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**Detection of hepatitis B virus infection: A systematic review**

Ghosh M *et al*. Detection of HBV

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**Abstract**

**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 (v2.0) 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:** Hepatitis B virus; Serology; Chemiluminescent immunoassay; Molecular assay; Automated detection

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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), b DNA 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.

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**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[2]. In 1940’s several experiments in human volunteers by Cameron (1943)[3], Mac Callam (1944)[2,3], Paul, Havens, Sabin and Philip (1945)[3,4] confirmed the viral etiology of hepatitis. 2 distinct clinicoepidemiological forms of viral hepatitis: serum hepatitis and infectious hepatitis was evidenced by the study of Krugman S. 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[5]. In 1968 Alfred Prince also described a serum antigen (SH Ag) in the serum of post transfusion patients[6]. These Au Antigen and SH Antigen were soon found to be identical[1]. In the year 1970, David S. Dane discovered 42 nm sized virus like particles while observing Au Ag immune complexes under Electron Microscope (EM)[7]. 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 HBeAg was identified by Magnius *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 (HCV, HIV, *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 and Susi to detect Phenylketonuria[30]. DBS collection is much easier than taking venous blood. More over 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 (NAAT) 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 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 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 an HBcAg was 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 hepatitis B virus infection***

The detection of HBV is very important in controlling its spread. After the discovery of Ausria 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 HBcAg, 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*[31], 2012 evaluated 3 ELISA kits (Span diagnostics Ltd., J. Mitra& 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& 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, PPV and NPVs depend widely on the prevalence of that infection. Different researchers have modified this method even. Yazdani *et al*[32], 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 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 diution 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 *E. Coli* and *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 immune assay 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 immune assay (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 eachspecific for epitopes of HBsAg. This method was 230 fold higher 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 0.01 IU/mL, when compared with the commercial light-initiated chemiluminescence assay. The correlation coefficient of this assay was as 0.921.

**Automated systems:**AxSYM (Abbott) is the first automated third generation immunoassaysystem. Abbott PRISM HBsAg assay is an *in vitro* chemiluminescent immunoassay. A new prototype assay based on magnetic micro particle was developed in this sysyem 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)andArchitect (Abbott) gave comparable results for quantitation of HBsAg when assessed by Gupta *et al*[41]. BeckmanCoulter’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, positive predictive value (PPV) and negative predictive value (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. It’s Sn and Sp was 100% and 99.5%. The automated chemiliminescent 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 & closed DiaSorinLIAISON(®)XL by [Krawczyk](http://www.ncbi.nlm.nih.gov/pubmed/?term=Krawczyk%20A%5BAuthor%5D&cauthor=true&cauthor_uid=24268764)  *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 days, respectively. Sn for the VIDAS HBsAg Ultra (L), (S) and AxSYM HBsAg v2 were 99.07%, 97.87% and 94.14%. The Sp were100% (VIDAS HBsAg Ultra L and S) and 99.6% (AxSYM HBsAg v2)[47].

**Other methods:** A biosensor based imaging ellipsometrywas developed and validated for 169 patients by Qi *et al*[48]. They concluded that this method could detect 5 markers within 1 hour with acceptable agreement when compared to ELISA. Another novel assay based on magnetic beads and time resolved fluroimmunoassay (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](http://www.ncbi.nlm.nih.gov/pubmed/?term=Myyryl%C3%A4inen%20T%5BAuthor%5D&cauthor=true&cauthor_uid=21108849) *et al*[51]. Burbelo *et al*[52] used Luciferase Immmunoprecipitation system (LIPS) 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. 615 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:** POCTare 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 compared to AxSYM HBsAg ELISA as reference test. Rapid kits (J. Mitra& Co. Pvt. Ltd., Span diagnostic Ltd., Standard Diag. Inc.) were also evaluated by Maity *et al*[31], 2012. Sn, Sp, PPV and NPV of all the kits were 100%.

***Comparison of different methods***

Liu *et al*[55] compared test results of 4 different types of serological tests in 116455 samples. Chemiluminescentmicroparticle 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 toCMIA 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, it lacks sensitivity. Yao *et al*[56] constructed a peptide nucleic acid (PNA) 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 technique scan be: (1)target amplification: polymerase chain reaction (PCR), nucleic acid sequence based amplification (NASBA), transcription mediated amplification (TMA), Strand Displacement amplification (SDA), *etc.*; (2) Signal amplification: branched DNA probe (bDNA); and (3) probe amplification: ligase chain reaction (LCR). 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 HBe Ag positive chronic HBV patients by [Ozdarendeli](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ozdarendeli%20A%5BAuthor%5D&cauthor=true&cauthor_uid=16506386)  *et al*[57].

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[63]. Commercial assays were more sensitive than in house assays.

Park *et al*[69] evaluated Magicplex™ HepaTrio Real-time Detection test, a multiplex PCR assay for the detection of HAV, HBV and HCV. Sn and Sp was 93.8% and 98.2%. Mojezi *et al*[70] 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*[71]. 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 closed circular DNA (cccDNA). Takkenberg *et al*[72] 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*[73]. Guo *et al*[74] 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, b DNA assays are principal methods used for this purpose. Automated systems are more sensitive when compared to in house assays. Among the molecular techniques version 2.0 (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 PCR, b DNA assays are principal methods for quantitation. Automated systems are more sensitive compared to in house assays. CobasAmpliprep/CobasTaqMan version 2.0 (v2.0) 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.

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**P-Reviewer:** Changotra H **S-Editor:** Tian YL

**L-Editor: E-Editor:**

**Figure 1 Flow Chart for selection of relevant articles**

Total 770 studies were identified in MEDLINE as per search strategy

247 studies were found to be relevant as per title and abstract

Studies excluded (n=175):

Reasons:

Studies not original (n1 = 33)

Studies detecting drug resistance or mutants only (n2 = 66)

Studies describing only non microbiological methods for detection of hepatitis (n3 = 10)

Studies describing diagnosis of HBV patients co infected with other pathogen (n4 = 30)

Full text or abstract not available (n5 = 2)

Studies describing non human experiments (n6 = 9)

Duplicate study (n7 = 5)

Article not in English (n8 =20)

**Studies included in review (n = 72)**

**Table 1 Studies describing detection of HBV from samples other than serum or whole blood**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Year of publication** | **Sample used** | **Method used** | **Comments** |
| Mendy *et al*[13] | 2005 | Dried blood spots along with serum | HBsAg detected by Determine (TM) HBsAg | Comparison of DBS results with serum testing results: Sensitivity (Sn) 96%, Specificity (Sp) 100% |
| Chen *et al*[21] | 2005 | Ovarian tissue | HBsAg and HBcAg detected by immunocytochemistry and HBV DNA by PCR | Positivity rate of HBV DNA was 58.3%. |
| [van der Laan](http://www.ncbi.nlm.nih.gov/pubmed/?term=van%20der%20Laan%20LJ%5BAuthor%5D&cauthor=true&cauthor_uid=17328969)  *et al*[17] | 2007 | hepatocytes | Flowcytometric quantitation | A significant correlation was found between the percentage of infected hepatocytes and the intracellular expression level of HBsAg (R = 0.841, *P* < 0.001). |
| Goh *et al*[22] | 2008 | Cerumen and otorrhoea samples along with serum | HBsAg and HBeAg were detected by Enzyme Immunoassay and HBV DNA was detected by quantitative Polymerase Chain Reaction (PCR). | HBV DNA was detected in 66.7% of cerumen samples and 100% of otorrhoea samples |
| Chen *et al*[25] | 2009 | Parotid tissue | Serological markers by immunocytrochemistry and HBV DNA by PCR | Overall positivity rate was 54.5% to 58.3%. |
| Nuriya *et al*[18] | 2010 | Hepatocyres | PCR - *In situ* Hybridisation | All hepatocytes were infected with HBV in chronic liver disease. |
| Villar *et al*[14] | 2011 | Dried blood spots | HBsAg, anti-HBc, and anti-HBs were detected by ELISA | Sn was 90.5%, 97.6%, and 78% for anti-HBc, HBsAg, anti-HBs assays, and Sp was 92.6%, 96.7%, and 97.3% for anti-HBc, HBsAg, and anti-HBs assays, respectively. |
| Wu *et al*[29] | 2012 | Paraffin embedded intrahepatic and extrahepaticcholangiocarcinoma tissue | HBV DNA by nested PCR and HBV related antigens by immunohistochemistry method. | HBV DNA and HBV antigens were detected significantly in cases of intrahepatic cholangiocarcinoma. |
| Arora *et al*[24] | 2012 | saliva | HBsAg was detected by ELISA | Sn 74.29% and Sp 100% |
| Cobo *et al*[27] | 2012 | Spent culture media and liquid nitrogen samples of oocytes and embryos | Reverse transcriptase PCR | Viral sequences were not detected in these samples from seropositive patients. |
| Ye *et al*[28] | 2013 | Discarded test tube embryos from mothers with chronic HBV infection undergoing Invitro fertilization treatment | Single cell Reverse transcriptase PCR | Detection rate was 13.2% |
| [Eftekharian](http://www.ncbi.nlm.nih.gov/pubmed/?term=Eftekharian%20A%5BAuthor%5D&cauthor=true&cauthor_uid=24131958)  *et al*[23] | 2013 | Cerumen along with serum | HBV DNA was detected by PCR | HBV DNA was detected in 6.6% of HBsAg positive patients. |
| [Kong](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kong%20D%5BAuthor%5D&cauthor=true&cauthor_uid=23474175)  *et al*[26] | 2013 | Frozen renal tissue | HBsAg and HBcAg detected by immunohistochemistry | Found positive in 9 out of 500 patients of glomerulonephritis without serological evidence. |
| Ross *et al*[15] | 2013 | Dried blood spots | HBsAG, Anti HBcAg, Anti HBsAg detected by Abbott Architect and HBV DNA by artus HBV LC PCR | Sensitivity was 98.6%, 97.1%, 97.5%, 93% |
| [Alidjinou](http://www.ncbi.nlm.nih.gov/pubmed/?term=Alidjinou%20EK%5BAuthor%5D&cauthor=true&cauthor_uid=24342801) *et al*[16] | 2014 | Dried plasma spots | HBsAg and HBV DNA detected by ELISA and PCR. | Sn and Sp 100% for serological markers and Sn 96%, Sp 100% for HBV DNA. |
| Zhong *et al*[19] | 2014 | Hepatocytes | Covalently closed circular HBV DNA detected by *in situ* PCR. | Helps to detect recurrence of HBV. |
| Huang *et al*[20] | 2015 | Formalin fixed paraffin embedded hepatocellular carcinoma tissue | Droplet digital PCR to detect HBV copy number | Highly sensitive method. |

HBsAg: Hepatitis B surface antigen; DBS: Dried blood spots; Sn: Sensitivity; Sp: Specificity; HBcAg: Hepatitis B core antigen; HBV DNA: Hepatitis B virus deoxy ribonucleic acid; PCR: Polymerase chain reaction; ELISA: Enzyme Linked immunosorbent assay; Anti HBc: Antibody to HBcAg; Anti HBs: Antibody to HBsAg.

**Table 2 Studies describing different quantitative molecular methods**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Year of Publication** | **Method of quantitation** | **Detection limit** |
| Garson *et al*[58] | 2005 | FRET based real time PCR assay | Sn at 95% detection level was 24.2 IU/mL |
| Welzel *et al*[59] | 2006 | Novel real time PCR | Sn at 95% detection level was 56 IU/mL |
| Mazet Wagner *et al*[60] | 2006 | Real time PCR assay to detect total HBV DNA and cccDNA from serum and peripheral blood mononuclear cells | 27 IU/mL |
| [McCormick](http://www.ncbi.nlm.nih.gov/pubmed/?term=McCormick%20MK%5BAuthor%5D&cauthor=true&cauthor_uid=16427802) *et al*[61] | 2006 | Procleix Ultrio Assay (Multiplex PCR) to detect Human Immunodeficiency Virus (HIV 1), Hepatitis C Virus (HCV RNA) and HBV DNA simultaneously | Sp ≥ 99.5%, Sn is > 95% with detection limit for HBV DNA of 15 IU/mL |
| Cai *et al*[62] | 2008 | Real time fluorogenic Loop Mediated Isothermal Amplification (RtF-LAMP) | At 95% detection level 210 copies/mL |
| Paraskevis *et al*[63] | 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](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chevaliez%20S%5BAuthor%5D&cauthor=true&cauthor_uid=20720031) *et al*[64] | 2010 | version 2.0 (v2.0) of the CobasAmpliPrep/CobasTaqMan (CAP/CTM) assay | Highly Sn, 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*[65] | 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*[66] | 2013 | ExiStation HBV diagnostic system | 9.55 IU/mL |
| Yang *et al*[67] | 2014 | Colorimetric PCR with DNA zyme containing probe | Broad range of lineariy and high sensitivity |
| Kania *et al*[68] | 2014 | 2 in house real time PCR targeting X (qPCR1) or S (qPCR2) genes. | qPCR1: 104IU/mL  qPCR2: 91IU/mL. |

FRET: Fluoresence resonant energy transfer; PCR: Polymerase chain reaction; cccDNA: Covalently closed circular DNA; Sn: Sensitivity; Sp: Specificity; HBV DNA: Hepatitis B virus deoxy ribonucleic acid.

**Table 3 Comparison of different methods**

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| --- | --- | --- | --- |
| **Ref.** | **Year of Publication** | **Comparison between** | **Remarks** |
| Hochberger *et*  *al*[75] | 2006 | Automated COBAS AmpliPrep/COBAS TaqMan system (real time PCR) and Versant HBV 3.0 | Good correlation between two. |
| Juman *et al*[76] | 2008 | Versant HBV 3.0 (Bayer, branched DNA mediated assay) and Biotitre B (real time PCR variant) | Both were highly specific, though reproducibility of Versant HBV 3.0 was higher. |
| Yang *et al*[77] | 2009 | Real Art HBV PCR Kit (Abbott, real time PCR) and VERSANT bDNA 3.0 | Abbott’s kit was more sn, detection limit 27 IU/mL |
| [Louisirirotchanakul](http://www.ncbi.nlm.nih.gov/pubmed/?term=Louisirirotchanakul%20S%5BAuthor%5D&cauthor=true&cauthor_uid=20336717) *et al*[78] | 2010 | fully automated ElecsysHBsAg II assay, Architect, AxSYM and Advia Centaur HBsAg assays | The later 2 tests appeared less sensitive in detecting early HBV infection |
| Berger *et al*[79] | 2010 | CobasAmpliPrep/CobasTaqMan (CAP/CTM) assay version 2.0 and version 1.0 | Comparable results for all 278 tested samples |
| [Lunel-Fabiani](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lunel-Fabiani%20F%5BAuthor%5D&cauthor=true&cauthor_uid=20857892) *et al*[80] | 2010 | Access immunoassay system from Beckman coulter with Abbott AxSYM and PRISM HBsAg assays. VIDAS was used to conclude discrepant results. | Sn: 100%, Sp: 99.96% |
| Caliendo *et al*[81] | 2011 | Abbott RealTime HBV IUO, the Roche CobasAmpliPrep/CobasTaqMan HBV test, the Roche CobasTaqMan HBV test with HighPure system, and the Qiagen artus HBV TM ASR. | Limit of deection of artus 1.5 log(10) IU/mL, of other 3 tests 1.0 log(10) IU/mL. |
| Ismail *et al*[82] | 2011 | Abbott HBV real-time PCR (Abbott PCR), artus HBV real-time PCR with QIAamp DNA blood kit purification (artus-DB), and artus HBV real-time PCR with the QIAamp DSP virus kit purification (artus-DSP). | Lower limit of detection against WHO standards were 1.43, 82 and 9 IU/mL respectively |
| Yeh *et al*[83] | 2014 | Abbott real time HBV (RealTime assay) and CobasAmpliPrep/CobasTaqMan HBV assays 2.0 (TaqMan assay). | Real time assay’s Sn: 98.2%, Sp: 100%. Good level of agreement between the two. |

PCR: Polymerase chain reaction; Sn: Sensitivity; Sp: Specificity; WHO: World Health Organization.