Truenat®

HBV

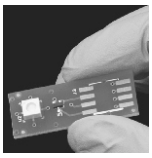
Chip-based Real Time PCR Test for Hepatitis B Virus

1. INTENDED USE

Truenat® HBV (REF 601090005 / 601090020 / 601090025 / 601090050 / 601090100 / 601090200) is an automated point-of-care or near patient chip-based Real Time Polymerase Chain Reaction (PCR) test for the quantitative estimation of the Hepatitis B Virus (HBV) in human blood/serum/plasma specimen and aids in the diagnosis of infection with Hepatitis B Virus and in the estimation of viral load. **Truenat® HBV** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® HBV** is a single-use *in vitro* diagnostics test meant for professional use in near-patient, laboratory or any healthcare settings, by healthcare professionals or any user appropriately trained by a representative of Molbio Diagnostics.

2. INTRODUCTION

Hepatitis B infection is caused by the Hepatitis B Virus (HBV) that affects around two billion people globally and causes around 6,00,000 deaths each year. The infection, which can be acute or chronic, occurs in the liver. Acute infections are mostly asymptomatic but sometimes may cause symptoms such as jaundice, extreme fatigue, vomiting and abdominal pain. A chronic infection can develop into cirrhosis of the liver and liver cancer. Transmission occurs from contact with infected blood or other body fluids through perinatal, transfusion, injection and sexual routes and through close contact with infected family members, especially in early childhood. Despite the availability of effective vaccination and antiviral drugs, Hepatitis B remains a major global health problem. The most common methods of diagnosing Hepatitis B are serology based assays and molecular tests. The HBV DNA is detectable about three weeks before the appearance of serological markers. HBV viral load quantitation by PCR is very useful for treatment initiation decisions and treatment monitoring. However PCR or Real Time PCR tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days. The **Truenat®** point-of-care real time PCR system enables decentralization and near patient diagnosis and treatment monitoring of Hepatitis B infection by making real time PCR technology rapid, simple, robust and user friendly, thereby offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated **Truelab®** Real Time Quantitative micro PCR Analyzer and **Trueprep® AUTO/AUTO v2** Universal Cartridge based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and **Trueprep® AUTO/AUTO v2** Universal Cartridge based Sample Prep kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site. **Truenat® HBV** is a disposable, room temperature stable, chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for quantitative estimation of the Hepatitis B Virus and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. All components of **Truenat®** pouch are nuclease-free. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information including standard values for quantitation. It also stores information of used test to prevent any accidental re-use of the test.



NOTE: Truelab® / Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® HBV works on the principle of Real Time Polymerase Chain Reaction (real time PCR) based on Taqman chemistry. The patient sample (blood/serum/plasma)is first pre-treated using the **Trueprep® AUTO** Universal Sample Pre-treatment Pack. The DNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The cartridge from the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit contains pre-loaded Internal Positive Control (IPC), it is a synthetic plasmid construct provided in a stable formulation preloaded into Cartridges which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. The DNA extract is analyzed using the **Truenat® HBV** Chip-based Real Time Polymerase Chain Reaction (PCR) test and the **Truelab®** Real Time Quantitative micro PCR Analyzer. **Truenat® HBV** chip is placed on the chip tray of the **Truelab®** Real Time Quantitative micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided calibrated micropipette and tip into the microtube containing freeze dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® HBV** chip and the test is inserted in the **Truelab®** Real Time Quantitative micro PCR Analyzer. A positive amplification causes the dual labelled fluorescent probe in the **Truenat® HBV** chip to release the fluorophore in an exponential manner which is then captured by the builtin opto-electronic sensor and displayed as amplification curve on the analyser screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e., exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, HBV "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and International Units (IU) per milliliter (IU/ml) is also displayed on the screen. Based on the detection of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed using the **Truelab®** micro PCR printer or transferred to the lab computer/or any remote computer via WIFI network or 4G/3G/GPRS network. Up to 20,000 test results in **Truelab® Uno Dx/Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this kit is part of the core/pre-core region of HBV genome. The region selected is specific to HBV and conserved across the HBV Genotypes.

5. CONTENTS OF THE Truenat® HBV KIT

A. Individually sealed pouches, each containing
1. **Truenat® HBV** micro PCR chip. (1 No.)
2. Microtube with freeze dried PCR reagents. (1 No.)
3. DNase and RNase free pipette tip. (1 No.)
4. Desiccant pouch. (1 No.)

B. Package Insert. (1 No.)

Pack sizes of **Truenat® HBV KIT**

REF	601090005	601090020	601090025	601090050	601090100	601090200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep® AUTO Universal Sample Pre-treatment Pack

A. Lysis buffer.
B. Disposable transfer pipette (graduated).
C. Package Insert.

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
Σ	5T	20T	25T	50T	100T	200T

7. CONTENTS OF THE Trueprep® AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit

A. The reagent pack contains the following reagents

No.	Contents	Purpose
1.	Wash Buffer A	To wash inhibitors from the sample
2.	Wash Buffer B	To wash inhibitors from the sample
3.	Elution Buffer	To elute nucleic acids
4.	Priming Waste	To purge residual liquid from tubing

B. The cartridge pack contains the following:

No.	Contents	Purpose
1.	Cartridge	Cartridges containing immobilized internal control (IPC) for extraction
2.	Elute collection tube (ECT)	Capped tubes for collection and storage of extracted nucleic acids
3.	Elute collection tube (ECT) label	To label Elute Collection Tube (ECT)
4.	Disposable transfer pipette	To pierce the seal of elute chamber and to transfer extracted nucleic acids from elute chamber of cartridge into the Elute Collection Tube (ECT)

C. Disposable transfer pipettes (graduated) - 3 mL
D. Reagent reset card-1 No.
E. Package insert

Pack sizes of **Trueprep® AUTO Universal Cartridge Based Sample Prep Kit**

REF	60203AR05	60203AR25	60203AR50	60203AR100	60203AR200
Σ	5T	25T	50T	100T	200T

Pack sizes of **Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit**

REF	60207AR05	60207AR25	60207AR50	60207AR100	60207AR200
Σ	5T	25T	50T	100T	200T

8. STORAGE HANDLING AND STABILITY

1. **Truenat® HBV** test is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.
2. **Trueprep® AUTO** Universal Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures upto 45°C. Do not freeze.
3. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit is stable for two (2) years from the date of manufacture if stored between 2°C to 40°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to sunlight.
4. Do not open the pouch until ready to test. ⚠ Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
5. Do not use the pouch if torn.
6. Do not use pouches that have passed the expiration date.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

REF **Truelab®** Real Time micro PCR Workstation (REF623010001/633010001/643010001/653010001) consisting of,

1. **Trueprep® AUTO/AUTO v2** Sample Prep Device (REF603041001/603042001)
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (603021001/603022001/603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100 / 60203AR200) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100 / 60207AR200), **Truenat®** Positive Control Kit - Panel II (REF 801020008), Powder free disposable gloves, waste disposal container with lid.

10. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Truenat® HBV requires purified nucleic acids from whole blood/plasma specimens collected in EDTA anticoagulant or serum specimen that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge based Sample Prep kit. Sample must be pre-treated using **Trueprep® AUTO** Universal Sample Pre-treatment pack. Transfer 250 µl of whole blood or 500 µl of plasma/serum specimen using the transfer pipette provided into the Lysis buffer tube provided and mix well. Use the entire content of lysis buffer tube containing the specimen for further procedure with the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep device and the package insert of Sample Prep kit for details).

Nucleic acid extraction: Use entire content from the Lysis Buffer tube containing specimen for further procedure with the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep device and the package insert of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep kit for details). ⚠ Dispose off lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 18).

11. SAFETY PRECAUTIONS

IVD 1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (2-30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the user manuals, package inserts and Material Safety Data Sheets (MSDS) of all the components of the **Truenat®** point-of-care real time PCR system before use.
5. Good laboratory practices are recommended to avoid contamination of specimens or reagents.
6. All materials of human origin should be handled as though potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
9. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.
10. Do not substitute assay components / reagents with any other components / reagents.
11. Each single-use **Truenat®** chip is used to process one test. Do not reuse chip.

12. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g., from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the **Truenat® HBV** micro PCR chip, microtube and the DNase and RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.
4. Ensure that the colour of the desiccant pouch is orange after opening a sealed **Truenat®** chip pouch. If the colour of the desiccant pouch changes from orange to white due to the absorption of moisture, do not use the contents of the **Truenat®** chip pouch.

13. PROCEDURAL LIMITATIONS

1. A specimen with a result of "Not detected" cannot be presumed to be negative for HBV DNA.
2. A negative result does not exclude the possibility of Hepatitis B infection because very low levels of infection or sampling error may cause a false-negative result.
3. Though rare, mutations within the highly conserved regions of the genomic DNA of HBV not covered by the **Truenat® HBV** Test's primers and/or probes may result in failure to detect the presence of the viral DNA.
4. As with any diagnostic test, results from the **Truenat® HBV** assay should be interpreted in conjunction with other clinical and laboratory findings.

14. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite [10 times dilution of 5% sodium hypochlorite (household bleach) before continuing work].
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially bio- hazardous waste e.g. in a biohazard waste container.

15. TEST PROCEDURE

(Please also refer the **Truelab®** Real Time Quantitative micro PCR Analyzer user manual)

1. Switch on the **Truelab®** Analyzer.
2. Select User and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "HBV" to be run from the Profiles Screen on the Analyzer screen. For **Truelab® Duo/Quattro**, select the Bay (Idle1/2) for Duo and (Idle1/2/3/4) for Quattro from the Status Screen to view the Profiles Screen. Select the test profile for "HBV" to be run from the Profiles Screen on the Analyzer screen.
4. Enter the patient details as prompted in the **Truelab®** Analyzer screen.
5. Press Start Test.
6. For **Truelab® Uno Dx**, Press the eject button to open the chip tray. For **Truelab® Duo/Quattro**, the chip tray opens automatically on tapping the "Start Test" button.
7. Open a pouch of **Truenat® HBV** and retrieve the micro PCR chip, microtube and Dnase and RNase free pipette tip.
8. Place the **Truenat® HBV** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
9. Place the microtube containing freeze dried PCR reagents in the microtube stand provided along with the **Truelab®** Real Time Quantitative micro PCR workstation after ensuring that white pellet of dried PCR reagents remains at the bottom of the microtube. Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 18). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA from the Elute Collected Tube into the microtube. Allow it to stand for 30-60 seconds to get a clear solution. ⚠ Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the **Truenat® HBV** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the microtip as per the section on "Disposal and Destruction" (Section 18).
10. For **Truelab® Uno Dx**, slide the chip tray containing the **Truenat® HBV** chip-

based Real Time PCR test loaded with the sample into the **Truelab**® Analyzer. Press Done on the "Please Load Sample" Alert message. For **Truelab**® **Duo/Quattro**, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.

11. Read the result from the screen.
12. After the reaction is completed, for **Truelab**® **Uno Dx**, push the Eject button to eject the chip tray. For **Truelab**® **Duo/Quattro**, tap the "Open/Close Tray" button to eject the chip tray.
13. Take out the **Truenat**® **HBV** chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 18).
14. Turn on **Truelab**® micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later.
    (Refer to the **Truelab**® Analyzer manual).
15. Switch off the **Truelab**® Analyzer.

16. RESULTS AND INTERPRETATIONS

Two amplification curves are displayed on the **Truelab**® Real Time Quantitative micro PCR Analyzer screen when optical plot is selected to indicate the progress of the test. Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Ct will depend on the number of viral genomes in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the Ct value and the IU/ml for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

17. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab**® Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The **Truenat**® Positive Control Kit-Pane II (REF 801020008) containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances:

- Whenever a new shipment of test kit is received.
- When opening a new test kit lot.
- If the temperature of the storage area falls outside of 2-30°C.
- By each new user prior to performing testing on clinical specimen

18. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat**® **HBV** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% Sodium hypochlorite for 10 volumes of contaminated fluid or water).
4. Do not autoclave materials or solutions containing Sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

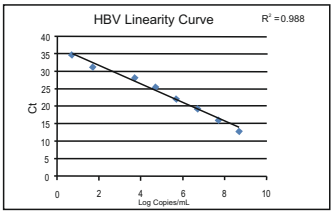
19. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusivity (Primer specificity): The following microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine potential cross-reactivity in the **Truenat**® **HBV** assay. Results obtained showed no cross reactivity of the **Truenat**® **HBV** test with the listed group of organisms.

Bacteria	Virus
Acinetobacter anitratus	Adenovirus
Candida albicans	Cytomagalovirus
Chlamydia trachomatis	Hepatitis C virus
Enterobacter cloacae	Human Immunodeficiency virus
Salmonella enterica	Epstein-Barr virus
Staphylococcus aureus	Herpes Simplex virus
Streptococcus mutans	Simian virus
Escherichia coli	
Gardnerella vaginalis	
Neisseria gonorrheae	
Trichomonas vaginalis	
Enterococcus faecalis	

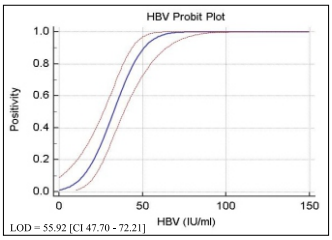
Linearity and Assay range:
The linearity assay was performed according to CLSI Guidelines. Serial dilutions of the HBV DNA cloned in a plasmid was made from 5.09E+09 Copies/ml to 5.09E+02 Copies/ml were made and nucleic acids were extracted on **Trueprep**® **AUTO** Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® **Uno Dx/Duo** Real Time micro PCR Analyzer. The assay is found to be linear over 8 orders of magnitude (from 5.09E+09 Copies/ml to 5.09E+02 Copies/ml) for HBV DNA.

Copies/ml	log Copies/ml	HBV Ct	IPC Ct
5.09E +09	8.7	13.11	28.14
5.09E +08	7.7	16.26	27.49
5.09E +07	6.7	19.28	28.63
5.09E +06	5.70	22.38	29.01
5.09E +05	4.70	25.57	27.4
5.09E +04	3.70	28.11	28.78
5.09E +03	1.70	31.35	26.55
5.09E +02	0.70	34.8	26.55



Limit of detection (Analytical Sensitivity):
The LoD was determined by testing dilutions of HBV 4th International Standard from NIBSC in plasma. Probit analysis of the data was used to determine the concentration of the respective DNA with 95% probability of detection. LoD was determined to be 55.92 IU/ml for HBV in plasma.

HBV IU/ml	Total runs	Percentage Positivity
0.00E +09	24	0%
2.55E +01	24	33.3%
5.09E +01	24	87.5%
5.09E +02	24	100%
5.09E +03	24	100%
5.09E +04	24	100%
5.09E +05	24	100%
5.09E +06	24	100%



Robustness:
To determine whether the **Truenat**® **HBV** chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive samples and negatives samples were performed. 10 positive samples and 10 negative samples were used for the study. The **Truenat**® **HBV** test did not exhibit detectable carryover between positive and negative sample PCR runs.

Reproducibility:
The purpose of this study is to compare the functional performance of the **Truenat**® **HBV** assay using three different titres of samples on **Truelab**® **Uno Dx** Real Time micro PCR Analyzer. High, Medium and low titre samples were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device and tested among three different users (Inter user), on three different devices (Inter device) and on 5 consecutive days (Inter day) to check the variability. Mean %CV values for all titres has been calculated as Inter User (1.79), Inter day (1.50) and Inter Device (1.32) which were in the accepted range of ≤15% CV for **Truenat**® **HBV** assay.

Interference:
The purpose of this study is to determine the effect of potentially interfering substances on the performance of **Truenat**® **HBV** assay. The experiments were performed with HBV positive samples spiked in negative human plasma. Interfering substances used in this study are: Albumin- 9 g/dL, Triglycerides-3.0 mg/dL, Human DNA-0.4 mg/dL and Hemoglobin- 500 mg/dL. The presence of these substances did not interfere with the performance of **Truenat**® **HBV** assay.

Precision of Truenat® HBV assay:
Precision was tested by performing **Truenat**® **HBV** assay of High, Medium and Low titre HBV DNA for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The CV values obtained for High titre (2.39), Medium titre (2.28) and low titre (1.49) were within the accepted range of ≤15% CV for **Truenat**® **HBV** assay.

Analytical reactivity or Inclusivity:
Analytical reactivity or Inclusivity of **Truenat**® **HBV** assay was performed on a clinical genotype panel consisting of 6 HBV Genotypes. The genotype panel was procured from Discovery life Sciences (DLS). The respective genotype DNA was extracted on **Trueprep**® **AUTO** Sample Prep Device in duplicates followed by PCR on **Truelab**® **Uno Dx** Real Time micro PCR Analyzer. **Truenat**® **HBV** assay effectively detected all the 6 HBV genotypes.

Validation of specimen types:
The validity of specimen types was demonstrated for the blood, plasma and serum. The panel comprised of 25 positive and 25 negative specimens for each claimed

specimen type. Positive specimens were spiked with 3X LLOQ of NIBSC (code:10/266) 4th WHO International Standard for Hepatitis B virus DNA. Each sample was subjected for nucleic acid extraction on **Trueprep® AUTO** Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR Analyzer by using **Truenat® HBV** test of one lot of reagents. The %CV values obtained for blood (5.10), plasma (6.46) and serum (3.76) were within the accepted range of ≤15% CV.

Whole system failure:
Whole system failure rate was performed utilizing blood sample spiked at 3X LLOQ of NIBSC (code:10/266) 4th WHO International Standard for Hepatitis B virus DNA. The study was performed on a sample panel of 100 numbers spread over 5 consecutive days. Every day 20 samples were utilized for nucleic acid extraction on **Trueprep® AUTO** Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR Analyzer by using **Truenat® HBV** test of one lot of reagents and by single user. Since the study had to be performed on high viscosity sample, blood was chosen as the sample matrix. The runs were not showed false results with 2.54 IU/ml Average log. The observed standard deviation across the 100 runs was 0.314 and a %CV of 12.38.

Trueness of measurement:
The Trueness of **Truenat® HBV** was demonstrated by comparison of the performance of the **Truenat® HBV** test with artus® HBV RG PCR comparator. The sample panel comprised of 100 positive specimens of plasma. The viral loads covered the entire linear range of the IVD covering all the HBV genotypes. Two lots of reagents were utilized for the study. All the 100 samples positive by comparator artus® HBV RG PCR were found to be positive by **Truenat® HBV** test also.

Clinical validations:
Totally 107 plasma samples comprising of 76 negative and 31 positive specimens were tested on three different manufacturing lots of **Truenat® HBV** assay at AIIMS (All India Institute of Medical Sciences, New Delhi) against the AIIMS in-house HBV PCR assay.
Specificity: 76 negative runs correlated between the methods, depicting **100%** specificity for the **Truenat® HBV** assay.
Sensitivity: Positive samples containing viral loads ranging from ~ 140 IU/ml to 8,50,00,000 IU/ml were tested. All 31 positive runs correlated between the methods giving a sensitivity of **100%** for the **Truenat® HBV** assay.
Concordance of viral loads:
Satisfactory concordance [over 95% within log variation] was seen between the viral load obtained using **Truenat® HBV** assay and AIIMS in-house HBV PCR assay.

20. REFERENCES

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3 Fryer JF, Heath AB, Wilkinson DE, Minor PD and the collaborative study group. Collaborative study to evaluate the proposed 3rd WHO International Standard for hepatitis B virus (HBV) for nucleic acid amplification technology (NAT)-based assays. WHO ECBS Report 2011;.2170 .
4 Abe, Aki, et al. Quantitation of hepatitis B virus genomic DNA by real-time detection PCR. Journal of Clinical Microbiology 37.9 (1999): 2899-2903.
5 Chen, Ren Wei, et al. Realtime PCR for detection and quantitation of hepatitis B virus DNA. Journal of medical virology 65.2 (2001): 250-256.
6 Brechtbuehl, K., et al. A rapid real-time quantitative polymerase chain reaction for Hepatitis B virus. Journal of virological methods 93.1 (2001): 105-113.

21. REVISION HISTORY

Section	Description of the changes
Throughout	Symbols are added as per Regulatory requirement
3	Principle of the test is updated
8	Storage Handling and stability is updated
9,13,19	Section is updated
21	Added Revision History table
Symbol keys	Symbol keys are updated

SYMBOL KEYS

Consult instruction for use	IVD *In vitro* Diagnostic Medical Device	LOT Batch number/ Lot number	REF Catalogue number	UDI Unique Device Identifier	This way up	Manufacturer	Caution	NON STERILE Non sterile.
Contains sufficient for <n> tests	Temperature limitations	Date of manufacture	Date of expiry	For single use only	Keep dry	Keep away from sunlight	Device for near-patient testing	

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