OIE Procedure for Validation and Certification of Diagnostic Assays

Abstract sheet

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

Disease: Salmonellosis

Pathogen Agent: *Salmonella* spp.

Type of Assay: The Check&Trace Salmonella 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 4,12: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 PTS.

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 PTS. 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 PTS test. If the result of the PTS 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 PTS.

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. The more technical questions can be sent to serovar@check-points.com.

2. Summary of validation studies

Analytical characteristics

Calibration: The PTS is based on the presence/absence pattern of a number of specific DNA markers. The pattern is translated into a numerical code (genovar), which may be associated with known serotypes.

Because the genovar is linked with DNA markers which are not always coding antigens used for the serotyping, it is possible to have different genovar (corresponding into different combination of DNA markers) linked with the same serotype. , This expresses the genetic variability inside a serotype.

The initial choice of DNA markers potentially allows having a big spectrum of different combinations.

In order to design the PTS, the selection of the targeted DNA sequences was done to have the possibility to differentiate the maximum of serotypes (in other words the targeted DNA sequences are the best to improve the chance to see the difference between two serotypes).

To develop the software and to build the PTS database, the main serotypes (most often encountered in the laboratories) were tested. For these serotypes, the genovar (combination of positive spots) became known and were included in the PTS software (and became the first PTS-list).

The "PTS database" can be expanded each time a new serotype can be linked with a specific (unique) genovar-code (combination of DNA markers).

Repeatability: As regards serotype identification the repeatability was:

- 91% in trial 1 (3 strains of 6 different serotypes tested twice by 2 different technicians);

- 96% in trial 4 (5 strains of 5 major serotypes tested twice by 5 different laboratories).

For spot reaction:

- Trial 1 was designed in order to test all the spots involved in serotype identification;

- For 17 spots 100% of the results were satisfactory and for 3 spots higher than 95% of the results were satisfactory;

- Finally 390 interpretable spots (for chosen serotypes) were tested twice and 387 repetitions were satisfactory: 99.2% of repeatability (lower Confidence Limit = 98.5%).

Analytical specificity and sensitivity of the kit

The serotypes tested were serotypes with more than 20 positives strains and were the following ones:

- The 9 regulatory serotypes;
- The 10 serotypes of the other top 20;
- 2 other serotypes.

Globally, it means that, for each serotypes, at least 20 “positive” strains and around 1700 “negative” strains (other serotypes) were tested with both methods (Kaufman-White [KW] and PTS).

		Number of positive strains	Number of negative strains
Agona		28	1691
Anatum		40	1679
Bredeney		26	1693
Derby		42	1677
Dublin		43	1676
Enteritidis	Regulatory (*)	75	1644
Hadar	Regulatory (*)	53	1666
Heidelberg	Regulatory (*)	30	1689
Indiana		25	1694
Infantis	Regulatory (*)	38	1681
Kottbus		28	1691
Mbandaka		39	1680
Montevideo	Regulatory (*)	36	1683
Newport	Regulatory (*)	30	1689
Paratyphi B		24	1695
Paratyphi B v Java		41	1678
Saintpaul		27	1692
Senftenberg		39	1680
Tennessee		47	1672
Typhimurium	Regulatory (*)	105	1614
Virchow	Regulatory (*)	39	1680
I4,12:i:-	Regulatory (*)	22	1697

(*) In European Union and/or United States.

Analytical specificity

The hypothesis tested was: H0: The percentage of same results between PTS and KW method is q=99%.

The following table summarises the results:

Serotype		False + (FP)	True - (TN)	Total (FP+TN)	%Sp	A= Maximum of number of discordant if q0=99%	Conclusion
Agona		0	1691	1691	100,0%	27	PTS is 99% equivalent to KW
Anatum		0	1679	1679	100,0%	27	PTS is 99% equivalent to KW
Bredeney		0	1693	1693	100,0%	27	PTS is 99% equivalent to KW
Derby		0	1677	1677	100,0%	27	PTS is 99% equivalent to KW
Dublin		4	1672	1676	99,8%	27	PTS is 99% equivalent to KW
Enteritidis	Regulatory	3	1641	1644	99,8%	27	PTS is 99% equivalent to KW
Hadar	Regulatory	1	1665	1666	99,9%	27	PTS is 99% equivalent to KW
Heidelberg	Regulatory	0	1689	1689	100,0%	27	PTS is 99% equivalent to KW
Indiana	Top 20	0	1694	1694	100,0%	27	PTS is 99% equivalent to KW
Infantis	Regulatory	0	1681	1681	100,0%	27	PTS is 99% equivalent to KW
Kottbus		0	1691	1691	100,0%	27	PTS is 99% equivalent to KW
Mbandaka		0	1680	1680	100,0%	27	PTS is 99% equivalent to KW
Montevideo	Regulatory	0	1683	1683	100,0%	27	PTS is 99% equivalent to KW
Newport	Regulatory	1	1688	1689	99,9%	27	PTS is 99% equivalent to KW
Paratyphi B		0	1695	1695	100,0%	27	PTS is 99% equivalent to KW
Paratyphi B v Java		0	1678	1678	100,0%	27	PTS is 99% equivalent to KW
Saintpaul		0	1692	1692	100,0%	27	PTS is 99% equivalent to KW
Senftenberg		0	1680	1680	100,0%	27	PTS is 99% equivalent to KW
Tennessee		0	1672	1672	100,0%	27	PTS is 99% equivalent to KW
Typhimurium	Regulatory	0	1614	1614	100,0%	27	PTS is 99% equivalent to KW
Virchow	Regulatory	0	1680	1680	100,0%	27	PTS is 99% equivalent to KW
I4,12:i:-	Regulatory	0	1697	1697	100,0%	27	PTS is 99% equivalent to KW

For the False Positives, the following comments were made by the applicant:

- For Dublin, the false positives concern 2 Salmonella Rostock and 2 Salmonella Kiel. These two serotypes have never been identified by CDC during ten years.
- For Enteritidis, the false positives also includes very rare serotypes: 2 Salmonella Blegdam and 2 Salmonella Gallinarum gallinarum, with a frequency of 0,008% and 0,003% in the CDC database.
- For Hadar, the FP is a confusion with Istanbul serotype (0,06% of the Salmonella database of CDC).
- For Newport, the FP is a confusion with an Hadar serotype (1,02% of the Salmonella in the CDC database).
- For Schwarzengrund, the FP is a confusion with a Panama serotype (0,43% of the Salmonella in the CDC database).
- The mistakes mainly concern rare serotypes. So, the frequency of False Positives would be lower in a routine laboratory.

Analytical sensitivity

In a first step, the hypothesis tested was H0: a minimum q0=99% PTS in accordance with KW with a risk α of 1% only with the mistakes (wrong serotype) in the "false negatives."

The following table summarises the results:

Serotype		False - (FN) with only mistake	True + (TP)	Total (TP+FN)	%Se with only mistake	A= Maximum of number of discordant if q0=99% (i)	Conclusion	Estimation of risk β(%) to accept a q1 < 80% (ii)
Agona		1	27	28	96%	1	PTS is 99% equivalent to KW	4,4%
Anatum		0	39	39	100%	2	PTS is 99% equivalent to KW	0,8%
Bredeney		0	25	25	100%	1	PTS is 99% equivalent to KW	5,3%
Derby		1	39	40	98%	2	PTS is 99% equivalent to KW	0,8%
Dublin		0	42	42	100%	2	PTS is 99% equivalent to KW	0,8%
Enteritidis	Regulatory	0	71	71	100%	3	PTS is 99% equivalent to KW	0,2%
Hadar	Regulatory	1	51	52	98%	2	PTS is 99% equivalent to KW	0,6%
Heidelberg	Regulatory	0	29	29	100%	2	PTS is 99% equivalent to KW	4,1%
Indiana		2	23	25	92%	2	PTS is 99% equivalent to KW	5,3%
Infantis	Regulatory	0	36	36	100%	2	PTS is 99% equivalent to KW	1,9%
Kottbus		0	26	26	100%	2	PTS is 99% equivalent to KW	5,0%
Mbandaka		0	38	38	100%	2	PTS is 99% equivalent to KW	1,3%
Montevideo	Regulatory	1	34	35	97%	2	PTS is 99% equivalent to KW	2,2%
Newport	Regulatory	0	26	26	100%	2	PTS is 99% equivalent to KW	5,0%
Paratyphi B		0	22	22	100%	2	PTS is 99% equivalent to KW	6,2%
Paratyphi B v Java		0	41	41	100%	2	PTS is 99% equivalent to KW	0,8%
Saintpaul		0	26	26	100%	2	PTS is 99% equivalent to KW	5,0%
Senftenberg		0	37	37	100%	2	PTS is 99% equivalent to KW	1,6%
Tennessee		0	47	47	100%	2	PTS is 99% equivalent to KW	0,7%
Typhimurium	Regulatory	0	103	103	100%	4	PTS is 99% equivalent to KW	0,0%
Virchow	Regulatory	0	38	38	100%	2	PTS is 99% equivalent to KW	1,3%
I4,12:i:-	Regulatory	0	21	21	100%	1	PTS is 99% equivalent to KW	6,5%

(i) A has been estimated following the Binomial Law (AFNOR NF X V 03-111).
(ii) A has been estimated following the Binomial Law (AFNOR NF X V 03-111).

The following table gives the results for the 22 serotypes.

- Hypothesis H0 is confirmed for all serotypes;
- The risk β is always less than 6,5%, and less than 5% for 18/22 serotypes.

In a second step, the hypothesis tested was H1: a minimum q0=95% PTS in accordance with KW with a risk α of 1% with the mistakes (wrong serotypes) and genovar scores included in the "false negatives".

The following table summarises the results:

Serotype		False - (FN) with mistake and genovar score	True + (TP)	Total (TP+FN)	%Se with mistake and genovar score	A= Maximum of number of discordant if q_0=95% (i)	Conclusion	Estimation of risk β(%) to accept a q_1 < 80% (ii)
Agona		1	27	28	96%	3	PTS is 95% equivalent to KW	31,1%
Anatum		1	39	40	98%	5	PTS is 95% equivalent to KW	16,1%
Bredeney		1	25	26	96%	3	PTS is 95% equivalent to KW	33,6%
Derby		3	39	42	93%	5	PTS is 99% equivalent to KW	15,4%
Dublin		1	42	43	98%	5	PTS is 95% equivalent to KW	15,1%
Enteritidis	Regulatory	4	71	75	95%	8	PTS is 95% equivalent to KW	4,6%
Hadar	Regulatory	2	51	53	96%	6	PTS is 95% equivalent to KW	11,8%
Heidelberg	Regulatory	1	29	30	97%	4	PTS is 95% equivalent to KW	28,6%
Indiana		2	23	25	92%	3	PTS is 95% equivalent to KW	34,9%
Infantis	Regulatory	2	36	38	95%	4	PTS is 95% equivalent to KW	18,6%
Kottbus		2	26	28	93%	3	PTS is 95% equivalent to KW	31,1%
Mbandaka		1	38	39	97%	4	PTS is 95% equivalent to KW	17,4%
Montevideo	Regulatory	2	34	36	94%	4	PTS is 95% equivalent to KW	21,1%
Newport	Regulatory	4	26	30	87%	4	PTS is 95% equivalent to KW	28,6%
Paratyphi B		2	22	24	92%	3	PTS is 95% equivalent to KW	36,1%
Paratyphi B v Java		0	41	41	100%	5	PTS is 95% equivalent to KW	15,8%
Saintpaul		1	26	27	96%	3	PTS is 95% equivalent to KW	32,4%
Senftenberg		2	37	39	95%	4	PTS is 95% equivalent to KW	17,4%
Tennessee		0	47	47	100%	5	PTS is 95% equivalent to KW	13,8%
Typhimurium	Regulatory	2	103	105	98%	10	PTS is 95% equivalent to KW	0,6%
Virchow	Regulatory	1	38	39	97%	4	PTS is 95% equivalent to KW	17,4%
I4,12:i:-	Regulatory	1	21	22	95%	3	PTS is 95% equivalent to KW	38,6%

(i) A has been estimated following the Binomial Law (AFNOR NF X V 03-111).
(ii) A has been estimated following the Binomial Law (AFNOR NF X V 03-111).

The table gives the results for the 22 serotypes.

- Hypothesis H1 is confirmed for all serotypes;
- The risk β is less than 36%, and less than 16% for 7/22 serotypes.

The detailed results for the “false negatives” are given as follow by the applicant:

For Derby:

- There is one mistake. The PTS gives the serotype Livingstone.
- In the two other cases, the PTS has given a genovar score without any identification.

For Enteritidis:

- The PTS has given a genovar score for the 4 False negatives.

For Newport:

- 4 strains have been identified with the genovar score. Three of them have the same genovar score.

Reproductibility

5 European laboratories were involved in the trial (Belgium, Germany, Netherlands, Spain, United Kingdom).

Each laboratory tested 26 samples (5 strains of *Salmonella* regulatory serotypes tested twice, 15 strains of other serotypes of *Salmonella* tested once and one Non *Salmonella tested once*) with two methods: reference method (KW) and PTS.

In average 97% of strains were well identified with the PTS (*Salmonella* + good serotype) and 86% with KW (because one of the laboratories had bad results).

Applications

The kit is currently used in different laboratories world-wide and in the food industry.

References

Publications:

- Wattiau P., Weijers T., Andreoli P., Schliker C., Veken H.V., Maas H.M.E., Verbruggen A.J., Heckc M.E.O.C, Wannetc W.J., Imberechts H., Vos P. Evaluation of the Premi®Test Salmonella, a commercial low-density DNA microarray system intended for routine identification and typing of Salmonella enterica. *International Journal of Food Microbiology*, 2008, 123 (3), pp. 293-298.
- Wattiau P., Van Hessche M., Schlicker C., Vander Veken H., Imberechts H. Comparison of Classical Serotyping and PremiTest Assay for Routine Identification of Common Salmonella enterica Serovars. *J. Clin. Microbiol*., 2008; 46, pp. 4037 - 4040.

Posters:

- Rapid detection of salmonella serotypes in feed and food using SST. Poster presented at the meeting “UW-RiverFalls Food Microbiology symposium” in 2006.
- Salmonella Molecular Serotyping with a DNA microarray: an approach for non agglutinable Salmonella enterica serotypes. Congrès ICEID, Atlanta, 17-19 mars 2008.
- The Effect of the Culture Medium on the Performance of “SST”: A Multiplex Molecular Serotyping Test using a DNA Microarray System. MedVetNet, June 08.
- A multiplex molecular serotyping test using a DNA microarray system as an alternative Salmonella serotyping method. Rapid Methods, January 2009.
- DNA Microarray evaluation for Salmonella serotyping. MedVetNet, June 09
- Short Evaluation of the Premi®Test Salmonella Method, Food Micro 2010, Copenhagen 30 August – 3 September 2010.

- Comparison of Two Molecular Methods for Serotyping Salmonella, abstract presented at Fern 2010 by Junia Jean-Gilles Beaubrun, Ph.D. Microbiologist, Food and Drug Administration.
- Interlaboratory study of a DNA Microarray for Salmonella Molecular Serotyping in comparison to the conventional serotyping method. i3S, International Symposium Salmonella and Salmonellosis, 28, 29 and 30th of June 2010 Saint-Malo – France by Anne Brisabois, AFSSA.