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Truenat®

Salmonella

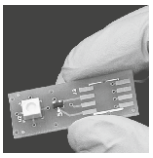
Chip-based Real Time PCR Test for *Salmonella* spp.

1. INTENDED USE

Truenat® Salmonella (REF 601080005 / 601080020 / REF 601080025 / 601080050 / REF 601080100 / REF 601080200) 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®** Real Time Quantitative micro PCR Analyzers.

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®** 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 kit 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.

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® Salmonella works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA 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® Salmonella** 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® Salmonella** chip and the test is started. 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 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 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. Microtube with freeze dried PCR reagents.
3. DNase & RNase free pipette tip.
4. Desiccant pouch.

B. Package Insert.

REF	601080005	601080020	601080025	601080050	601080100	601080200
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 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 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 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 (REF 603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 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) 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, 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 12 for nucleic acid extraction with **Trueprep® AUTO/AUTO v2.**

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

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 250 µL can 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: Use the entire content of lysis buffer tube containing blood culture sample 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 lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section19).

12. 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.

13. 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.

14 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.

15. 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.

16. TEST PROCEDURE

(Please also refer the **Truelab®** Real Time Quantitiative 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 "Salmonella" 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 "Salmonella" 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® Salmonella** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat® Salmonella** 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 ensure that white pellet of dried PCR reagents remain at the bottom of the microtube.** Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section19). 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® Salmonella** 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" (Section19).
10. For **Truelab® Uno Dx,** slide the chip tray containing the **Truenat® Salmonella** 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® Salmonella** chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section19).
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.

17. 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.

18. 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 - 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.

19. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® Salmonella** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube, syringe 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.

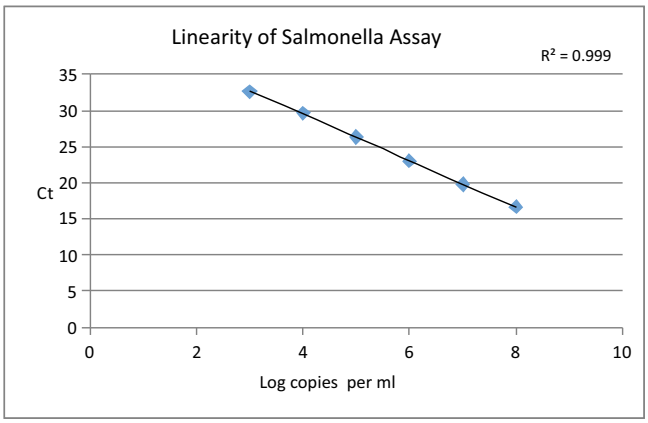
20. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusitivity (Primer specificity): The following viruses and 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® Salmonella** assay. Results obtained showed no cross reactivity of the **Truenat® Salmonella** assay with the listed organisms.

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

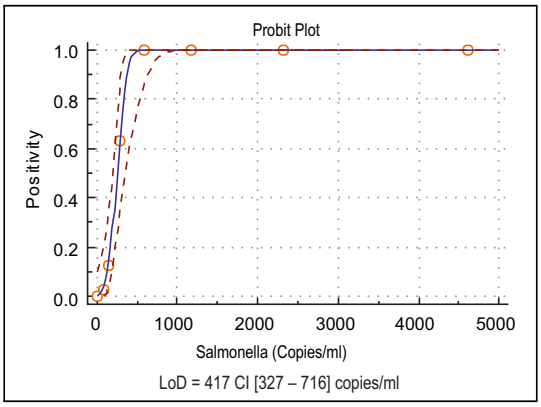
Linearity:

The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of *Salmonella enterica* subsp. *enterica* (*ex* Kauffmann and Edwards) Le Minor and Popoff serovar Paratyphi B (ATCC® 8759™) strain DNA from 2.31E+08 copies/mL to 2.31E +03 copies/mL 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 micro PCR analyzer. The assay is found to be linear over 6 orders of magnitude (from 2.31E+08 copies/mL to 2.31+03 copies/mL) for *Salmonella enterica* subsp *enterica* (ex Kauffmann and Edwards) Le Minor and Popoff serovar Paratyphi B (ATCC®8759™) strain DNA as depicted in the given graph.



Limit of detection (LoD):

The LoD was determined by making dilutions of *Salmonella* DNA into negative blood and performing nucleic acid extractions on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device for each of the dilution 10 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 of detection. The LoD was found to be 417 copies/mL for *Salmonella enterica* subsp. *enterica* (*ex* Kauffmann and Edwards) Le Minor and Popoff serovar Paratyphi B (ATCC® 8759™) strain DNA by **Truenat® Salmonella** assay.



Robustness:

To determine whether the **Truenat® Salmonella** 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. 20 positive samples and 20 negative samples were used for the study. The **Truenat® Salmonella** 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® Salmonella** 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 for Salmonella as Inter User (1.55), Inter day (2.30) and Inter Device (2.12) which were in the accepted range of ≤15% CV for **Truenat® Salmonella** assay.

Interfering Substances:

The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat® Salmonella** assay. For this study medium load samples were used. To the samples, different concentrations of Albumin (9 g/dl), Triglycerides (3.0 mg/dl) and Human DNA (0.4mg/dl) 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 Quantitative micro PCR analyzer. The presence of any of these substances did not interfere with the performance of **Truenat® Salmonella** assay. The CV values obtained were within the accepted range of ≤15% for **Truenat® Salmonella** assay.

Precision:

Precision was tested by performing **Truenat® Salmonella** assay with extracted DNA of High (7.6E+07 copies/mL), Medium (7.6E+05 copies/mL) and Low (7.6E+04 copies/mL) titres for Salmonella for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for High titre (1.05), Medium titre (1.63) and low titre (1.20) for Salmonella were within the accepted range of ≤15% CV for **Truenat® Salmonella** assay.

21. REFERENCES

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