1. INTENDED USE
Truenat™ Salmonella (REF 60100005 / 60100002) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the qualitative detection and diagnosis of Salmonella in human blood and aids in the diagnosis of infection with Salmonella. Truenat™ Salmonella runs on the Trueprep™ Uno and Trueprep™ Uno Dx Real Time micro PCR Analyzer.

2. INTRODUCTION
Typhoid fever is a symptomatic condition caused by infection of Salmonella serotypes including S. Typhi, S. Paratyphi A, S. Paratyphi B. The symptoms of the illness include high fever, headache, fatigue, sore throat, loose stools or diarrhea, vomiting, weight loss and appearance of skin rashes. About 12 million people, including children, throughout the world suffer from typhoid fever. Accurate diagnosis of typhoid fever at an early stage is not only important for etiological diagnosis to initiate prompt treatment and disease management but also to identify and treat potential carriers and prevent acute typhoid fever outbreaks. Current methods of diagnosis include serology based tests like conventional WIDAL test, and RDTs, culture and molecular techniques. The WIDAL test detects antibodies to S. Typhi in human serum or plasma. Both these methods are known to have limitations of sensitivity and specificity, and with little to no practical value in geographical settings where the disease is endemic. Blood culture is currently the gold standard. Conventional blood culture is time consuming and takes several days. Rapid blood culture followed by molecular techniques such as Polymerase Chain reaction (PCR) or Real Time PCR are much more sensitive and confirm infection with Salmonella, immediately upon onset of symptoms. However, these techniques have so far been restricted to centralized reference laboratories as they require skilled manpowers and infrastructure. Also, the turnaround time for results could take a few days.

The Trueprep™ Real Time micro PCR System operates on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA is first extracted using Trueprep™ MAG micro PCR chips and Trueprep™ AUTO Sample Prep kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technican can easily perform these tests in their facilities and report PCR results for Salmonella in clinical specimen as in early as 6 hours.

Truenat™ Salmonella is a disposable, room temperature stable, micro PCR chip with dried down PCR printing technology, simple, robust and user friendly and offering “sample to result” capability even at resource limited settings. The fast and highly sensitive blood culture Real Time PCR test can detect the salmonella in blood culture within about 6 hours if the amplification curve crosses the threshold (i.e. exceed the background signal) which is achieved through a combination of lightweight, portable, mains / battery operated Trueprep™ Uno / Trueprep™ Uno Dx Real Time micro PCR Analyzer and Trueprep™ MAG / AUTO Sample Prep Device and room temperature stable Trueprep™ micro PCR chips and Trueprep™ AUTO Sample Prep kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests in their facilities and report PCR results for Salmonella in clinical specimen as in early as 6 hours.

The Trueprep™ Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 and corresponding claims of any foreign counterpart(s) thereof. The Trueprep™ 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™ Salmonella works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA is first extracted using Trueprep™ MAG Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit or using the Trueprep™ AUTO Universal Card based Sample Prep Device and the Trueprep™ AUTO Universal Card based Sample Prep Kit. Six (6) μl of purified DNA is then dispensed into the reaction mixture of the Truenat™ Salmonella chip. This chip is then inserted into the Trueprep™ Real Time micro PCR Analyzer where thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the Truenat™ Salmonella chip to release the fluorochrome in an exponential manner which is then captured by the built-in optic-electronic sensor and displayed on the screen as the accumulation curve on the analyzer screen. In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen 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 values are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct the greater is the amount of target nucleic acid in the sample). At the end of the test run, Salmonella “DETECTED” or “NOT DETECTED” result is displayed. Based on the detection of Internal Positive Control (IPC), the full positive control test run is also displayed. The IPC is a full positive control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of shift of IPC signal beyond a pre-set range in case of negative sample indicates that the detection process was valid. The IPC detection allows to avoid inconclusive cases and, in some specimens having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed via Bluetooth using the Trueprep™ micro PCR printer or transferred to the lab computer or any remote computer via Wifi network or 3G/ GPRS network. Upto 5000 results in Trueprep™ Uno to 20000 results in Trueprep™ Uno Dx can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION
The target sequence for this kit has been taken from ‘pem’ gene encoding the “Pilus maintenance protein’. The sequence is highly conserved and specific for the species S. Typhi and S. Paratyphi.

5. CONTENTS OF THE Truenat™ Salmonella Kit
A. Individually sealed pouches, each containing
   1. Truenat™ Salmonella micro PCR chip.
   2. DNase & RNase free pipette tip.
   3. Desiccant pouch.
   4. Package insert.

6. CONTENTS OF THE Trueprep™ AUTO Universal Sample Pre-treatment Pack (only for Trueprep™ AUTO users)
A. Lysis buffer.
B. Disposable transfer pipette (reusable).

7. CONTENTS OF BILE BROTH
1. Bile Broth (Contains pre-dispensed sterile culture media).

8. STORAGE AND STABILITY
Truenat™ Salmonella is stable for one year from the date of manufacture if stored between 2-30°C. It is also stable for up to 5 weeks at 2-30°C. It is stable also for 4 weeks at temperatures up to 45°C. Do not freeze.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT
Trueprep™ Real Time micro PCR Instrument (REF 60301000/181300001/182300001/183300001) consisting of
1. Trueprep™ MAG/Trueprep™ AUTO Sample Prep Device (REF 603340001/603401001).
2. Trueprep™ Uno/ Trueprep™ Uno Dx Real Time micro PCR Analyzer (REF 60302000/1803020001).
3. Trueprep™ micro PCR Printer (REF 603350001).

10. PRECAUTIONS FOR PRECISION Micro pipettes
Also required additionally are: Trueprep™ MAG Blood Sample Prep kit (REF 60201005). Truelab™ Universal Control Kit (REF 601100001), DNase and RNase-free pipette tips with filter barrier, which may also be procured from Mobio, Powder free disposable gloves, waste disposal container with lid, 2ml Disposable syringe, Orbital Shaker (e.g. Lead Instruments Model No. LI-6041/2016).

11. NUCLEIC ACID EXTRACTION WITH TRUEPREP™ MAG
Step 1: Transfer 100μl of the contents of the culture tube to the extraction Tube (EXT) provided with the Trueprep™ MAG Blood Sample Prep kit. If culture has to be extended, then a small volume can be first drawn using a sterile syringe, transferred to a test tube from which 100μl can be pipetted into the EXT. If the Trueprep™ Salmonella test is negative at 5 hours of culture and Salmonella infection is suspected, continue incubating the culture tube for up to 24 hours and then repeat Step 1.

Nucleic acid extraction: Proceed with extraction using the Trueprep™ MAG Blood Sample Prep kit (Refer to the User Manual of Trueprep™ MAG Sample Prep Device and the package insert of Trueprep™ MAG Blood Sample Prep kit for details).

12. NUCLEIC ACID EXTRACTION WITH TRUEPREP™ AUTO
Step 1: Transfer 250μl of the contents of the culture tube to the lysis buffer tube using the graduated transfer pipette provided with the Trueprep™ AUTO Universal Sample Pre-treatment Pack. If culture has to be extended, then a small volume can be first drawn using a sterile syringe, transferred to a test tube from which 250μl can be pipetted into the lysis buffer tube.

If the Trueprep™ Salmonella test is negative at 5 hours of culture and Salmonella infection is suspected, continue incubating the culture tube for up to 24 hours and then repeat Step 1.

Nucleic acid extraction: Transfer entire contents of the lysis buffer bottle containing blood culture sample to sample chamber of cartridge (provided with Trueprep™ AUTO Universal Card based Sample Prep Kit).

Follow extraction procedure (section-13) of Trueprep™ AUTO Universal Card based Sample Prep kit package insert (Refer to the User Manual of Trueprep™ AUTO Universal Card based Sample Prep Device and the package insert of Trueprep™ AUTO Universal Card based Sample Prep Kit for details).

13. 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 and package insert(s) of all the components of the Trueprep™ Real Time micro PCR System (including lysis buffer and extraction buffer).
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not 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.

14. PROCEDURAL PRECAUTIONS
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.

Do not perform the test in the presence of reactive vapours (e.g., from sodium hypochlorite, acids, alkalis or aldehydes) or dust.

While retrieving the Truenat™ Salmonella micro PCR test and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests are used.

15. PROCEDURAL LIMITATIONS
There is a risk of false negative test results due to the presence of sequence variants in the gene target of the assay, procedural errors, recent antibiotic use by patient, amplification inhibitors in
Blood Culture

5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off.

16. Carry Over Effect: A Carry-Over Contamination Study was conducted to demonstrate the Carry-Over Cross Contamination that may occur when High Positive samples were processed alongside True Negative samples during nucleic acid extraction on the Trueplex™ and during subsequent Truenat™ Salmonella Assay on the Trueplex™. To determine whether the Truenat™ Salmonella micro chip PCR assay showed any signs of carry-over of PCR products between runs, alternating runs of positive Salmonella samples and negatives were performed in duplicates. Ten positives samples and 10 negative samples were used for the study.

15. Take out the Truenat™ Salmonella micro PCR chip at the end of the test and dispose it off as per the guideline.

16. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper products. The area should be decontaminated by flushing with 0.5% sodium hypochlorite solution for 10 volumes of contaminated fluid or water)

2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area surface and not to spill elute on the outside of the well.

3. This test is a qualitative test and does not provide the quantitative value of detected organism.

2. Sodium hypochlorite solution at 0.5% (5% sodium hypochlorite for 10 volumes of contaminated fluid or water).

3. Analyte target (bacterial nucleic acid) may persist throughout the test duration and the IPC curve will take an exponential path in case of negative samples.

4. The results were indicative of no carry-over of PCR products between runs using Truenat™ Salmonella micro PCR chip.

5. To determine whether the Truenat™ Salmonella micro chip PCR assay showed any signs of carry-over of PCR products between runs, alternating runs of positive Salmonella samples and negatives were performed in duplicates. Ten positives samples and 10 negative samples were used for the study.

6. All 59 blood culture negative samples were detected as negative, leading to sensitivity of 100%.

7. Effect of elevated biochemical blood parameters:

8. Effect of interferences of elevated serum parameters such as lipid, cholesterol and triglycerides were evaluated in this study. Samples (total 10 samples) showing higher level of serum parameters were spiked with known amount of Salmonella positive samples. These samples were run on Truenat™ Salmonella.

9. Results of carry-over evaluation:

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Sample ID</th>
<th>Cholesterol</th>
<th>HDL</th>
<th>Triglycerides</th>
<th>LDL</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ST1</td>
<td>230</td>
<td>35</td>
<td>259</td>
<td>140</td>
<td>DETECTED</td>
</tr>
<tr>
<td>2.</td>
<td>ST2</td>
<td>276</td>
<td>35</td>
<td>177</td>
<td>194</td>
<td>DETECTED</td>
</tr>
<tr>
<td>3.</td>
<td>ST3</td>
<td>325</td>
<td>32</td>
<td>114</td>
<td>114</td>
<td>DETECTED</td>
</tr>
<tr>
<td>4.</td>
<td>ST4</td>
<td>304</td>
<td>35</td>
<td>186</td>
<td>186</td>
<td>DETECTED</td>
</tr>
<tr>
<td>5.</td>
<td>ST5</td>
<td>168</td>
<td>32</td>
<td>342</td>
<td>58</td>
<td>DETECTED</td>
</tr>
<tr>
<td>6.</td>
<td>ST6</td>
<td>269</td>
<td>46</td>
<td>258</td>
<td>176</td>
<td>DETECTED</td>
</tr>
<tr>
<td>7.</td>
<td>ST7</td>
<td>220</td>
<td>42</td>
<td>230</td>
<td>124</td>
<td>DETECTED</td>
</tr>
<tr>
<td>8.</td>
<td>ST8</td>
<td>94</td>
<td>27</td>
<td>56</td>
<td>52</td>
<td>DETECTED</td>
</tr>
<tr>
<td>9.</td>
<td>ST9</td>
<td>225</td>
<td>39</td>
<td>138</td>
<td>148</td>
<td>DETECTED</td>
</tr>
<tr>
<td>10.</td>
<td>ST10</td>
<td>219</td>
<td>24</td>
<td>196</td>
<td>128</td>
<td>DETECTED</td>
</tr>
<tr>
<td>11.</td>
<td>Normal blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>DETECTED</td>
</tr>
</tbody>
</table>

No interference was observed on Truenat™ Salmonella from elevation of any of the biochemical parameter as tabulated above.

18. RESULTS & INTERPRETATION

Two Amplification curves are displayed on the Trueplex™ Real Time micro PCR 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 Ct will depend on the number of bacterial genome 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 also displays the validity of the test run as “VALID” or “INVALID”.

20. DISPOSAL AND DESTRUCTION

1. Submerge the used Truenat™ Salmonella micro PCR chip in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.

2. Dissolve the solutions and/or solid waste containing biological samples before discarding them according to local regulations.

3. Specimen collection equipment 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% sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of contaminated fluid or water).

4. Do not store 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.

21. SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection: The Truenat™ Salmonella test has a lower limit of detection of 1-2 CFU/ml of blood which can be detected after a patient blood sample is subjected to culture for a minimum of 5 hours as per the conditions described in Section 10.

CLINICAL SENSITIVITY/CLINICAL SPECIFICITY

An external clinical evaluation was conducted at a Tertiary Care hospital in India. A panel of 68 samples was analyzed by both blood culture, the gold standard and the Truenat™ Salmonella protocol. The agreement between both methods was 100%. All 9 blood culture positive samples were detected as positive, leading to sensitivity of 100%. All 59 blood culture negative samples were detected as negative, leading to specificity of 100%.

<table>
<thead>
<tr>
<th>Blood Culture</th>
<th>Truenat™ Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

Analytical Specificity (Primer specificity): The primers and probes were tested for reactivity to bacterial strains including S. Waterer, S. Benfotmben, S. Vidi, E. coli, Klebsiella, Campylobacter, Shigella and the results obtained showed that the primers were specific in detecting only Salmonella. No cross-reactivity was observed. The primers and probes were tested for reactivity to the following viruses and Parasitologonivirus, Rotavirus, Cryptogpandirum and Giardia. Results obtained showed that the primers were specific in detecting only Salmonella. No cross-reactivity was observed.

22. REFERENCES


