



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
121001

The AOAC Research Institute hereby certifies the method known as:

Check&Trace Salmonella (previously marketed as Premi[®]Test Salmonella)

manufactured by

Check-Points

Binnenhaven 5

6709 PD Wageningen

The Netherlands

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. 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.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

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

Issue Date

December 13, 2024

Expiration Date

December 31, 2025

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| AUTHORS ORIGINAL VALIDATION: Ron von Santen, Joost Thijssen, and Anne Brisadois MODIFICATION FEBRUARY 2021: Joep van Bortel and Pieter Vos MODIFICATION AUGUST 2023: Joep van Bortel and Pieter Vos | SUBMITTING COMPANY DSM Premi®Test B.V. P.O. Box 1163 6160 DB Geleen The Netherlands | CURRENT SPONSOR Check-Points Binnenhaven 5 6709 PD Wageningen The Netherlands |
| METHOD NAME Check&Trace Salmonella Previously marketed as Premi®Test Salmonella | CATALOG NUMBER 10-0010 | |
| INDEPENDENT LABORATORY Agence Nationale de Sécurité de l'alimentation De l'environnement et du travail (ANSES) Laboratoire de Sécurité des Aliments de Maisons-Alfort 23 Avenue du Général de Gaulle 94706 Maisons-Alfort France | APPLICABILITY OF METHOD Target organism – <i>Salmonella</i> spp. Matrices – Pure cultures Performance claims – The Check&Trace Salmonella has been validated for two claims: 1) Confirmation of <i>Salmonella</i> isolates to <i>Salmonella</i> genus, and 2) correct allocation to one of 139 serotypes. | |
| ORIGINAL CERTIFICATION DATE December 16, 2010 | CERTIFICATION RENEWAL RECORD Renewed through December 2025. | |
| METHOD MODIFICATION RECORD 1. December 2011 Level 1 2. March 2014 Level 2 3. December 2018 Level 1 4. March 2020 Level 1 5. February 2021 Level 2 6. August 2023 Level 2 | SUMMARY OF MODIFICATION 1. Test kit name change. 2. Expanded the number of serotypes identified by software. 3. Version number and date change on user manual. 4. Editorial changes. 5. Addition of algorithm to improve ability to correctly recognize background and label isolates with more background as non-Salmonella. 6. Software update to definition file and addition of 4 serovars: Kingston, Molade, Jerusalem, and 4, 12, i:-. | |
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PRINCIPLE OF THE METHOD (1)

The Check&Trace Salmonella (formerly Premi®Test Salmonella) identifies the serotype of a pure *Salmonella* culture, based on bacterial DNA. The test can use any (suspect) *Salmonella* isolate, regardless of the method used to isolate the culture. The PTS combines multiplex ligation dependent PCR with simultaneous detection of the PCR products on a micro array. The hybridization pattern on the micro array is read using a camera and interpreted using dedicated software.

DISCUSSION OF THE VALIDATION STUDY (1)

The data presented in this report indicate that the Check&Trace Salmonella is a reliable and suitable tool for the confirmation (claim 1) and subsequent serotyping (claim 2) of *Salmonella* cultures. Both the observed sensitivity and specificity are 100%. In addition, the non-target *Salmonella* strains were correctly not assigned to a claimed serotype. The ruggedness data indicate that the results are not affected by small deviations in the execution of the test. In addition, the lot-to-lot stability data prove that the test performs well over its entire shelf life and yields reproducible results over different lots.

As a result, the Check&Trace Salmonella is a suitable alternative for traditional serotyping. It provides laboratories with a rapid and robust way to identify and serotype pure, presumptive *Salmonella* isolates. Because of its high specificity, preliminary tests to determine whether the isolate belongs to the *Salmonella* genus are not required. Closely related non *Salmonella* isolates are typed as “No *Salmonella*”. The methodology used to isolate the organism is not important, allowing the Check&Trace Salmonella to be used in combination with any commonly used *Salmonella* detection method. The only requirement is to have a pure culture. The Check&Trace Salmonella protocol recommends the use of a non selective agar, but the test has proven to yield good results with other media as well [2]. The sensitivity and specificity data presented in this report are in line with data published previously. Wattiau *et al.* reported 87% sensitivity for a previous version of the test [2]. At that time, the DNA extraction procedure had not been standardized. The importance of a proper DNA extraction procedure was indicated in the same publication. When executed with purified genomic DNA, the sensitivity increased to 95%. Since then, the DNA extraction procedure has been standardized and the required materials have been included in the test kit. A more recent study confirms the enhanced sensitivity score (98%) since standardization of the DNA extraction [3].

The Check&Trace Salmonella provides users with an easy to use alternative for traditional serotyping. The test doesn’t require an extensive range of sera and extensive experience for reliable execution. Each kit contains the required reagents and minimal training is required for execution. New users receive an on site training for 2 days, after which they are normally able to execute the test themselves. Automated reading, without user interpretation, significantly reduces the level of experience required for reliable execution. Therefore, the test is especially interesting for laboratories considering to start with *Salmonella* serotyping themselves.

The Check&Trace Salmonella is also able to serotype *Salmonella* strains that cannot be typed using the conventional serotyping method: so called non agglutinable or rough strains. An external study has indicated that the PTS results correlate very well with results obtained by Pulsed Field Gel Electrophoresis for these difficult strains [4]

The current list of serotypes recognized by the Premi®Test Salmonella includes 100 serotypes. Even though more than 2500 *Salmonella* serotypes have been described, the current list contains the ones most frequently encountered in practice. The list includes all serotypes from the 20 most frequently reported *Salmonella* serotypes from human sources [5]. Moreover, 87% of all strains isolated by CDC from human sources between 1996 and 2006 belong to the 100 serotypes present on the PTS list (338214 out of 390676 isolates) [4].

Experience has shown that serotypes not yet present on the PTS list often yield unique and reproducible DNA patterns (reflected in a genovar score without a specific serotype). Once these genovar scores have been found for 3 independent strains, the serotype is added to the database. Using this approach, the number of serotypes recognized by the test has steadily increased from 64 to 100 in the last three years. It is expected to grow even more in the near future.

Table 4: Summary of results obtained for inclusivity and exclusivity testing (1)

| Strains | Number tested | Number of strains correctly assigned to | | Specificity | Sensitivity |
|------------------------------------|------------------|---|----------|-------------|-------------|
| | | <i>Salmonella</i> | Serotype | | |
| Inclusivity: target strains | | | | | |
| Claimed serotypes | 300 ^a | 300 | 300 | 100% | 100% |
| <i>S. bongori</i> | 3 | 3 | 3 | 100% | 100% |
| Exclusivity: non targets | | | | | |
| Non claimed serotypes | 10 | 10 | n.a. | 100% | n.a. |
| Other subspecies ^b | 7 | 7 | n.a. | 100% | n.a. |
| Non-Salmonella | 30 | 0 | n.a. | 100% | n.a. |

^a : 3 strains for each of the 100 claimed serotypes. 90 and 10 serotypes covered by internal and independent data respectively.

^b : Subspecies tested: arizonae, diarizonae, houtenae, indica and salamae

n.a.: Not applicable for non target strains

DISCUSSION OF MODIFICATION APPROVED AUGUST 2023 (6)

Existing data from previous validations, method updates and QC data was reanalyzed with the current version of definitions and a proposed update of the definitions file that includes improvements to existing patterns and the addition of new patterns, or “genotypes”, to recognize more *Salmonella* with the same method. Some unresolved results in the existing data were attributed to 1) corrupt raw data, 2) manual interpretation of data at the time original validation and 3) samples that were rerun but had no definitive calling by the software. Results like “DNA recognition not OK” or “Reference spots not Found” were not used for calculations. The dataset creates an accurate image of the impact of the changes to the *Salmonella* definitions in our software and shows no evidence that the alterations to current pattern definitions have an effect. This shows the more stringent pattern definitions have no detectable effect on pattern recognition. Not seen in this dataset as these conditions were not present, but in high background conditions or cross-contaminations events, the more stringent definitions will reduce the likelihood of false identifications.

Table 1 Strains used for extension of recognized strains. (6)

| Serotype | Sample type | Source |
|------------|------------------------|-------------|
| Kingston | Production environment | dust sample |
| Kingston | Production environment | dust sample |
| Kingston | Production environment | dust sample |
| Kingston | | |
| Kingston | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Molade | | |
| Jerusalem | | |
| Jerusalem | | |
| Jerusalem | | |
| Jerusalem | | |
| Jerusalem | | |
| 4, 12, i:- | | |
| 4, 12, i:- | | |
| 4, 12, i:- | | |
| 4, 12, i:- | | |
| 4, 12, i:- | | |
| Derby | new genetic variant | |
| Derby | new genetic variant | |
| Derby | new genetic variant | |
| Derby | Food | Chicken |
| Derby | Collection | |
| Derby | Collection | |

Table 2 Exclusivity for extension (6)

| Serotype | Sample type | Source |
|-------------|----------------------|---------------|
| Aba | AOAC Validation data | |
| Potsdam | Lab sample | |
| Elomrane | AOAC Validation data | Factory swab |
| Goelzau | AOAC Validation data | Meat |
| Hithergreen | AOAC Validation data | ex Coda |
| Hofit | AOAC Validation data | Monkey faeces |
| Telaviv | Lab sample | |
| Teddington | Lab sample | |
| Irumu | collection | |
| Ordonez | collection | |
| Arizonae | AOAC Validation data | |
| Diarizonae | AOAC Validation data | |
| Diarizonae | AOAC Validation data | |
| Houtenae | AOAC Validation data | |
| Indica | AOAC Validation data | |
| Salamae | AOAC Validation data | |

Table 3. Results for added genotypes (6)

| Serovar | Correct results out of total |
|------------|------------------------------|
| Kingston | 5/5 |
| Molade | 8/8 |
| Jerusalem | 5/5 |
| 4, 12, i:- | 5/5 |
| Derby | 6/6 |

Table 4. Summary of results obtained for comparison of results with the current definitions and with the new definitions. (6)

| Strains | Number tested | No. Errors ³ | Inconclusive | Number of serovars assigned | Sensitivity ⁴ |
|------------------------------------|---------------|-------------------------|---------------------------|-----------------------------|--------------------------|
| Inclusivity: target strains | | | | | |
| Claimed serotypes + Bongori | 507 | 3 | 10 | 494 | 100% |
| proposed new serotypes | 29 | 0 | 0 | 29 | 100% |
| Total | 536 | 3 | 10 | 524 | 100% |
| Exclusivity non targets | | | | | |
| Strains | Number tested | No. Errors ³ | Inconclusive ¹ | Number of serovars assigned | Specificity |
| Non claimed serotypes | 10 | 0 | 10 | 0 | 100% |
| Other subspecies ² | 6 | 0 | 6 | 0 | 100% |
| Total | 16 | 0 | 16 | 0 | 100% |

¹ All inconclusive results for the non targets were "genovar score" results and considered as the correct result

² Includes *Salmonella enterica* subspecies *arizonae*, *diarizonae*, *houtenae*, *indica* and *salamae*

³ Errors defined as software showing a 'DNA recognition not OK' warning or 'Hybridization error' warning. These were not used in calculations.

⁴ To calculate Sensitivity; a disagreement between reference method and Check&Trace was seen as a False Negative result.

REFERENCES CITED

1. von Santen, R., Thijssen, J., and Brisadois, A. Evaluation of the Premi[®]Test Salmonella for confirmation and serotyping of *Salmonella* isolates, AOAC Performance Tested MethodsSM certification number 121001.
2. Brisadois, A. et al. 2008. The effect of the culture medium on the performance of the Premi[®]Test Salmonella: a multiplex molecular serotyping test using a DNA micro array system. Poster presented at MedVetNet.
3. Hansen, F. et al. 2010. Short evaluation of the Premi[®]Test *Salmonella* method. Poster presented at Food Micro.
4. Brisadois, A. et al. 2008. *Salmonella* molecular serotyping with a DNA micro array: an approach for non-agglutinable *Salmonella enterica* serotypes. Poster at ICEID
5. CDC. *Salmonella* Surveillance: Annual Summary, 2006. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2008.
6. von Boret, J. and Vos, P., Modification for Determination of *Salmonella* isolates from pure colonies, AOAC Performance Tested MethodsSM certification number 121001. Approved August 16, 2023.