OIE Procedure for Validation and Certification of Diagnostic Assays
OIE approval of application for a new, shortened protocol
for Check & Trace Salmonella (CTS)

Validation Studies Abstract – Supplementary Data

Name of the diagnostic kit: Check&Trace Salmonella
Manufacturer: Check-Points
OIE Registration Number: 20110106
Date of Registration: May 2011 / Renewal May 2016 / Amended May 2020

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General information of Check&Trace Salmonella

Disease: Salmonellosis
Pathogen Agent: *Salmonella* spp.
Type of Assay: The Check&Trace Salmonella (CTS) kit is a multiplex LDR PCR reaction followed by detection on a diagnostic micro array.

Purpose of Assay: Certified by the OIE as fit for rapid (molecular) confirmation and serotyping of presumptive *Salmonella* spp. of the following 22 serotypes:
Agona, Anatum, Bredeney, Derby, Dublin, Enteritidis, Hadar, Heidelberg, Indiana, Infantis, Kottbus, Mbandaka, Montevideo, Newport, Paratyphi B, Paratyphi B v. Java, Saintpaul, Senftenberg, Tennessee, Typhimurium (and its monophasic variant 1,4,[5],12:i:) and Virchow.

Species and Specimen: for the species see purpose of the assay above. The test can be used to confirm and identify strains considered to be *Salmonella*. These isolates may have been obtained using a range of regularly applied *Salmonella* tests.

If the ISO method for *Salmonella* is used (ISO 6579), the isolates must be sampled from a pure culture on the Nutrient Agar medium (step 9.5.2. on the ISO standard) to confirm "*Salmonella*" and to type with CTS.

The medium on which they are cultured is not relevant. The validation has been carried out using non selective agar (Nutrient Agar), but the outcome of the test is independent from the medium used (see reference: A. Brisabois – MedVetNet June 2008).
Only pure cultures can be submitted to typing with CTS. If there are two strains in the same isolate, the results will be an unknown genovar combining the results of two different serotypes. A purification of the culture is necessary before to carry out again the CTS test. If the result of the CTS is still an unknown genovar, it is recommended to send the culture to a reference laboratory for serotyping because it is probable a serotype not included in the database of the CTS.
The strains should be cultured freshly prior to the test. Regular long-term storage and shipping conditions for *Salmonella* are also suitable here.

1. **Information on the kit:** General information on the kit can be asked to info@check-points.com. More technical questions can be sent to serovar@check-points.com.

2. **Summary of supplementary validation studies**

2.1 Background

Check&Trace Salmonella (CTS) is a molecular diagnostic kit that allows for rapid and accurate Salmonella serotyping. CTS, after extended validation, obtained OIE certificate in 2011. This document provides supplementary data to the original validation study to revise the user instructions for the kit as registered with the OIE Secretariat for Registration of Diagnostic Kits. The experiments in this document demonstrate there is no observed differences in the kit's performance (e.g., sensitivity and repeatability) when adopting the shortened protocol.

2.2 Objectives of this study

The objective of this study was to provide supplementary validation data for Check&Trace *Salmonella* (CTS). In this study, a new, shortened user instruction for the kit was compared to the original previously approved user instruction for the kit. This new shortened protocol changes several steps across the entire user instruction, to save a total of 59 minutes in comparison to the original user instruction (see Table 1).

Table 1: List of the changes in the protocol

	Normal protocol	Short protocol	Time saving
Lysis	15 min	5 min	10 min
A	24 x 5 min	25 x 4 min	20 min
B	45 min	30 min	15 min
C	Not changed	Not changed	
D: 1st wash	2 x 2 min	2 x 1 min	2 min
D: 1st blocking	5 min	3 min	2 min
D: 2nd blocking	10 min	5 min	5 min
D: Conjugation	15 min	12 min	3 min
D: 2nd wash	2 x 2 min	2 x 1 min	2 min
			59 min

2.3 Materials and Methods

This study was performed using the CTS kit and all its components as described in the original validation study of the OIE in 2011 and CTS manual. All experiments in this study were done using the same batch of these components.

2.4 Results: Comparison study of the protocols

2.4.1 Comparison of sensitivity of both protocols to identify *Salmonella* serovars

The first goal of this trial was to compare the sensitivity of both protocols to correctly identify the *Salmonella* serovars. The isolates used for this trial were serotyped according to the White-Kauffman-LeMinor scheme in an accredited reference laboratory. Twenty-two unique serotypes represented by 110 strains were selected to reach this goal (Table 2). Each strain was tested twice, resulting in 220 independent tests. A summary of the results is shown in table 3. From this table, the following conclusions can be drawn: 110 out of 110 tests (100%) gave correct results with the normal protocol and 110 out of 110 tests (100%) with the short protocol.

Table 2: List of the serovars used for the study

Serotype approved in original OIE application (2011)	Serotype	Included in this study	Number of Strains
1,4,[5],12:i:-	1	Yes	5
Agona	2	Yes	5
Anatum	3	Yes	5
Bredeney	4	Yes	5
Derby	5	Yes	5
Dublin	6	Yes	5
Enteritidis	7	Yes	5
Hadar	8	Yes	5
Heidelberg	9	Yes	5
Indiana	10	Yes	5
Infantis	11	Yes	5
Kottbus	12	Yes	5
Mbandaka	13	Yes	5
Montevideo	14	Yes	5
Newport	15	Yes	5
Paratyphi B	16	Yes	5
Paratyphi B v. Java	17	Yes	5
Saintpaul	18	Yes	5
Senftenberg	19	Yes	5
Tennessee	20	Yes	5
Typhimurium	21	Yes	5
Virchow	22	Yes	5
	Total		**110**

Table 3: Comparison of sensitivity of normal protocol (NP) and shortened protocol (SP) on the serotype level

Serotype KW	Number of Strains	Number of Tests	Correct Results NP	Correct Results % NP	Correct Results SP	Correct Results % SP
1,4,[5],12:i:-	5	5	5	100%	5	100%
Agona	5	5	5	100%	5	100%
Anatum	5	5	5	100%	5	100%
Bredeney	5	5	5	100%	5	100%
Derby	5	5	5	100%	5	100%
Dublin	5	5	5	100%	5	100%
Enteriditis	5	5	5	100%	5	100%
Hadar	5	5	5	100%	5	100%
Heidelberg	5	5	5	100%	5	100%
Indiana	5	5	5	100%	5	100%
Infantis	5	5	5	100%	5	100%
Kottbus	5	5	5	100%	5	100%
Mbandaka	5	5	5	100%	5	100%
Montevideo	5	5	5	100%	5	100%
Newport	5	5	5	100%	5	100%
Paratyphi B	5	5	5	100%	5	100%
Paratyphi B v. Java	5	5	5	100%	5	100%
Saintpaul	5	5	5	100%	5	100%
Senftenberg	5	5	5	100%	5	100%
Tennessee	5	5	5	100%	5	100%
Typhimurium	5	5	5	100%	5	100%
Virchow	5	5	5	100%	5	100%
Total	**110**	**110**	**110**	**100%**	**110**	**100%**

2.4.2 Repeatability trial of the shortened protocol

The same twenty-two unique serovars, as used in the sensitivity study, were used to determine the repeatability of the shortened protocol. The isolates used for this trial were serotyped according to the White-Kauffman-LeMinor scheme in an accredited reference lab. Three technicians performed the testing, in accordance with standardised testing protocols. The results are as shown in the table 4. Each strain was tested at least twice by each technician. All technicians identified correctly 100% of all tested strains.

Table 4: Comparison between technicians using the shortened protocol

		Technician A			Technician B			Technician C		
Serotype KW	**Nr. strains**	**Nr. tests**	**Correct Results**	**Correct Results %**	**Nr. tests**	**Correct Results**	**Correct Results %**	**Nr. tests**	**Correct Results**	**Correct Results %**
1,4,[5],12:i:-	5	5	5	100%	3	3	100%	3	3	100%
Agona	5	5	5	100%	3	3	100%	3	3	100%
Anatum	5	5	5	100%	3	3	100%	3	3	100%
Bredeney	5	5	5	100%	3	3	100%	3	3	100%
Derby	5	5	5	100%	3	3	100%	3	3	100%
Dublin	5	5	5	100%	3	3	100%	3	3	100%
Enteriditis	5	5	5	100%	3	3	100%	3	3	100%
Hadar	5	5	5	100%	3	3	100%	3	3	100%
Heidelberg	5	5	5	100%	3	3	100%	3	3	100%
Indiana	5	5	5	100%	3	3	100%	3	3	100%
Infantis	5	5	5	100%	3	3	100%	3	3	100%
Kottbus	5	5	5	100%	2	2	100%	2	2	100%
Mbandaka	5	5	5	100%	2	2	100%	2	2	100%
Montevideo	5	5	5	100%	2	2	100%	2	2	100%
Newport	5	5	5	100%	2	2	100%	2	2	100%
Paratyphi B	5	5	5	100%	2	2	100%	2	2	100%
Paratyphi B v. Java	5	5	5	100%	2	2	100%	2	2	100%
Saintpaul	5	5	5	100%	2	2	100%	2	2	100%
Senftenberg	5	5	5	100%	2	2	100%	2	2	100%
Tennessee	5	5	5	100%	2	2	100%	2	2	100%
Typhimurium	5	5	5	100%	2	2	100%	2	2	100%
Virchow	5	5	5	100%	2	2	100%	2	2	100%
Total	**110**	**110**	**110**	**100%**	**55**	**55**	**100%**	**55**	**55**	**100%**

3. Conclusions

In this study, there was no observed difference in the kit's performance (in regards to sensitivity and repeatability) when the samples were tested with the original submitted protocol (normal protocol) and the new protocol (shortened protocol). As the shortened protocol enables technicians to complete the CTS test in 59 minutes less time, it offers advantages in terms of work scheduling.

4. Publications

- Diep B., Barretto C., Portmann A., Fournier C., Karczmarek A., Voets G., Li S., Deng X., Klijn A. Salmonella Serotyping; Comparison of the Traditional Method to a Microarray-Based Method and an in silico Platform Using Whole Genome Sequencing Data *Frontiers in Microbiology*, 2019, 10 (2554), pp. 1-8.

- Brisabois A. Fremy S, Moury F, Marault M, van Santen R, Dekker A, Fabre JM, Vos P, de Goeijen F. The Effect of the Culture Medium on the Performance of the Premi®Test Salmonella: A Multiplex Molecular Serotyping Test using a DNA Microarray System. Presented at: 4th Med-Vet-Net Meeting; 2008 Jun 11-14; Saint-Malo, France.