CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

072301

The AOAC Research Institute hereby certifies the method known as:

Check & Trace Salmonella 2.0

manufactured by

Check-Points
Binnenhaven 5
6709 PD Wageningen
The Netherlands

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

AUTHOR
P.A. Boleij

SUBMITTING COMPANY
Check-Points
Binnenhaven 5
6709 PD Wageningen
The Netherlands

METHOD NAME
Check & Trace Salmonella 2.0

CATALOG NUMBER
18-2020

INDEPENDENT LABORATORY
WFC Analytics
Arkel, The Netherlands

APPLICABILITY OF METHOD
Target organism – *Salmonella* spp.

Matrixes – Nutrient agar (NA) or Xylose Lysine Deoxycholate agar (XLD)

Performance claims – The Check & Trace Salmonella 2.0 can be used to confirm *Salmonella* species from non-selective Nutrient Agar and selective Xylose Lysine Deoxycholate agar and type of the following 59 *Salmonella* serovars: 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, Sandiego, Schwarzengrund, Senftenberg, Stanley, Tennessee, Thompson, Typhimurium, Uganda, Virchow, Worthington, Yoruba, monophasic variant of Salmonella Typhimurium (1,4,[5],12:i:-), and 4,[5],12:d:-.

ORIGINAL CERTIFICATION DATE
July 10, 2023

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **December 2023 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes.**

Under this AOAC *Performance Tested Methods*^{SM} License Number, 072301 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)

DNA is extracted from a single Nutrient Agar (NA) or Xylose Lysine Deoxycholate agar (XLD) colony by means of a quick boiling method. Extracted DNA is mixed with Mastermix (containing PCR-enzyme) and added to a PCR-strip, containing 6 different dried primer/probe-solutions. The strips are put in the CFX-96 or-OPUS instruments for target amplification.

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 PCRs in parallel. Control markers will assess the presence or absence of *Salmonella* and detect reaction failure. The presence or absence of each of the remaining 21 DNA markers generates a unique “genetic fingerprint”, which is used to determine the *Salmonella* type.

The CTS 2.0 assay uses dedicated software to determine the results in a 2-step process. After initial installation to setup the customers portal account with the correct settings, the presence or absence of each of the 27 DNA markers is established resulting in 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 identify the serovar from the CTS 2.0 assays; if no match is found but the *Salmonella* genus is confirmed, a genovar result will be generated.

DISCUSSION OF THE VALIDATION STUDY (1)

Salmonella strains were resuscitated from frozen cryoprotective beads on Nutrient Agar (NA) and Xylose Lysine Deoxycholate agar (XLD). A crude DNA lysate of the strains was mixed with Mastermix and transferred to a PCR reaction strip, prefilled with dried targets, primers, and probes. The PCR Reagent Strip was placed in the real-time PCR instrument and the amplification process was started. After the run, the results were analyzed using cloud-based software. Samples were tested on the Bio-Rad CFX-96 and -Opus real-time PCR cycler and the experimental setup and testing of variables, including testing by an independent lab, was performed as described in ISO 16140-6:2019 [1].

Salmonella spp. inclusivity (600/600) and exclusivity (394/394) testing yielded 100% correct results. Typing of *Salmonella* spp. yielded 99.8% correct results for inclusivity (1228/1230) and 99% for exclusivity (408/412).
For confirmation of *Salmonella* no deviating results were found. For typing the range of correct results was 99.7-100% for inclusivity strains and 100% for exclusivity strains. One S. Poona strain was misidentified as S. Abaetetuba on both XLD and NA agar due to an erroneous result of a single marker.

Ruggedness experiments performed by the method developer indicate that the results are not affected by small deviations in execution of the test. In addition, the lot-to-lot stability study yielded reproducible results over lots of different ages. Based on these results, the CTS 2.0 provides a quick and reliable alternative to traditional serotyping for the confirmation and serotyping of suspected *Salmonella* colonies on XLD or Nutrient Agar.

Because of the molecular nature of the test, CTS 2.0 is also able to serotype *Salmonella* auto-agglutinable or rough strains. These strains cannot be typed by the conventional method. Each strain receives a unique score and if this score is in the serovar database, the corresponding serovar name will be assigned to the result. Further expansion of the database will provide additional serovar confirmations without changing the test itself.

Another advantage of the test is that analysis of the results uses cloud-based software, negating the need for any software updates by customers.

Table 3: Summary of results for confirmation and typing (1)

	Medium	**PCR system**	**Correct results confirmation**	**Correct results confirmation %**	**Correct results typing**	**Correct results typing %**
Inclusivity	NA	Bio-Rad CFX 96	**150/150**	**100%**	**310/310**	**100%**
	XLD		**150/150**	**100%**	**304/304**	**100%**
	NA	CFX Opus 96	**150/150**	**100%**	**310/311**[a]	**99.7%**
	XLD		**150/150**	**100%**	**307/308**[a]	**99.7%**
Exclusivity	NA	Bio-Rad CFX 96	**101/101**	**100%**	**104/104**	**100%**
	XLD		**94/94**	**100%**	**102/102**	**100%**
	NA	CFX Opus 96	**100/100**	**100%**	**103/103**	**100%**
	XLD		**96/96**	**100%**	**102/102**	**100%**

[a] Strain CTS_239, Salmonella Poona, was identified as S. Abaetetuba

Table 4: *Salmonella* serovars used in the typing study (1)

Inclusivity Serovar	# Strains tested[a]	Correct serovar result on CFX96/XLD	Correct serovar result on CFX96/NA	Correct serovar result on CFX Opus/XLD	Correct serovar result on CFX Opus/NA
S. Abaetetuba	4	4	4	4	4
S. Agona	5	5	5	5	5
S. Alachua	5	5	5	5	5
S. Albany	5	5	5	5	5
S. Anatum	5	5	5	5	5
S. Bovismorbificans	5	5	5	5	5
S. Braenderup	6	6	6	6	6
S. Brandenburg	5	5	5	5	5
S. Bredeney	5	5	5	5	5
S. Cerro	5	5	5	5	5
S. Choleraesuis	6	6	6	6	6
S. Corvallis	5	5	5	5	5
S. Cubana	4	4	4	4	4

S. Derby	5	5	5	5	5
S. Dublin	5	5	5	5	5
S. Enteritidis	5	5	5	5	5
S. Gall. Gallinarum	5	5	5	5	5
S. Gall. Pullorum	5	5	5	5	5
S. Give	5	5	5	5	5
S. Goldcoast	5	5	5	5	5
S. Hadar	5	5	5	5	5
S. Havana	5	5	5	5	5
S. Heidelberg	5	5	5	5	5
S. Idikan	5	5	5	5	5
S. Infantis	6	6	6	6	6
S. Javiana	5	5	5	5	5
S. Kentucky	5	5	5	5	5
S. Livingstone	5	5	5	5	5
S. London	5	5	5	5	5
S. Mbandaka	5	5	5	5	5
S. Minnesota	5	5	5	5	5
S. Molade	4	4	4	4	4
S. Montevideo	5	5	5	5	5
S. Muenchen	6	6	6	6	6
S. Muenster	6	6	6	6	6
S. Newport	5	5	5	5	5
S. Ohio	5	5	5	5	5
S. Oranienburg	5	5	5	5	5
S. Orion	5	5	5	5	5
S. Ouakam	5	5	5	5	5
S. Panama	5	5	5	5	5
S. Paratyphi B (Java)	5	5	5	5	5
S. Poona	5	5	5	4	4
S. Reading	5	5	5	5	5
S. Rissen	5	5	5	5	5
S. Saintpaul	4	4	4	4	4
S. Sandiego	5	5	5	5	5
S. Schwarzengrund	5	5	5	5	5
S. Senftenberg	5	5	5	5	5
S. Stanley	5	5	5	5	5
S. Tennessee	5	5	5	5	5
S. Thompson	5	5	5	5	5
S. Typhimurium	5	5	5	5	5
mST[b]	5	5	5	5	5
S. Uganda	4	4	4	4	4
S. Virchow	5	5	5	5	5
S. Worthington	5	5	5	5	5
S. Yoruba	5	5	5	5	5
S. 4,[5],12:d:-	5	5	5	5	5
Total	295	295	295	294	294

[a]Because of availability for a few serovars only 4 strains have been tested. To compensate for this, other serovars been tested with 6 strains

[b]mST = monophasic variant of S.Typhimurium (1,4,[5],12:i:-)

REFERENCES CITED

1. Boleij, P.A., Validation of Check & Trace Salmonella 2.0 for Confirmation and Typing of *Salmonella* spp., AOAC *Performance Tested Methods*[SM] certification number 072301.