology; Automated detection; Molecular assay; Hepatitis B virus

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Core tip: The article was aimed to review published
methods of detection of hepatitis B virus (HBV) infection. A thorough search on medline database was conducted and 72 studies were included. It was observed that HBV can be detected reliably from dried blood spot (sensitivity > 90%). Serological and Molecular assays are predominant and reliable methods. Chemiluminescent immunoassay is more sensitive than Enzyme linked immunosorbent assay. Rapid tests are useful for screening. Real time polymerase chain reaction (PCR), branched DNA probe assays are principal methods for quantitation. Automated systems are more sensitive compared to in house assays. Abbott real time PCR was found to be most sensitive with a lower detection limit of only 1.48 IU/mL.

INTRODUCTION
The enigma of hepatitis started long back in 3rd millennium B.C. in Sumeria with the first description of jaundice. Epidemic icterus was reported initially by Hippocrates (460 to 375 B.C.) followed by various vague descriptions by Greeks and Romans. But the perception of transmissibility came into acceptance with the spread of syphilis by Columbus and crew in 1494[1]. Further innumerable epidemics occurred in recipients of vaccines containing human serum or lymph. The largest was in 1942 among United States Army personnel, who received yellow fever vaccine containing human serum[3]. In 1940’s several experiments in human volunteers by Cameron (1943)[3], Mac Callam (1944)[2,3], Paul et al[6] (1945) confirmed the viral etiology of hepatitis. 2 distinct clinciopneumological forms of viral hepatitis: Serum hepatitis and infectious hepatitis was evidenced by the study of Krugman[3] in the late 1950’s and 1960’s at Willowbrook State Schools, NewYork[4]. But the most important exploration in the history of viral hepatitis was of Sir B. Blumberg in the year 1960’s. He observed an unusual reaction between the serum of hemophiliac patient and that of Australian aborigine in immunodiffusion gel and named this unusual protein Australia Antigen (Au Ag) which was further linked to viral hepatitis[3]. In 1968 Alfred Prince also described a serum antigen (SH Ag) in the serum of post transfusion patients[6]. These Au Antigen and SH Ag were soon found to be identical[3]. In the year 1970, Dane et al[7] discovered 42 nm sized virus like particles while observing Au Ag immune complexes under Electron Microscope. It was obvious that Au Ag was the surface antigen, whereas the Dane particles were actual virus. Hence the Au Ag was named hepatitis B surface antigen (HBsAg). By treating these “Dane particles” with mild detergents core particles were released by Almeida et al[8]. Antibody present in post hepatitis serum reacted with these inner/core particles. Researchers could comprehend soon that to assess the infectivity of the disease mere presence of HBsAg is not sufficient. In 1972 hepatitis B e antigen (HBeAg) was identified by Magnus et al[9] which helped to differentiate between highly infectious and less infectious forms. Simultaneously hepatitis B virus (HBV) DNA was identified by Robinson et al[10]. In earlier days infection with HBV was detected by demonstration of antibody titer by Complement Fixation Test[2]. The first solid phase sandwich radio immunoassay named Ausria 125 was developed by Ling et al[11] at Abbott Laboratories (North Chicago). This highly sensitive detection method became a major discovery in the diagnosis of viral transfusion hepatitis and screening of blood donors[2]. Since then innumerable serological and molecular methods have been developed for diagnosing HBV. This article provides an overview of detection of HBV infection employing different techniques.

MATERIALS AND METHODS

Literature search
The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[12]. A protocol was developed and pertinent studies were identified as per inclusion and exclusion criteria (Figure 1). A thorough search on Medline database was conducted for articles related to diagnosis of HBV infection. The search was based on the following keywords or medical subject heading terms in the database: (detection[All Fields] AND (“hepatitis B virus” [MeSH Terms] OR “hepatitis B virus”[All Fields]) AND (“infection”[MeSH Terms] OR “infection”[All Fields])) AND (“2005/04/02”[PDat] : “2015/03/30”[PDat]).

Inclusion and exclusion criteria
The inclusion criteria were (1) articles describing methods or techniques of diagnosis of HBV; (2) published in English language; and (3) published in last 10 years. Articles were excluded if (1) study not original (review or editorial or case report); (2) studies describing methods of detection of drug resistance or mutants; (3) studies describing non microbiological serum biomarkers for diagnosing hepatitis only; (4) studies describing diagnosis of patients coinfected with other viruses [hepatitis C virus (HCV), human immunodeficiency virus, etc.] or bacteria (Mycobacterium tuberculosis); and (5) full text or abstract not available in Medline.

RESULTS

Detection of HBV from samples other than serum or whole blood
HBV is most commonly detected in serum or whole blood. But we retrieved total 17 studies, which have been published in MEDLINE in last 10 years, discussing about detection of HBV from samples other than
serum or whole blood. Researchers have detected HBV from dried blood/plasma spots[13-16], hepatocytes[17-20], ovarian tissue[21], cerumen[22,23], saliva[24], parotid tissue[25], renal tissue[26], oocytes and embryos[27,28], cholangiocarcinoma tissue[29], etc. (Table 1).

Dried blood spots were first used in medical diagnostics by Guthrie et al[30] to detect Phenylketonuria. Dried blood spot (DBS) collection is much easier than taking venous blood. Moreover, different antibodies, medications, metabolites, and nucleic acids remain stable for a longer period in these samples[15]. As researchers have validated this sample in diagnosis of HBV, it has been used much conveniently in field settings or resource-poor settings. This review highlights that serological markers and nucleic acid of HBV can be detected from this sample by Point Of Care Tests (POCT), Enzyme Linked Immunosorbent Assay (ELISA) or Nucleic Acid Amplification Techniques with high sensitivity (Table 1). The combination of DBS and POCT is even more advantageous to use in resource-poor settings. Sn of detection of HBsAg from saliva was 74.29% in the study of Arora et al[24]. Presence of viral antigen in saliva makes dentistry personnel more vulnerable. Saliva can also be collected very easily without technical expertise and with the help of POCTs diagnosis can be made in resource-poor settings rapidly.

In certain cases of chronic infection with low level viremia or seronegative patients, HBV DNA has been detected from hepatocytes by polymerase chain reaction (PCR) - In situ hybridization, while couldn’t be detected from blood[18]. Other novel and highly sensitive techniques like flowcytometric quantitation, droplet digital PCR has increased the sensitivity of HBV detection from hepatocytes even more. This is especially important in diagnosing the etiology of chronic hepatitis/hepatocellular carcinoma in seronegative or low viremic patients. Again persistent detection of covalently closed circular DNA (cccDNA) helps to predict recurrence of the disease[19]. Detection of serological markers and HBV DNA from ovarian tissue, oocytes or embryo becomes important in case of in vitro fertilization[21,28]. Though in one study nucleic acid couldn’t be detected after culture and vitrification of oocytes or embryos from seropositive mothers during the procedure[27]. In the study of Kong et al[26], HBsAg and HBeAg were detected in frozen renal tissue by immunohistochemistry in 1.9% of seronegative patients with glomerulonephritis. As it is a common extrahepatic manifestation of viral hepatitis, in occult infections renal tissues can be used to detect the presence of virus.

Different methods of detection of HBV infection
The detection of HBV is very important in controlling its spread. After the discovery of Austria 125 various serological, molecular, and automated detection methods have been introduced and validated by different researchers. While searching Medline database in last 10 years total 55 studies were found describing different methods of detection.

Serological methods
Serological methods are most common, rapid and cost effective methods to detect different markers like HBsAg, anti-HBsAg, anti-HBeAg, HBeAg, anti-HBeAg, etc.

ELISA: ELISA is a type of solid phase immunoassay in which antigens or antibodies are covalently bound with suitable enzymes that can catalyze the conversion of a substrate into colored products. It is a validated method.
to detect different serological markers. Various ELISA kits are commercially available. Maity et al. (2012) evaluated 3 ELISA kits (Span diagnostics Ltd., J. Mitra and Co. Pvt. Ltd., and Transasia Biomedicals Ltd.) in 300 samples. All the kits were found to be good at screening having higher specificity. Positive predictive value (PPV) and negative predictive value (NPV) were 100% when panels were tested by kits of J. Mitra and Co. Pvt. Ltd. and Transasia Biomedicals Ltd, though little less in case of kit of Span Diagnostics Ltd. Though in most of the cases kits are evaluated against a pretested panel, when the results are projected to a population, PPVs and NPVs tend to change depending on the prevalence of the infection. In a study by Yazdani et al. (2010), used novel monoclonal antibodies as capture layer and a polyclonal biotinylated antibody as detector phase to develop one new ELISA system.

Sensitivity and specificity of the assay were 98.98% and 92.6%. Overall positivity rate was 54.5% to 58.3%.

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and 99.6%, respectively when compared to established commercial kit. The performance of ELISA depends on concentration of coating antibody, conjugates and sera. Using different concentrations by checkerboard titration method Fatema et al [33], found that, optimal concentration of coating antibody to be 0.25 ng/mL and 1 in 9 dilution of both conjugate and sera. Poly L lysine coated magnetic beads were used to concentrate the virus by Satoh et al [34]. HBsAg and Anti Hbc were tested by Enzyme Immuno Assay (AxSYM, Abbott), and haemaglutination inhibition test. By HBsAg EIA they were able to detect 27 out of 40 occult HBV infection. Antigen/ antibody quality is very important for diagnostic accuracy. Recombinant HBCAg is expressed in Escherichia coli and Pichia pastoris (P. pastoris) by Li et al [35], 2007 and used in ELISA for detection of anti HbcAg. P. pastoris derived antigen was more specific and sensitive in detection than the other counterpart.

Chemiluminescent enzyme immunoassay and its modifications: This rapid immunoassay method uses antigen or antibodies labeled with luminescent molecules. This is more sensitive than ELISA. In comparative studies with PCR the sensitivity of chemiluminescent enzyme immunoassay (CLEIA/ CLIA) is 96%[36]. Its sensitivity is even more enhanced by different modifications by researchers. Matsubara et al [37], 2009 developed a highly sensitive CLEIA method for quantitative detection of HBsAg by a combination of monoclonal antibodies each specific for epitopes of HBsAg. This method was 230 fold more sensitive than existing CLIA methods. Incorporating firefly luciferase as labelling enzyme a bioluminescent enzyme immunoassay was developed by Minekawa et al[38]. This became 50 fold more sensitive than conventional CLIAS. Liu et al[39], 2013 developed an amplified luminescent proximity homogeneous assay (AlphaLISA) for HBsAg. The detection sensitivity was as low as 0.01 IU/mL, when compared with the commercial light-initiated chemiluminescence assay. The correlation coefficient of this assay was 0.921.

Automated systems: AxSYM (Abbott) is the first automated third generation immunoassay system. Abbott PRISM HBsAg assay is an in vitro chemiluminescent immunoassay. A new prototype assay based on magnetic micro particle was developed in this system to increase its sensitivity and ability to detect mutants. Lou et al[40] demonstrated that it can detect more commercially available seroconversion panel members (185 of 384) than PRISM (181). Researchers have evaluated different automated CLIA systems across the world. Elecsys (Roche) and Architect (Abbott) gave comparable results for quantitation of HBsAg when assessed by Gupta et al [41]. Beckman Coulter’s anti-HBs chemiluminescence immunoassay (Access AbHBSII) was evaluated in 1207 routine samples prescreened with AxSYM (Abbott) for detection of anti HBsAg by Motte et al [42]. Sn, Sp, PPV and NPV were 97.8%, 98.1%, 96%, and 99%, respectively. ADVIA centaur CP Immunoassay System is based on chemiluminescent with advanced acridinium ester technology. van Helden et al [43] compared its performance with AxSYM, Abbott. Its Sn and Sp was 100% and 99.5%. The automated chemiluminescent micro particle immunoassay of Abbott (Architect) detects anti-Hbc. Borderline reactivity in this system was reassessed by 2 other tests: Microparticle enzyme immunoassay (MEIA, AxSYM, Abbott), and enzyme linked fluorescent assay (ELFA, VIDAS Anti-Hbc Total II, bioMérieux) by Ollier et al [44]. 42.99% of borderline reactive samples were found to be positive by MEIA, ELFA. So, other confirmatory tests should be done in this scenario. This commonly used Abbott’s Architect system was also compared with another fully automated and closed DiaSorinLIAISON(TM)XL by Krawczyk et al [45] and Kinn et al [46]. The two tests were in > 95% agreement in both the studies. In a multicentre study, automated VIDAS HBsAg Ultra [long (L) and short (S)] incubation protocol (Biomérieux) was compared to AxSYM (Abbott) by Weber et al [47]. Sn of the VIDAS HBsAg Ultra (L), (S) and the AxSYM HBsAg v2 were 99.07%, 97.87% and 94.14% respectively. Sp was 100% for VIDAS. The mean time of the diagnostic window was shortened with the VIDAS HBsAg Ultra (L) and (S) when compared with the AxSYM HBsAg v2 by 1.06 and 0.66 d, respectively. Sn for the VIDAS HBsAg Ultra (L), (S) and AxSYM HBsAg v2 were 99.07%, 97.87% and 94.14%. The Sp were 100% (VIDAS HBsAg Ultra L and S) and 99.6% (AxSYM HBsAg v2) [47].

Other methods: A biosensor based imaging ellipsometry was developed and validated for 169 patients by Qi et al [48]. They concluded that this method could detect 5 markers within 1 h with acceptable agreement when compared to ELISA. Another novel assay based on magnetic beads and time resolved fluoroimmunoassay (TR FIA) was developed by Ren et al [49]. 2014. The detection antibodies were europium labeled and capturing monoclonal antibodies were immobilized on magnetic beads. The test results had correlation with CLIA (Y = 1.182X - 0.017, R = 0.989). The same TRFIA method was also used to detect HBV Pre S1 antigen by Hu et al [50] and HBsAg by Myyryläinen et al [51]. Burbelo et al [52] used Luciferase Immunoprecipitation system to detect HBV infection. This could correctly predict the HBV status in all but 2 of 99 assays. Fletcher et al [53] standardised an in house neutralization test for confirmation of HBsAg. Six hundred and fifteen HBsAg samples were subjected to the test. 100% of high reactive samples and 93% of low reactive samples were neutralized by this method, whereas 100% of grey zone reactive samples were negative.

POCT: POCTs are developed to make diagnosis more rapid and accessible to patients. Njai et al [54] validated 3 POCTs (Determine, Vikia and Espline) for detecting HBsAg in field or laboratory setting in Gambia, Western Africa. All the 3 tests gave acceptable result when
Table 2  Studies describing different quantitative molecular methods

| Ref. | Year of publication | Method of quantitation | Detection limit |
|------|---------------------|------------------------|----------------|
| Carson et al[32] | 2005 | FRET based real time PCR assay | Sn at 95% detection level was 24.2 IU/mL |
| Welzel et al[33] | 2006 | Novel real time PCR | Sn at 95% detection level was 56 IU/mL |
| Mazet-Wagner et al[34] | 2006 | Real time PCR assay to detect total HBV DNA and cccDNA from serum and peripheral blood mononuclear cells | 27 IU/mL |
| McCormick et al[35] | 2006 | Procleix Ulitro Assay (Multiplex PCR) to detect HIV 1, HCV RNA and HBV DNA simultaneously | HCV DNA of 15 IU/mL |
| Cai et al[36] | 2008 | Real time fluorescent Loop Mediated Isothermal Amplification (RF-LAMP) | At 95% detection level 210 copies/mL |
| Paraskevis et al[37] | 2010 | New ultrasensitive in house real time PCR assay | Sn at 95% and 50% detection level: 22.2 IU/mL and 8.4 IU/mL |
| Chevaliez et al[38] | 2010 | v2.0 of the CAP/CTM assay | Highly sensitive, could even detect 6.25 IU/mL HBV DNA; Sp is 99%. Intra-assay and interassay coefficients of variation ranged from 0.21% to 2.67% and from 0.65% to 2.25%, respectively |
| Sun et al[39] | 2011 | Duplex real-time PCR assay using two sets of primers/probes and a specific armored DNA as internal control | Detection limit 29.5 IU/mL; Sp 100% |
| Cha et al[40] | 2013 | ExiStation HBV diagnostic system | Broad range of linearity and high sensitivity |
| Yang et al[41] | 2014 | Colorimetric PCR with DNAzyme containing probe | qPCR1: 104 IU/mL; qPCR2: 91 IU/mL |
| Kania et al[42] | 2014 | 2 in house real time PCR targeting X (qPCR1) or S (qPCR2) genes | |

FRET: Fluorescence resonant energy transfer; PCR: Polymerase chain reaction; cccDNA: Covalently closed circular DNA; Sn: Sensitivity; Sp: Specificity; HBV DNA: Hepatitis B virus deoxy ribonucleic acid; v2.0: Version 2.0; HCV: Hepatitis C virus; CAP/CTM: CobasAmpliPrep/CobasTaqMan.

Comparison of different methods

Liu et al[33] compared test results of 4 different types of serological tests in 116455 samples. Chemiluminescent microparticle immunoassay (CMIA), electrochemiluminescent immunoassay (ECLIA), ELISA and golden immunochromato-graphic assay (GICA) were used to test the HBsAg level. For qualitative results GICA was significantly less specific than the other 3 tests. Compared to CMIA the false negativity rate of ECLIA, ELISA and GICA were 0.2%, 1.3%, 12.3%.

Molecular methods: Molecular methods used in diagnosis can be categorized as nucleic acid hybridization, nucleic acid amplification, sequencing and enzymatic digestion of nucleic acids.

Hybridization technique: Conventional hybridization technique, though highly specific, lacks sensitivity. Yao et al[43] constructed a peptide nucleic acid probe which combined with target DNA sequences more efficiently than DNA probes. The detection limit was 8.6 pg/L and Sp was 94.4%.

Nucleic acid amplification technique: Amplification techniques can be: (1) target amplification: PCR, nucleic acid sequence based amplification, transcription mediated amplification, Strand Displacement amplification, etc.; (2) Signal amplification: Branched DNA probe (bDNA); and (3) probe amplification: Ligase chain reaction. These techniques can qualitatively or quantitatively detect minute amount of HBV DNA present in the sample. Some researchers have even combined 2 different methods to increase Sn. Combination of bDNA and HBV PCR helped in detection of HBcAg positive chronic HBV patients by Ozdarendeli et al[44].

Quantitative detection is very important for monitoring of HBV infection. Molecular methods have been used for quantitation by different researchers (Table 2). In this review, of the entire in house and automated molecular techniques version 2.0 (v2.0) of the CobasAmpliPrep/CobasTaqMan (CAP/CTM) assay was found to be most sensitive, with a lower detection limit of only 6.25 IU/mL[45]. Commercial assays were more sensitive than in house assays.

Park et al[46] evaluated MagicplexTM HepaTrio Real-time Detection test, a multiplex PCR assay for the detection of hepatitis A virus, HBV and HCV. Sn and Sp was 93.8% and 98.2%. Morjezi et al[47] developed a Taq Man real time detection assay based on the concept of phage display mediated immune PCR for the detection of HbcAg. This method was able to detect about 10 ng of HbcAg.

A rapid real time micro scale chip based PCR system consisting of 6 individual thermal cycling modules was developed by Cho et al[48]. It took less than 20 min to complete 40 thermal cycles. They conducted large clinical evaluation study to detect HBV infection. The sn and sp was 94% and 93% respectively.

The persistence of HBV can be detected by demonstration of covalently cccDNA. Takkenberg et al[49] compared to AxSYM HBsAg ELISA as reference test. Rapid kits (1. Mitra and Co. Pvt. Ltd., Span diagnostic Ltd., Standard Diag. Inc.) were also evaluated by Maity et al[50]. Sn, Sp, PPV and NPV of all the kits were 100%.
developed a sensitive, specific and reproducible Real Time PCR to detect and quantitate cccDNA in chronic HBV patients. The lower limit of detection was 15 copies/PCR. cccDNA is detected by Southern blot analysis in cell cultures by Cai et al(23), Guo et al(24) developed magnetic capture hybridization and quantitative PCR assay to detect cccDNA with a detection limit of 90 IU/mL.

Studies have been conducted to compare different methods (Table 3). Abbott's real time PCR kit was most sensitive with lower limit of detection of only 1.48 IU/mL. In comparison most of the automated systems had good agreement.

**DISCUSSION**

HBV can be detected reliably from DBS (Sn > 90% in all cases). In certain cases of occult infections or seronegative patients, HBV have been detected from hepatocytes or renal tissues also. Serological and Molecular assays are predominant and reliable methods for HBV detection. ClIA is more sensitive than ELISA. Rapid tests are also dependable and useful for screening purpose, especially in resource poor settings. Quantitation is important for monitoring. Real time PCR, bDNA assays are principal methods used for this purpose. Automated systems are more sensitive when compared to in house assays. Among the molecular techniques v2.0 of the CAP/CTM assay and Abbott real time PCR were found to be most sensitive with a lower detection limit of only 6.25 IU/mL and 1.48 IU/mL respectively.

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**COMMENTS**

**Background**

In earlier days infection with hepatitis B virus (HBV) was detected by demonstration of antibody titer by Complement Fixation Test. The first solid phase sandwich radio immunoassay named Ausria 125 was developed by Ling et al at Abbott Laboratories (North Chicago). This highly sensitive detection method became a major discovery in the diagnosis of viral transfusion hepatitis and screening of blood donors. Since then innumerable serological and molecular methods have been developed for diagnosing HBV.

**Research frontiers**

This article provides an overview of detection of HBV infection employing different techniques.

**Innovations and breakthroughs**

Beside serum/plasma, HBV can be detected reliably from dried blood spots (DBS) (Sn > 90% in all cases). In occult infections or seronegative patients, HBV was detected from hepatocytes or renal tissues. Serological and Molecular assays are predominant and reliable methods. Chemiluminescent immunoassay is more sensitive than enzyme Linked Immunosorbent Assay. Rapid tests are useful for screening. Real time polymerase chain reaction (PCR), branched DNA assays are principal methods for quantitation. Automated systems are more sensitive compared to in house assays. CobasAmpliprep/CobasTaqMan version 2.0 assay and Abbott real time PCR were found to be most sensitive with a lower detection limit of only 6.25 IU/mL and 1.48 IU/mL respectively. Rapid tests are also highly sensitive and specific as evaluated by different researchers.

**Applications**

Use of DBS and validated rapid tests can aid in initial diagnosis in resource poor settings. Quantitation is important for monitoring and prognostic evaluation and automated systems are highly sensitive and efficient for this purpose.

**Peer-review**

The authors have performed a good study, the manuscript is interesting.
Detection of hepatitis B virus markers using a biosensor based on magnetic beads for the detection of hepatitis B virus surface antigen in human serum. 

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