# 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 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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|---|---|
| Issue Date | October 3, 2022 |
| Expiration Date | December 31, 2023 |

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**METHOD NAME**
Check&Trace Salmonella
Previously marketed as Premi®Test Salmonella

**CATALOG NUMBER**
10-0010

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Modification February 2021 reviewed internally by AOAC Research Institute.

**APPLICABILITY OF METHOD**
**Target organism – *Salmonella***

**Matrices – Pure cultures**

**Performance claims - Claim 1: Confirmation of presumptive *Salmonella* isolates to *Salmonella* genus**
**- Claim 2: Allocation to one of 100 serotypes, encompassing the most commonly encountered ones, and *Salmonella bongori* (second *Salmonella* species in addition to *Salmonella enterica*)**
**The results indicate that the Check&Trace Salmonella (previously marketed as Premi®Test Salmonella) is a reliable tool for both confirmation and serotyping of presumptive *Salmonella* isolates belonging to 100 of the most frequently encountered serotypes. The reported specificity (claim 1) and sensitivity (claim 2) are both 100%.**

**ORIGINAL CERTIFICATION DATE**
**December 16, 2010**

**CERTIFICATION RENEWAL RECORD**
**Renewed annually through December 2023.**

**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**

**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.**

**Under this AOAC *Performance Tested Methods℠* License Number, 121001 this method is distributed by:**
**NONE**

**Under this AOAC *Performance Tested Methods℠* License Number, 121001 this method is distributed as:**
**NONE**

**PRINCIPLE OF THE METHOD (1)**
The Check&Trace Salmonella (formerly Premi®Test Salmonella) identifies the serotype of a pure *Salmonella* culture, based on the 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 |
|---|---|---|---|---|---|
| | | *Salmonella* | Serotype | | |
| Inclusivity: target strains | | | | | |
| Claimed serotypes | 300[a] | 300 | 300 | 100% | 100% |
| *S. bongori* | 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

**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 Methods*℠ certification number 121001.
2. Brisabois, 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. Brisabois, 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.