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Truenat™

Salmonella

Chip - based Real Time PCR test for *Salmonella* spp.

1. INTENDED USE

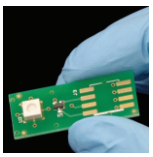
Truenat™ Salmonella (REF 601080005 / 601080020) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the qualitative detection and diagnosis of *Salmonella* in human blood and aids in the diagnosis of infection with *Salmonella*. **Truenat™ Salmonella** runs on the **Truelab™ Uno** and **Truelab™ 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, abdominal pain, diarrhea or constipation, 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 to also identify and treat potential carriers and prevent acute typhoid fever outbreaks. Current methods of diagnosis include serology based tests like conventional WIDAL test, and RDT's, culture and molecular techniques. The WIDAL test detects antibodies to *S.* Typhi and *S.* Paratyphi in the patient serum, and is positive only from the second week of onset of symptoms. RDT's qualitatively detect presence of IgM or IgM+IgG antibodies specific to *S.*Typhi in human serum or plasma. Both these methods are known to have limitations of sensitivity and specificity, 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 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 diagnosis of *Salmonella* by making real time PCR technology rapid, 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 method employed for *Salmonella* detection allows same-day initiation of treatment. This is achieved through a combination of lightweight, portable, mains / battery operated **Truelab™ Uno / Truelab™ Uno Dx** Real Time micro PCR Analyzer and **Trueprep™ MAG / AUTO** Sample Prep Device and room temperature stable **Truenat™** micro PCR chips and **Trueprep™MAG / AUTO** 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 for *Salmonella* in clinical specimen in as early as 6 hours.

Truenat™ Salmonella is a disposable, room temperature stable, micro PCR chip with dried down PCR reagents for performing a Real Time PCR test for detection of *Salmonella* and runs on the Truelab™ Real Time 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 including standard values for quantitation. The **Truenat™ Salmonella** chip also stores information of used chip to prevent any accidental re-use of the chip.



NOTE : Truelab™ / Truelab™ Uno / Truelab™ Uno Dx / Trueprep™ MAG / Trueprep™ AUTO / Truepet™ / Truenat™ are all registered trademarks of Molbio Diagnostics (P) Limited.

The Truelab™ 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 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™ 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 Cartridge Based Sample Prep Device and **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit. Six (6) µL of the purified DNA is then dispensed into the reaction well of the **Truenat™ Salmonella** chip. The **Truenat™ Salmonella** chip is then inserted in the **Truelab™** 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 fluorophore 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. 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 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). 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 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 signal 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 via Bluetooth using the **Truelab™** micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 3G/ GPRS network. Upto 5000 results in **Truelab™ Uno** to 20000 results in **Truelab™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 '*pmp*' gene encoding the "Plasmid 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.

B. Package Insert.

REF	601080005	601080020
Σ	5T	20T

6. CONTENTS OF THE Trueprep™ AUTO Universal Sample Pre-treatment Pack (only for Trueprep™ AUTO users)

A. Lysis buffer.

B. Disposable transfer pipette(graduated).

REF	60205AB05	60205AB20
Σ	5T	20T

7. CONTENTS OF BILE BROTH

1. Bile Broth (Contains pre-dispensed sterile culture media).

REF	10503006
Σ	20 x 5 ml

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 upto one (1) month at temperatures up to 40°C. Avoid exposure to light or elevated temperatures (above recommended levels).

Trueprep™ AUTO Universal Sample Pre-Treatment Pack is stable for 2 years from the date of manufacture if stored between 2-30°C . It is also stable for 4 weeks at temperatures upto 45°C. Do not freeze.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab™ Real Time micro PCR Workstation (REF 603010001/613010001/623010001) consisting of
1. **Trueprep™ MAG /Trueprep™ AUTO** Sample Prep Device (REF 603040001/603041001).
2. **Truelab™Uno/ Truelab™Uno Dx** Real Time micro PCR Analyzer (REF 603020001/6030210001).
3. **Truelab™** micro PCR Printer (REF 603050001).
4. **Truepet™** Precision Micropipettes .

Also required additionally are**: Trueprep™ MAG** Blood Sample Prep kit (REF 602010050), **Truenat™** Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips with filter barrier, which may also be procured from Molbio , Powder free disposable gloves, waste disposal container with lid, 2ml Disposable syringe, Orbital Shaker (e.g, Lead Instruments Model No. LI/IS-401/2016).

10. SPECIMEN COLLECTION AND PREPARATION WITH BILE BROTH

Truenat™ Salmonella requires purified nucleic acids from blood culture specimen.
1. Collect 1 ml of blood from the patient using a 2 ml sterile disposable syringe.
2. Slowly inject the blood into the provided culture tube containing pre-dispensed culture media through the rubber cap after lifting the aluminium flap. Do not remove or damage the aluminium cap.
3. Discard the empty syringe and cover the cap of the culture tube with aluminium flap and label it with patient ID and date/time of collection.
4. Place the culture tube in an orbital shaker, for between 5-24 hours with temperature set to 37°C and speed set between 150-200 RPM.
   Note : 5 hours is the minimum incubation time and 24 hours is the maximum incubation time before extraction of nucleic acids.
5. At the end of the incubation period, follow section 11 for nucleic acid extraction with **Trueprep™ MAG** or follow section 12 for nucleic acid extraction with **Trueprep™ AUTO.**

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 **Truenat™ Salmonella** test is negative at 5 hours of culture and *Salmonella* infection is suspected, continue incubating the culture tube for upto 24 hours and then repeat Step 1.

Nucleic acid extraction : Proceed with extraction using the **Trueprep™ MAG** Sample Prep Device and **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 250µLcan be pipetted into the lysis buffer tube.

If the **Truenat™ Salmonella** test is negative at 5 hours of culture and *Salmonella* infection is suspected, continue incubating the culture tube for upto 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 Cartridge based Sample Prep kit).

Follow Extraction procedure (section-13) of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep kit package insert (Refer to the User Manual of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep™ AUTO** Universal Cartridge 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 inserts 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.

14. 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™ 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 run are used.

15 PROCEDURAL LIMITATIONS

1. 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

specimens, or inadequate numbers of bacteria for amplification.
2. Analyte target (bacterial nucleic acid) may persist *in vivo*, independent of bacterial organism viability. Detection of analyte target does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
3. This test is a qualitative test and does not provide the quantitative value of detected organism present.The performance of the test has been evaluated for use with human blood specimen only.

16. 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.

17. TEST PROCEDURE

(Please also refer the **Truelab™ Uno/ Truelab™ Uno Dx** Real Time micro PCR Analyzer user manual)

1. Switch on the **Truelab™** Analyzer .
2. If using the **Truelab™ Uno** device, also switch on the touch screen. If using the **Truelab™ Uno Dx** proceed to step 3.
3. Select user and enter password.
4. Select the test profile for "SALMONELLA" on the Analyzer screen.
5. Enter the patient details as prompted in the **Truelab™** Analyzer screen.
6. Press Start Reaction.
7. Press the eject button to open the chip tray.
8. Open a pouch of **Truenat™ Salmonella** and retrieve the chip-based Real Time PCR test .
9. Label the chip with the patient ID using a marker pen at the space provided on the back side of the chip.
10. Place the **Truenat™ Salmonella** micro PCR 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.
11. Using the filter barrier tip provided in the pouch, pipette six (6) µL of the purified DNA from the Elute Collection Tube into the centre of the white reaction well. Take care not to scratch the internal well surface and not to spill elute on the outside of the well.
12 Slide the chip tray containing the **Truenat™ Salmonella** chip-based Real Time PCR test loaded with the sample, into the **Truelab™** Analyzer.
13. Press Done on the "Please Load Sample" Alert message.
14. Read the result from the screen.
15. Take out the **Truenat™ Salmonella** micro PCR chip at the end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 20).
16. 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 **Truelab™** Analyzer manual).
17. Switch off the **Truelab™** Analyzer .

18. RESULTS & INTERPRETATIONS

Two Amplification curves are displayed on the **Truelab™** 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". Invalid samples have to be repeated with fresh specimen from the sample preparation stage.
Note : 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.

19. 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™** Universal Control Kit 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.

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. 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.

21. SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection: The **Truenat™ Salmonella** test has a lower limit of detection of 1-2 cfu per 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.

CLINICALSENSITIVITY/ CLINICALSPECIFICITY

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%.

		Blood Culture	
		Positive	Negative
Truenat™ Salmonella	Positive	9	0
	Negative	0	59

Analytical Specificity (Primer exclusivity):The primers and probes were tested for reactivity to bacterial strains including S. Weltervreder, S.Senftenberg, S. Virdi, 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 Parasitesorovirus, Rotavirus, Cryptosporidum and Giardia. Results obtained showed that the primers were specific in detecting only *Salmonella*. No cross-reactivity was observed.

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 **Trueprep™ MAG** and during subsequent **Truenat™ Salmonella** Assay on the **Truelab™**. 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 samples were performed in duplicates. Ten positives samples and 10 negative samples were used for the study.

Results of carry-over evaluation:

Sr.No.	Sample ID	Results
1.	Positive	DETECTED
2.	Negative	NOT DETECTED
3.	Positive	DETECTED
4.	Negative	NOT DETECTED
5.	Positive	DETECTED
6.	Negative	NOT DETECTED
7.	Positive	DETECTED
8.	Negative	NOT DETECTED
9.	Positive	DETECTED
10.	Negative	NOT DETECTED

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

Interference Studies:

Effect of interferences of elevated serum parameters such as lipid, cholesterol and triglycerides were evaluated in this study. Specimens (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.**

Effect of elevated biochemical blood parameters:

Sr. No.	Sample I.D.	Cholesterol	HDL	Triglycerides	LDL	Ct
1.	ST1	230	38	259	140	DETECTED
2.	ST2	276	35	177	194	DETECTED
3.	ST3	225	32	353	114	DETECTED
4.	ST4	304	35	394	186	DETECTED
5.	ST5	168	32	342	58	DETECTED
6.	ST6	269	46	258	176	DETECTED
7.	ST7	220	42	230	124	DETECTED
8.	ST8	94	27	56	52	DETECTED
9.	ST9	225	39	138	148	DETECTED
10.	ST10	219	24	196	128	DETECTED
11.	Normal blood	-	-	-	-	DETECTED

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

22. REFERENCES

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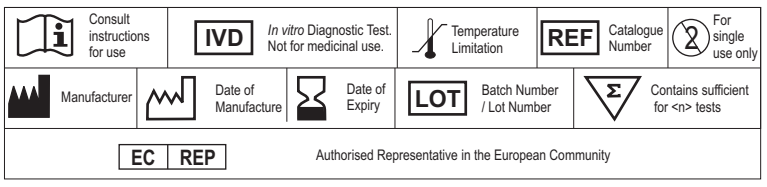
SYMBOL KEYS



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TNST/0417/VER-04