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Truenat®

GBS

Chip-based Real Time PCR Test for Group B Streptococcus

1. INTENDED USE

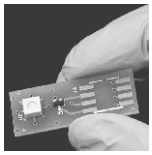
Truenat® GBS (REF 601240005 / 601240020 / 601240025 / 601240050 / 601240100 / 601240200) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi quantitative detection and diagnosis of group B streptococcus in vagino-rectal swabs, cerebrospinal fluid (CSF) and Blood / Serum / Plasma specimen and aids in the diagnosis of infection with group B streptococcus. **Truenat® GBS** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® GBS** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Group B streptococcus (GBS) infection is caused by bacterium *Streptococcus agalactiae*, a Gram positive, beta hemolytic cocci which causes sepsis and meningitis in infants <3 months. GBS can cause serious illness and sometimes death, especially in newborns, the elderly and people with compromised immune systems. GBS is an innocuous commensal bacterium that colonizes the gastrointestinal and genitourinary tract of humans. Though GBS colonization is asymptomatic, in general does not cause problems, it can sometimes cause serious illness for the mother and the baby during gestation and after delivery. GBS infections in the mother can cause chorioamnionitis (intra- amniotic infection or severe infection of the placental tissues) infrequently and postpartum infections (after birth). GBS urinary tract infections may induce labour and cause premature delivery. Vaginal colonization with GBS during labour is the primary risk factor for the development of early-onset disease (GBS-EOD) (0-7 days of birth). GBS is one of the main causes of bacterial infections in newborns such as septicemia, pneumonia and meningitis which can lead to death or long-term after effects.

An exam of the blood and cerebrospinal fluid is often necessary to rule out Bacteremia, Sepsis and meningitis caused by GBS. Antenatal screening and testing women for GBS colonization using vaginal or rectal swabs at 35–37 weeks of gestation is recommended to prevent GBS-EOD through vertical transmission.

The standard method for the diagnosis of GBS colonization consists of culturing combined vaginal and anal swab in a selective broth medium that inhibits the growth of non-GBS microorganisms. However, this method requires at least 48h for GBS identification. Moreover, negative culture results are observed in some women whose infants subsequently develop GBS infection. Molecular techniques such as polymerase chain reaction (PCR) or Real Time PCR are much more sensitive and specific. 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 **Truelab®** Real Time micro PCR System enables decentralization and near patient detection and monitoring of GBS by making real time PCR technology rapid, simple, robust and user friendly and 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 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® GBS is a disposable, room temperature stable, micro PCR test with dried $MgCl_2$ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for GBS and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. 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. The **Truenat® GBS** chip also stores information of used chips to prevent any accidental re-use of the chip.

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® GBS works on the principle of Real Time Polymerase Chain Reaction (PCR) based on Taqman chemistry. 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 **Truenat® GBS** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided 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® GBS** chip and the test is started. A positive amplification causes the labeled fluorescent probes in the **Truenat® GBS** chip to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer 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, a GBS "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, semi quantitative result 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 3G/GPRS network. Upto 20000 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 assay is *cAMP* factor gene.

5. CONTENTS OF THE Truenat® GBS KIT

A. Individually sealed pouches, each containing
   1. **Truenat® GBS** micro PCR chip.
   2. Microtube with freeze dried PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

REF	601240005	601240020	601240025	601240050	601240100	601240200
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 Transport Medium for Swab Specimen Pack

A. Transport Medium for Swab specimen tubes (contains transport medium).
B. Package Insert.

REF	60206TS05	60206TS20	60206TS25	60206TS50	60206TS100	60206TS200
Σ	5T	20T	25T	50T	100T	200T

8. STORAGE AND STABILITY

Truenat® GBS chip is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Universal Sample Pre-treatment Pack and **Trueprep® AUTO** Transport Medium for Swab Specimen 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.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF603021001 / 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** Transport Medium for Swab Specimen Pack (REF60206TS05 / REF60206TS20 / REF60206TS25 / REF60206TS50 / REF60206TS100 / REF60206TS200), **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep**® **AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat**® Positive Control Kit - Panel III (REF 801030008), Powder free disposable gloves, waste disposal container with lid.

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

A. Blood/Serum/Plasma or CSF specimen:

Truenat® **GBS** requires purified nucleic acids from whole blood/plasma 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 250µl of whole blood or 500µl of plasma/serum/CSF 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 **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep kit for details).

B. Swab specimen:

Swab specimen must be collected as per standard procedures using a standard nylon flocked swab. Insert the swab with specimen into the Transport Medium for Swab Specimen Tube provided and mix well by repeatedly twirling the swab in the buffer solution. After mixing, squeeze out the excess liquid from the swab by pressing it a few times against the inside wall of the tube. ⚠ Dispose off the swab as per the section on "Disposal and Destruction" (Section 18). Tightly close the cap of the Transport Medium for Swab Specimen Tube.

Sample Storage and Transportation:

Transport Medium for Swab Specimen decontaminates the specimen and makes it ready for storage/ transportation/extraction. The specimen in this form is stable for up to 3 days at 40ºC and 1 week at 30ºC.

Nucleic acid extraction: Transfer 500 µL from the Transport Medium for Swab Specimen Tube into the Lysis Buffer Tube. Use the entire content of lysis buffer tube containing Transport Medium for Swab 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 the Transport Medium for Swab Specimen Tube, lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 18).

11. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 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 **Truelab**® Real Time micro PCR System before use.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

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**® **GBS** 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.

13. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat**® assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truenat**® assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat**® assay should be interpreted in the context of 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 "GBS" 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 "GBS" 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 Reaction.
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 Reaction" button.
7. Open a pouch of **Truenat**® **GBS** and retrieve the chip-based Real Time PCR test and the microtube.
8. Place the **Truenat**® **GBS** 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 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 Collection 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**® **GBS** 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**® **GBS** 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**® **GBS** chip-based Real Time PCR test 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 & INTERPRETATIONS

Two amplification curves are displayed on the **Truelab**® Analyzer screen 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 time taken (Ct) of the specimen will depend on the number of target DNA in the sample. The 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 microbial load as "HIGH (Ct<20)","MEDIUM" (20≤Ct<25)", "LOW (25≤Ct<30)" or " VERY LOW (Ct ≥ 30)" 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. 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 Quantitative micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The **Truenat**® Positive Control Kit - Panel III (REF 801030008) 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 kits 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.

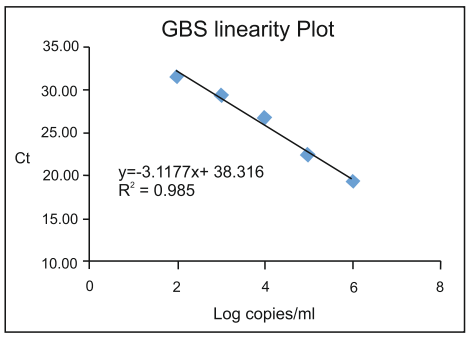
18. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® GBS** chip, microtube, microtube cap, transfer pipette, pipette tips, nylon flocked swab, Transport Medium for Swab Specimen Tube, 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 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

Linearity and Assay range:

The linearity analysis was performed according to CLSI guidelines. Serial dilutions of *Streptococcus agalactiae* Lehmann and Neumann (ATCC® BAA-611D-5™) strain DNA from 9.48 x 10^6 to 9.48 x10^2 were made and nucleic acids were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab® Uno Dx** Real Time Quantitative micro PCR Analyzer. The assay is found to be linear over 5 orders of magnitude and quantitates nucleic acids from 9.48E+06 copies/ml to 9.48E+02 copies/ml *Streptococcus agalactiae* Lehmann and Neumann (ATCC® BAA-611D-5™) strain DNA as depicted in the given graph.

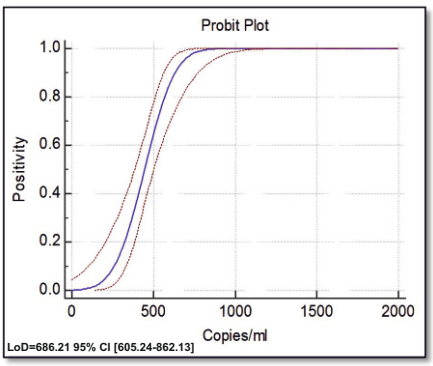


Analytical Exclusitivity (Primer specificity): The following microorganisms were evaluated *in silico* from the the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat® GBS** assay. No cross reactivity in the performance of the **Truenat® GBS** assay was observed with the listed microrganisms.

Abiotrophia defective	*Shigella flexneri*	*Acinetobacter baumanii*	*Klebsiella oxytoca*	*Shigella sonnei*	*Actinobacillus pleuropneumoniae*	*Lactobacillus acidophilus*
Staphylococcus aureus	*Aeromonas hydrophila*	*Lactobacillus casei*	*Staphylococcus epidermidis*	*Anaerococcus lactolyticus*	*Lactobacillus delbrueckii lactis*	*Staphylococcus intermedius*
Anaerococcus prevotii	*Lactobacillus gasseri*	*Staphylococcus haemolyticus*	*Anaerococcus tetradius*	*Lactobacillus plantarum*	*Staphylococcus lugdunensis*	*Arcanobacterium pyogenes*
Staphylococcus saprophyticus	*Bacillus cereus*	*Listeria monocytogenes*	*Staphylococcus simulans*	*Bacteriodes fragilis*	*Micrococcus luteus*	*Stenotrophomonas maltophilia*
Bifidobacterium brevis	*Moraxella atlantae*	*Streptococcus acidominimus*	*Bordetella pertusis*	*Moraxella catarrhalis*	*Steptococcus anginosus*	*Bulkholderia cepacia*
Morganella morganii	*Streptococcus bovis*	*Candida albicans*	*Neisseria gonorrhoeae*	*Streptococcus canis*	*Candida glabrata*	*Pantoea agglomerans*
Streptococcus constellatus	*Candida tropicalis*	*Pasteurella aerogenes*	*Streptococcus cricetus*	*Citrobacter freundii*	*Peptinophilus assacharolyticus*	*Streptococcus cristatus*
Clostridium difficile	*Peptostreptococcus anaerobius*	*Streptococcus downei*	*Corynebacterium urealyticum*	*Porphyromonas asaccharolytica*	*Streptococcus dysgalactiae*	*Enterobacter aerogenes*
Prevotella melaninogenica	*Streptococcus equi*	*Enterobacter cloacae*	*Prevotella oralis*	*Streptococcus mutans*	*Enterococcus faecium*	*Proteus vulgaris*
Streptococcus gordonii	*Enterococcus durans*	*Propionibacterium acnes*	*Streptococcus mitis*	*Enterococcus faecalis*	*Proteus mirabilis*	*Streptocoocus oralis*
Enterococcus gallinarum	*Providencia stuartii*	*Streptococcus parasanguinis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*	*Finegold magna*
Pseudomonas fluorescens	*Streptococcus pseudoporcinus*	*Fusobacterium nucleatum*	*Rhodococcus equi*	*Streptcoccus pyogenes*	*Gardnerella vaginalis*	*Salmonella dublin*
Streptococcus ratti	*Haemophilus influenzae*	*Vibrio chlolerae*	*Yersinia enterocolitica*	Human DNA		

Limit of detection:

The LoD was determined by testing dilutions of *Streptococcus agalactiae* Lehmann and Neumann (ATCC® BAA-611D-5™) strain DNA and performing nucleic acid extractions on **Trueprep® AUTO** sample prep device for each of the dilution 20 times followed by PCR on **Truelab® Uno Dx** Real Time micro PCR Analyzer. Probit analysis of the data was used to determine the concentration of the DNA with 95% probability. LoD was determined to be 686.21 copies/ml for Group B *Streptococcus agalactiae* Lehmann and Neumann (ATCC® BAA-611D-5™) strain DNA.



Robustness:

To determine whether the **Truenat® GBS** chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternate runs of positive samples and negatives samples were performed. 15 positive and 15 negative samples were used for the study. The **Truenat® GBS** test did not exhibit detectable carryover contamination between positive to negative sample runs.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat® GBS** 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) and on three different devices (Inter device) to check the variability. Mean %CV values for all titres has been calculated for Inter User (1.86) and Inter Device (1.77) which were in the accepted range of ≤15% CV for **Truenat® GBS** assay.

Interference:

The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat® GBS** assay. For this study medium load samples were used. To the samples different concentrations of blood ranging from 5%, 10% and 30% were spiked and then the samples were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and PCR was performed on **Truelab® Uno Dx** Real Time micro PCR analyzer. The presence of blood till 30% did not interfere with the performance of **Truenat® GBS** assay. The standard deviation values obtained were within the accepted range of ≤15 % CV.

Accuracy of Truenat® GBS assay :

Accuracy was determined by performing DNA extractions and **Truenat® GBS** PCR for varying titres of samples over 5 consecutive days. The standard deviation values obtained were within the accepted range of ≤15 % CV for GBS.

Precision of Truenat® GBS assay:

Precision was tested by performing **Truenat® GBS** assay with extracted DNA of High, Medium and Low titres for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for High titre (2.07), Medium titre (3.00) and low titre (2.7) were within the accepted range of ≤15% CV for **Truenat® GBS** assay.

20. REFERENCES

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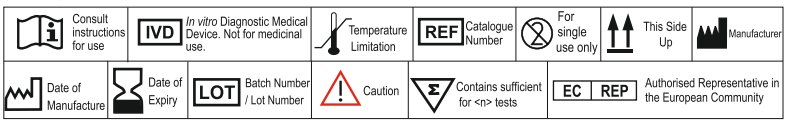
SYMBOL KEYS



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