

Check&Trace Salmonella 2.0

For use with the Bio-Rad CFX 96 and CFX Opus 96 real-time PCR Cycler



1 INTENDED USE

The Check&Trace Salmonella 2.0 Assay is a qualitative, semi-automated real-time PCR test designed for the confirmation and typing of presumptive *Salmonella* isolates from enriched culture media. The confirmation procedure advances a suspected (presumptive) *Salmonella* result (according to ISO-6579-1) to a confirmed *Salmonella spp.* result or a *Salmonella* negative result. The typing procedure generates a serovar result or a “genovar” code both uniquely defining each bacterial isolate tested.

Testing is performed after a simple sample preparation step of bacteria from culture. The sample is then used as input material for a 6-tube multiplex real-time PCR assay performed on the Bio rad CFX 96 or CFX Opus 96 Systems. Presence and absence of target sequences for confirmation and typing are assessed for each multiplex PCR and a final test result is generated automatically using dedicated software.

2 SUMMARY AND EXPLANATION OF THE PROCEDURE

Salmonella is one of the main causes of food poisoning. Detection of *Salmonella* in food and feed stuff is therefore mandatory in many countries. Standard protocols for detection of *Salmonella* are time-consuming taking several days to generate a final positive or negative test result. (ISO-6579/1). If positive for *Salmonella* many countries require further typing of *Salmonella* for epidemiological and health risk assessments. Traditionally this is done using serotyping for the somatic (O) lipopolysaccharide and flagellar (H) protein antigens (ISO-6579/3). However, *Salmonellae* are antigenically complex with over 2 400 serovars having various combinations of the 46 O-antigens and 85 H-antigens (3). So, once positive, further typing of a positive *Salmonella* may again take several days to complete requiring many O- and H-antisera for full typing.

DNA typing differs from serotyping. With serotyping the presence of antigens on the cell surface and flagella are detected. This is based on expression of genes located on two specific segments of the Salmonella genome. The CTS 2.0 assay detects genetic variation at 21 loci scattered over the whole Salmonella genome: this generates specific Salmonella genotypes, also called Genovars. The CTS 2.0 database links Genovars to a collection of well-characterized Salmonella Serovars. A Genovar will be given as test result if there is no established Serovar for the Genovar generated by the CTS 2.0 assay.

With the CTS 2.0 assay confirmation and typing of Salmonella can be performed in approximately 2.5 hours starting from bacteria from a XLD or Nutrient Agar (NA) plate. The presence of DNA target sequences for typing and confirmation are detected in parallel with various internal controls. CTS 2.0 uses a quick and simple sample preparation process and minimizes operator intervention once the samples are placed onto the real-time PCR system.

PRINCIPLES OF THE PROCEDURE

A worklist is generated on the CTS 2.0 analysis portal. Bacterial specimens are collected from XLD or NA media using “colony samplers” included in the CTS 2.0 kit. Colony samplers are then placed in 1.5 mL tubes prefilled with 200 µL Sample Buffer. Bacteria are resuspended in this buffer and tubes are closed and transferred to a heating block pre-heated at 98 °C. Tubes are incubated for 10 minutes at 98 °C and allowed to cool down to room temperature for 5 minutes. This procedure generates a crude lysate from the specimens releasing the bacterial DNA in the Sample Buffer. This crude lysate is suitable for the CTS 2.0 real-time amplification process without further purification.

140 µL of the crude lysate is transferred to a fresh tube prefilled with 35 µL PCR Master Mix and mixed by pipetting up and down 3 times. Next 25 µL of the crude lysate/Master Mix solution is added to each of 6 tubes of the CTS 2.0 PCR Reagent Strip. This Reagent Strip contains all PCR primers and probes for target sequence amplification and detection using real-time PCR. Finally, the PCR Reagent Strip is placed in the real-time PCR instrument and the amplification process is started.

The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. Probes labeled with different fluorophores are used to detect the various target sequences; up to 5 fluorophores may be used per reaction tube. The CTS 2.0 can detect a total of 27 DNA markers including 6 controls using 6 multiplex real-time PCR's in parallel. Control markers will assess presence or absence of *Salmonella* and detect reaction failure. The absence or presence of each of the remaining 21 DNA markers generates a unique "genetic fingerprint", which is used to determine *Salmonella* type.

The CTS 2.0 assay uses dedicated software for result calling in a 2-step process. In the first step absence and presence of each of the 27 DNA markers is established. This generates a unique presence/absence pattern for all DNA markers, i.e., the "genetic fingerprint". In a second step this "genetic fingerprint" is matched to a database containing the genetic fingerprints of many *Salmonella* serovars. If a match is found the software will call a serovar result from the CTS 2.0 assays; if no match is found but *Salmonella* genus is confirmed a genovar result will be called.

Kit contents	Quantity
CTS 2.0 PCR Reagent Strips <i>PCR strips (with caps) having 12 tubes each, and containing 2 times 6 sets of target-specific primers and fluorescent probes in dry format</i>	3 pouches each having 8 PCR reagent strips with 12 tubes
CTS 2.0 PCR Master Mix <i>PCR Master Mix with DNA polymerase, dNTP's, reaction buffer and stabilizers</i>	2 tubes with 1 mL each
Sample Buffer	1 vial with 12 mL
Colony Samplers	90

3 EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- Bio-Rad CFX 96 or CFX Opus 96 real-time PCR System
- Vortex Mixer
- Heating Block for Eppendorf Tubes
- Centrifuge for Eppendorf Tubes
- Centrifuge for microtiter plates
- Holder for Eppendorf Tubes
- Eppendorf Tubes
- Holder for PCR Reagent Strips
- Pipettes & disposable hydrophobic filter tips for volumes of 20 – 200 µL
- Lab coat and powderless disposable gloves

4 WARNINGS AND PRECAUTIONS

- The CTS 2.0 assay is for laboratory use only.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not remove desiccant from reagent pouches.
- Do not use reagents if desiccant is not present or is broken inside reagent pouches.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not use expired reagents and/or materials.
- Do not expose the PCR Reagent Strips to excessive light to prevent degradation of fluorophores by photo-bleaching.
- Use powder-free gloves and avoid fingerprints and writing on PCR Reagent Strip caps.
- Good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all materials and reagents.
- To avoid contamination by amplicons, do not open the CTS 2.0 PCR Reagent Strips after use, but dispose immediately.
- Always handle specimens in accordance with safe laboratory procedures for Microbiological Laboratories.
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with local, state, provincial and/or federal regulations.

5 STORAGE AND STABILITY

CTS 2.0 Assay reagents and components are stable at 2–8 °C through the stated expiration date. Do not use expired components. CTS 2.0 PCR Reagent Strips are provided in sealed pouches. To protect product from humidity, immediately re-seal after opening.

PCR Reagents Strips are stable for up to 30 days at 2–8 °C after initial opening and re-sealing of the pouch.

6 INSTRUCTIONS FOR USE

Generate Work List

1. Log in to Check-Points CTS 2.0 Analysis portal with e-mail address and password.
2. Open menu by clicking the ☰ icon on the top left.
3. Open the run sessions tab by clicking “Run sessions”.
4. Add a new run session with clicking the + icon on the top right. Global Identifier is automatically generated and cannot be changed. Custom run identifier may be created under “Details”.
5. To add samples, click the pipette icon 🍷 and enter sample information. “Samples names” are mandatory, other information like ‘Sample location’, Production batch”, Sample origin”, “Sampling device” and “Sampling date” may also be entered.
6. Click “Save + New” to go to the next sample and continue until the final sample and then click “Save”.
7. Alternatively, click the import samples icon 📄 to upload a CSV or XLSX file with sample information. File format may be downloaded using the export button 📄.
8. Continue to select further details in “Details” section. “Real-time PCR Cycler ID” and “Kit lot number” are mandatory, “Custom run identifier” and “Run session notes” are optional.
9. Click “Save run session” to save all run information.
10. Reopen the run session and click “Download cfx run file”. This generates the run file to start the real-time PCR run in the “PCR System Operation” step.
11. Next, download and print a work list for use in the laboratory by clicking the 📄 icon on the top right.
12. Proceed to Sample preparation.

Sample preparation

NOTE: One (1) PCR Reagent Strip is used for two (2) samples. In case of uneven sample numbers supplement with samples from a control (reference) strain.

1. Turn on heating block at **98°C**.
2. Dispense **200 µL** Sample Buffer into a 1.5 mL tube. Use a separate tube for each sample and write sample ID on the tube.
3. Pierce through a single colony into the agar using a colony sampler. Briefly touch the bottom of the plate and take the sampler out again. Always keep the colony sampler in a vertical position as shown in Figure 1.

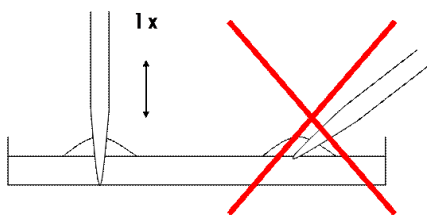


Figure 1: Correct colony sampling

4. Place the colony sampler in 1.5 mL tube with **200 µL** of Sample Buffer and roll the sampler between thumb and index finger at least 5 times back and forth while remaining in the buffer.
5. Remove and discard the colony sampler.
6. Close the tube and vortex briefly.
7. Place tubes in pre-heated heating block and incubate for **10 min at 98°C**.
8. Vortex tubes and place them on laboratory bench to cool down to room temperature for at least 5 minutes. If the samples are not used directly spin down briefly and store tubes at -20°C.
9. Proceed to “Real-time PCR Set-Up”.

Real-time PCR Set-Up

NOTE: Samples from -20°C should be placed at room temperature for 15 minutes, vortexed and spun down briefly.

1. Dispense **35 µL** PCR Master Mix into a 1.5 mL tube. Use a separate tube for each sample and write sample ID on the tube.
2. Briefly spin down the samples from the previous step (Sample preparation), and add **140 µL** sample to the PCR Master Mix. Mix by pipetting up and down gently 3 times.
3. Take the required number of PCR Reagent Strips from their protective pouches. Remove excess air, and close pouches with the zip seal. Label the end of each PCR Reagent Strip with the appropriate specimen identification.
4. Remove strip caps carefully and keep separate.

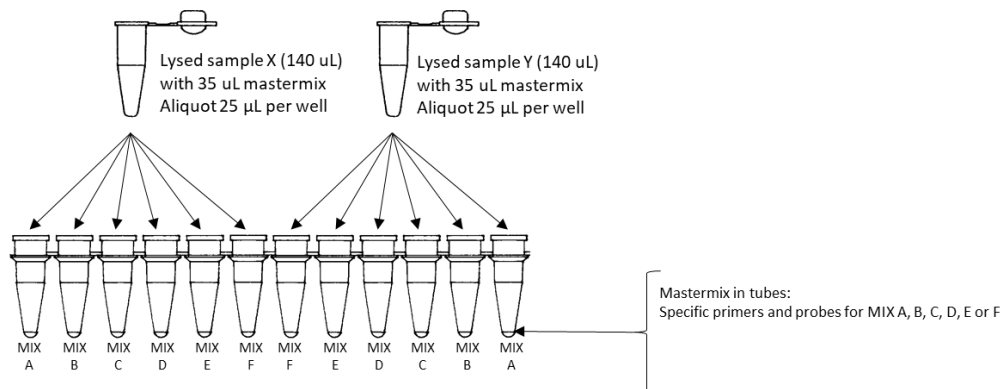


Figure 2 Pipetting scheme of Sample/Master mix specimens.

5. Transfer **25 µL** of the first Sample/Master Mix specimen to tube 1 of PCR Reagent Strip (Figure 2). Repeat this for tubes 2 – 6.
6. Transfer **25 µL** of the second Sample/Master Mix specimen to tube 7 its PCR Reagent Strip. Repeat this for tubes 8 – 12 (Figure 2).
7. Proceed until all Sample/Master Mix specimens have been transferred to their PCR Reagent Strips.
8. Carefully place the strip caps back on the strips. Make sure strip caps are sealed airtight to prevent evaporation during PCR, and spin down briefly.
9. Power on the PCR System and computer.
10. Open the lid of the real-time PCR cycler.
11. Place PCR Reagent Strips in the PCR machine using the worklist created in “Generate work list” section. If needed place “dummy” PCR reagent strips according to the worklist to distribute strips evenly over the PCR heating block.
12. Close the lid of the real-time PCR cycler.
13. Proceed to “PCR System Operation”.

PCR System Operation

NOTE: a computer connected to the cycler is required to use the LIMS file.

1. Start the Bio-Rad CFX Manager software.
2. Click “File” go to “Open” and click “Open LIMS File”. Then import CFX runfile from Check-Points CTS 2.0 Analysis portal.
3. Click “Start” to initiate run.
4. At the end of the run save the data file (.PCRD extension) to a dedicated network location or USB device.

Data Processing and Results




1. Log in to the Check-Points CTS 2.0 Analysis portal.
2. Open the Run session generated in the “Generate work list” section.
3. Click the “**Upload CFX Result file**” tab and upload the pcrd file containing the PCR run data.
4. Click on the banner that pops up or on the  icon to start data processing.
5. The data processing runs in the background and it is not necessary to keep the window open. Processing will take 5 – 10 minutes and results will pop-up automatically. If results do not appear, refresh the browser once (F5) and check the “**Messages**” tab.
6. Reports can be printed by clicking the “**Reporting**” tab. Use the tick boxes to select results for printing and use  to download individual reports or  to download a summary report.
7. Table 1 below shows the results and interpretations that may be obtained.

Table 1 CTS Assay Result Interpretation

Assay Result Reported	Interpretation of Result	Next Step
Salmonella Serovar	Salmonella positive and Serovar result	Final result
Salmonella Serovar 1 or Serovar 2	Salmonella positive result with two possible Serovar results	Final result
Salmonella positive Genovar	Salmonella positive result with Genovar code	Repeat sample once with fresh isolate
Salmonella positive, Inconclusive typing result	Salmonella positive, but no typing result	Repeat sample once with fresh isolate
No Salmonella	Gram-negative bacteria detected but not Salmonella	Final result
No DNA Detected	No gram-negative bacteria detected	Repeat sample once with fresh isolate
Reaction not OK	Reaction failed – inhibitory sample or reagent failure; no Internal Control amplification	Repeat sample once with fresh isolate

Repeat procedure

In the following circumstances it is advised to repeat the sample once with a fresh isolate:

- Salmonella positive with Genovar code
- Salmonella positive with inconclusive typing result
- No DNA detected
- Reaction not OK

A Salmonella positive with Genovar code or Inconclusive typing result may be obtained in case one (1) of the 21 typing markers has given an erroneous result generating a Genovar code, for which there is no association with a known Serovar in the CTS 2.0 database. This is a rare event for individual markers, but significant for the sum of the 21 typing markers. Repeating the sample once will generate a Serovar result if this specific issue has occurred and should be noted as the final result. The initial Genovar result or Inconclusive typing result may also be the proper result and repeating the assay will generate the same result in these cases.

In case of "Reaction not OK" or "No DNA detected" it is also advised to repeat the sample once with a fresh isolate. These results are often caused by sub-optimal reaction conditions and/or impurities in the sample. Repeating the reaction with a fresh isolate will generate a proper result the second time in many cases.

It may be that the Check&Trace Salmonella 2.0 Assay does not generate a conclusive confirmation and/or typing result, even after repeating the test. In such cases it is advised to use an alternative method to obtain a final test result. (1,2).

7 QUALITY CONTROL

Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type and frequency of testing control materials according to guidelines or requirements of local, provincial, state and/or federal regulations or accreditation organizations in order to monitor the entire analytical process.

External Positive and Negative Controls are not used by the CTS 2.0 Assay for the purpose of test result interpretation. The CTS 2.0 Assay also has a total of 6 internal controls to monitor for potential reaction failure. However, it is advised to test control samples daily until adequate process validation is achieved. Reduced frequency of control testing should be in accordance with applicable regulations.

External Controls should yield the expected results: *Salmonella* serovar for External Positive Control, and "No *Salmonella*" for External Negative Control. Any other result for the external controls is indicative for system failure.

Table 2 below specifies a number of reference strains that may be used for as external positive or negative controls. Other controls strains of which the test result is well documented may also be used.

Table 2 Commercially Available Strains for External Positive and negative Control

External Control Strain	Test Result
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis (NCTC 12694)	Salmonella Enteritidis
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium (NCTC 10413)	Salmonella Typhimurium
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium (Monophasic); I 4,5,12:i:- (NCTC 13952)	Salmonella Typhimurium Monophasic
<i>Escherichia coli</i> (ATCC 25922)	No Salmonella

8 LIMITATIONS OF THE PROCEDURE

- This product can only be used on the Bio-Rad CFX-96 or CFX Opus 96 Systems.
- Erroneous results may occur from improper sample collection, handling, storage, technical error, sample mix-up,
- If the CTS 2.0 results is "*Salmonella* positive with genovar code", "*Salmonella* positive inconclusive typing result", "No DNA detected" or "Reaction not OK", then the test should be repeated with a fresh isolate.
- Inclusivity testing and analysis have determined that the following *Salmonella* serovars are detected: Abaetetuba, Agona, Alachua, Albany, Anatum, Bovismorbificans, Braenderup, Brandenburg, Bredeney, Cerro, Choleraesuis, Corvallis, Cubana, Derby, Dublin, Enteritidis, Gallinarum Gallinarum, Gallinarum Pullorum, Give, Goldcoast, Hadar, Havana, Heidelberg, Idikan, Infantis, Javiana, Kentucky, Livingstone, London, Mbandaka, Minnesota, Molade, Montevideo, Muenchen, Muenster, Newport, Ohio, Oranienburg, Orion, Ouakam, Panama, Paratyphi B (possibly Java), Poona, Reading, Rissen, Saintpaul, San Diego, Schwarzengrund, Senftenberg, Stanley, Tennessee, Thompson, Typhimurium, Uganda, Virchow, Worthington, Yoruba, monophasic variant of *Salmonella* Typhimurium (1,4,[5],12:i:-), 4,[5],12:d:-. Other *Salmonella* serovars will yield a genovar code. In some cases CTS 2.0 may report "*Salmonella* positive serovar A or serovar B".
- Mutations or polymorphisms in primer or probe binding regions may affect detection of certain sequence variants (Sequence Types) of the serovars included in the CTS 2.0 assay. In such cases CTS 2.0 will report a genovar code instead of the serovar result.
- CTS 2.0 is not able to discriminate *Salmonella* species or subspecies, i.e. species *bongori* and *enterica* and *enterica* subspecies *arizonae*, *diarizonae*, *enterica*, *houtenae*, *indica* and *salamae*. In all these cases the CTS 2.0 result will be "*Salmonella* positive genovar code".
- CTS 2.0 is a qualitative test and does not provide quantitative values nor indicate the quantity of organisms present.

- The performance of the CTS 2.0 has been evaluated on Nutrient Agar and XLD agar. Other media will need to be evaluated separately for any laboratory to verify adequate performance on such media.

9 PERFORMANCE CHARACTERISTICS

General

CTS 2.0 Assay validation was carried out by Microval Expert Laboratory WFC, Arkel, the Netherlands, under supervision of the Microval Technical Committee (MVTC). ISO-16140 part 6 was used as standard for drafting the protocol and preparing the report including the acceptability criteria. ISO-6579/1 and ISO-6579/3 were used as reference methods for confirmation and typing, respectively. Assay validation consisted on two main parts: 1. A Methods Comparison Study (MCS), and 2. An Inter-Laboratory Study (ILS). The MCS was carried out using bacteria from two media, Nutrient Agar (NA) and XLD Agar (XLD) on two real-time PCR platforms, Bio-Rad CFX 96 and Bio-Rad CFX Opus 96. The ILS was carried out on a single medium, XLD, and using either Bio-Rad CFX 96 or Bio-Rad CFX Opus 96, whatever was available at the various ILS sites.

Inclusivity Confirmation Study

An inclusivity study was performed for confirmation of *Salmonella* according to ISO-16140_6. A total of 150 previously characterized *Salmonella* strains was used. Table 3 below depicts the results for Confirmation of *Salmonella* from these strains.

Table 3 Results of 150 Salmonella strains for confirmation of Salmonella, i.e. result is "Salmonella serovar" or "Salmonella genovar" or "Salmonella positive, inconclusive typing result", on two media, Nutrient Agar (NA) and XLD agar and two PCR platforms, Bio-Rad CFX 96 and Bio-Rad CFX Opus 96.

Number of Strains	qPCR System	Medium	Inclusivity Agreement	Inclusivity Disagreement
150	Bio-Rad CFX 96	NA	150	0
		XLD	150	0
	Bio-Rad CFX OPUS 96	NA	150	0
		XLD	150	0

In summary, all 150 *Salmonella* strains were confirmed as *Salmonella* by the CTS 2.0 Assay on both systems and with both media.

Exclusivity Confirmation Study

An exclusivity study was performed for confirmation of non-*Salmonella* according to ISO-16140_6. A total of 102 previously characterized non-*Salmonella* strains was used. Table 4 below depicts the results for confirmation of non-*Salmonella* from these strains.

Table 4 results of 102 strains for confirmation of non-Salmonella, i.e. result is "No Salmonella", on two media, Nutrient Agar and XLD agar and two PCR platforms, Bio-Rad CFX 96 and Bio-Rad CFX Opus 96. Note, numbers may vary slightly in case of non-growth on XL

Number of Strains	qPCR System	Medium	Exclusivity Agreement	Exclusivity Disagreement
102	Bio-Rad CFX 96	NA	102	0
		XLD	95	0
	Bio-Rad CFX OPUS 96	NA	101	0
		XLD	96	0

In summary, all non-*Salmonella* strains with a conclusive test result were confirmed as non-*Salmonella* by the CTS 2.0 Assay on both systems and with both media.

Inclusivity Typing Study

An inclusivity study was performed for typing of *Salmonella* according to ISO-16140_6. A total of 315 previously characterized *Salmonella* strains of 59 different serovars was used. Table 5 below depicts the results for typing of *Salmonella* from these strains.

Table 5 results for 315 Salmonella serovar strains for typing of Salmonella, i.e. result is "Salmonella serovar", on two media, Nutrient Agar (NA) and XLD agar and two PCR platforms, Bio-Rad CFX 96 and Bio-Rad CFX Opus 96. Some strains gave an inconclusive result. Note "Salmonella genovar" was considered as an inconclusive typing result for Inclusivity Typing.

Number of Strains	qPCR System	Medium	Inclusivity Agreement	Inclusivity Disagreement
315	Bio-Rad CFX 96	NA	311	0
		XLD	300	0
	Bio-Rad CFX OPUS 96	NA	308	1
		XLD	309	1

In summary, 1208 of 1210 *Salmonella* serovar test results were in agreement with the expected test results. Two (2) *Salmonella* serovar test results were in disagreement with the expected results, a *Salmonella* Poona strain was reported as *Salmonella* Abaetetuba with the CFX Opus 96 on both media.

Exclusivity Typing Study

An exclusivity study was performed for typing of *Salmonella* according to ISO-16140_6. A total of 104 strains were used, 78 *Salmonella* exclusivity serovars and 26 non-*Salmonella* *Enterobacteriaceae*. Table 6 below depicts the test results for these strains.

Table 6 combined test results for 78 Salmonella serovar exclusivity strains and 26 non-Salmonella Enterobacteriaceae strains on two media, Nutrient Agar (NA) and XLD agar and two PCR platforms, Bio-Rad CFX 96 and Bio-Rad CFX Opus 96. Some strains gave an inconclusive result. Note Salmonella exclusivity serovars should give a genovar results for "Exclusivity Agreement".

Number of Strains	qPCR System	Medium	Exclusivity Agreement	Exclusivity Disagreement
104	Bio-Rad CFX 96	NA	103	1
		XLD	102	0
	Bio-Rad CFX OPUS 96	NA	101	2
		XLD	102	1

In summary, 408 of 412 test results were in agreement with the expected result, "*Salmonella* genovar" for exclusivity *Salmonella* serovars, and "No *Salmonella*" for non-*Salmonella* *Enterobacteriaceae*. 4 *Salmonella* exclusivity serovars gave an incorrect result, i.e. "*Salmonella* serovar" instead of "*Salmonella* genovar". *Salmonella* Coeln gave "*Salmonella* Saintpaul" on NA with both PCR systems. *Salmonella* Matopeni gave "*Salmonella* Braenderup" on CFX Opus 96 with both media.

Inter-Laboratory Study

30 bacterial strains were sent to 15 collaborators at 13 sites participating in the Inter-Laboratory Study (ILS) and included 16 *Salmonella* Inclusivity serovars, 4 *Salmonella* Exclusivity serovars, one (1) *Salmonella enterica* subsp. *arizonae*, one (1) *Salmonella bongori* and 8 non-*Salmonella* *Enterobacteriaceae*. 24 of 30 test results were used for the ILS Confirmation Study and another set of 24 of 30 test results were used for the ILS Typing Study, hence a set of 18 test results was used for both typing and confirmation. Reference testing was only done for the confirmation part of the study. Testing was done from XLD Agar only using the PCR systems at the collaborators, i.e. 11 Bio-Rad CFX 96 systems and 4 Bio-Rad CFX Opus 96 systems.

ILS Confirmation Study

Data from 13 of 15 collaborators were included in the ILS, one collaborator did not report the data in time, the other collaborator deviated for the Instruction for Use (IFU). Table 7 below depicts the result of the ILS Confirmation Study for these 13 collaborators.

Table 7 test results for ILS Confirmation, 16 Inclusivity strains and 8 Exclusivity strains, all non-*Salmonella* *Enterobacteriaceae*.

Collaborator	Confirmation Inclusivity		Confirmation Exclusivity	
	Reference method	CTS 2.0	Reference Method	CTS 2.0
1	16/16	15/16	8/8	8/8
2	16/16	16/16	8/8	8/8
3	16/16	16/16	7/8	8/8
4	16/16	16/16	8/8	8/8
5	16/16	16/16	8/8	8/8
6	16/16	16/16	8/8	8/8
7	16/16	16/16	8/8	8/8
8	16/16	15/16	7/8	8/8
9	16/16	16/16	8/8	8/8
10	16/16	16/16	8/8	8/8
11	16/16	16/16	8/8	8/8
12	16/16	16/16	8/8	8/8
13	16/16	16/16	8/8	8/8
Total	208/208	206/208	102/104	104/104

In summary, 310 of 312 test results for Confirmation of CTS 2.0 were concordant. Two (2) of 312 samples did not give a conclusive result. No discordant test results were reported.

ILS Typing Study

Data from 13 of 15 collaborators were included in the ILS, one collaborators did not report the data in time, the other collaborator deviated for the Instruction for Use (IFU). Table 8 below depicts the result of the ILS Typing Study for these 13 collaborators.

Table 8 test results for Typing, 16 Inclusivity strains and 8 Exclusivity strains, i.e. 4 Salmonella Exclusivity serovars and 4 non-Salmonella Enterobacteriaceae.

Collaborator	Typing Inclusivity		Typing Exclusivity	
	Reference method	CTS 2.0	Reference Method	CTS 2.0
1	n.a.	15/16	n.a.	8/8
2	n.a.	16/16	n.a.	8/8
3	n.a.	16/16	n.a.	7/8
4	n.a.	16/16	n.a.	8/8
5	n.a.	14/16	n.a.	8/8
6	n.a.	16/16	n.a.	8/8
7	n.a.	16/16	n.a.	8/8
8	n.a.	14/16	n.a.	8/8
9	n.a.	16/16	n.a.	8/8
10	n.a.	16/16	n.a.	8/8
11	n.a.	16/16	n.a.	8/8
12	n.a.	16/16	n.a.	8/8
13	n.a.	16/16	n.a.	8/8
Total	n.a.	203/208	n.a.	103/104

In summary, 306 of 312 test results for Typing of CTS 2.0 were concordant. Six (6) of 312 samples did not give a conclusive test result. No discordant test results were reported.

Summary of the MicroVal Certificate



MicroVal hereby declares that the certification assessment has demonstrated that Check & Trace Salmonella 2.0 (CTS 2.0) has been validated and revealed to be at least equivalent to the reference method as demonstrated by the validation study report. The summary of the validation report is available on the Microval website: www.microval.org

Reference methods: ISO-6579-1:2017 and ISO/TR 6579-3:2014.

Scope: Confirmation of presumptive Salmonella isolated on non-selective NA and selective XLD agar and typing of 59 Salmonella serovars.



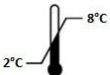



The validation and certification has been performed in accordance with ISO 16140-6:2019 and the MicroVal Rules and Certification Scheme version 9.1

Certificate no: 2021LR07

10 REFERENCES

1. ISO 6579-1. Microbiology of the food chain – Horizontal method for detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of *Salmonella* spp. [ISO 6579-1:2017].
2. ISO 6579-3. Microbiology of the food chain – Horizontal method for detection, enumeration and serotyping of *Salmonella* - Part 3: Guidelines for serotyping of *Salmonella* spp. [ISO 6579-3:2014].
3. Antigenic formulae of the *Salmonella* serovars. Grimont, P.A.D. and Weill, F.X. Vol.9, 2007.
4. ISO 16140-1:2016 Microbiology of the food chain – Method validation – Part 1: Vocabulary.
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11 SYMBOLS GLOSSARY

Symbol	Meaning
	Manufacturer
	Contains sufficient for <n> tests
	Temperature limit
	Consult instructions for use For electronic Instructions for use, the URL accompanies the symbol.
	Keep dry
	Keep away from light

