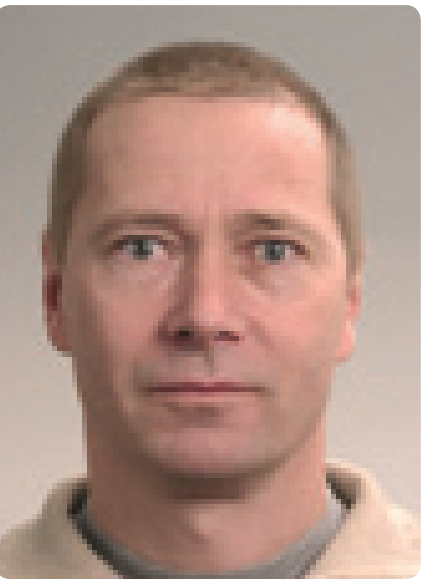




# Short Evaluation of the Premi® Test Salmonella Method

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## Objective:

In Denmark, special legislation claiming absence of *S. Dublin* and multidrug resistant (MDR) *S. Typhimurium* DT104 in fresh meat has previously been in force. Thus, the Danish meat companies needed a method for rapid detection of these organisms in order to avoid distributing contaminated meat. The Premi® Test Salmonella method (DSM PremiTest) is a rapid method for detecting and serotyping of *Salmonella* and could ideally be used for this purpose. In this study a brief evaluation of the Premi® Test Salmonella method was carried out including testing of 67 pure cultures of *Salmonella* and non-*Salmonella* and spiked samples of chicken skin, meat and fecal samples. The regular assay was supplemented with three specific DNA markers, to allow the detection of MDR *S. Typhimurium* DT104.

## Conclusion:

The Premi® Test Salmonella method performed excellent when testing pure cultures of *Salmonella*. All 59 *Salmonella* were correctly identified as *Salmonella*, and 58 (98.3%) were correctly serotyped. However, when testing samples taken directly from MSRV-plates, many false positive results were obtained from control samples not containing *Salmonella*. Also a high number of spiked samples were incorrectly serotyped. It can be concluded, that the Premi® Test Salmonella method is a valuable, flexible and reliable tool for rapid serotyping of *Salmonella* isolates, but less useful for testing sample material taken from MSRV plates.

## Materials & Methods:

Briefly, 59 *Salmonella* covering primarily MDR *S. Typhimurium* DT104, other *S. Typhimurium*, *S. Enteritidis* and *S. Dublin* and 8 strains of Gram Negative bacteria not belonging to genus *Salmonella* were included in the pure culture study. The 67 selected strains were cultured overnight in BHI at 37°C and subsequently grown on PCA. Single, isolated colonies were selected and the Premi® Test method (DNA extraction, PCR and Micro-array analysis) was subsequently performed according to the manufacturer's instructions.

Skin from chicken, slices of beef and pork and feces from pigs were inoculated in two levels (5 - 10 cfu respectively 50 - 100 cfu per 25 g) using three different *Salmonella* (*S. Dublin*, *S. Enteritidis* and MDR *S. Typhimurium* DT104). The samples were incubated for 18 - 20 hrs at 37°C in 10 x BPW. The next day 0.1 ml was inoculated in three different spots onto one plate of MSRV + 10 mg/ml novobiocine and incubated at 41.5°C for 24 hrs. Next day 5 µl was taken from the rim of the growth zone and the Premi® Test method (DNA extraction, PCR and Micro-array analysis) was subsequently performed according to the manufacturer's instructions.

Table 3: Data from testing inoculated chicken skin, meat and fecal samples

n = 12 in all levels	Correct serotype	Salmonella detected	Suspect Salmonella	Salmonella not detected
High level	8	3	1	0
Low level	6	5	1	0
Controls	0	3	5	4

From high level inoculated samples (pork/beef, chicken skin and feces), *Salmonella* was correctly detected in 11 samples. The remaining sample was reported as "suspect - please re-test". In 8 samples, the serotype was correctly identified as well.

From the low level inoculated samples (pork/beef, chicken skin and feces), *Salmonella* was also correctly detected in 11 samples, but only 6 of them were correctly serotyped. The remaining sample was reported as "suspect - please re-test".

From the 12 un-inoculated control samples, the expected answer "no *Salmonella*" was reported in only 4 cases. 5 of the control samples were reported as "suspect - please re-test" and in 3 cases "Salmonella presence" was reported, although no *Salmonella* was found using a reference cultural method.

## Results:

Table 1: Identification/serotype for 67 strains as obtained with the Premi® Test Salmonella Method

Strain/serotype	Premi® Test result	Agreement
E. coli O:rough	No Salmonella	OK
E. coli O157 tox+	Salmonella suspected, reprocess sample from the sampling step	(false positive)
E. coli O157:H42	Salmonella suspected, reprocess sample from the sampling step	(false positive)
K. oxytoca	No Salmonella	OK
P. mirabilis	No Salmonella	OK
P. vulgaris	No Salmonella	OK
Ps. aeruginosa	No Salmonella	OK
Y. enterocolitica O:3	Salmonella suspected, reprocess sample from the sampling step	(false positive)
S. 4:12:b	Salmonella, genovar 48	Species level - OK
S. adabraka	Salmonella, genovar 4478	Species level - OK
S. albania	Salmonella Albany (Genovar 3124)	OK
S. bailldon	Salmonella, genovar 14573	Species level - OK
S. berta	Salmonella Berta (Genovar 7529)	OK
S. choleraesuis	Salmonella Choleraesuis (Genovar 13545)	OK
S. dublin	Salmonella, genovar 3003	(OK)
S. dublin	Salmonella, genovar 3003	(OK)
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Manhattan (Genovar 2490) or Dublin (probability 99.92%)(Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. eastbourne	Salmonella, genovar 12916	Species level - OK
S. eastbourne	Salmonella, genovar 12908	Species level - OK
S. dublin	Salmonella Dublin (probability 99.92%) (Genovar 2488)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. enteritidis	Salmonella Enteritidis (Genovar 2994.G)	OK
S. frintrop	Salmonella, genovar 14960	Species level - OK
S. infantis	Salmonella Infantis (Genovar 9381)	OK
S. infantis	Salmonella Infantis (Genovar 9381)	OK
S. infantis	Salmonella Infantis (Genovar 9381)	OK
S. javiana	Salmonella Javiana (Genovar 12901)	OK
S. livingstone	Salmonella Livingstone (Genovar 15016)	OK
S. muenchen	Salmonella Muenchen (Genovar 11942)	OK
S. napoli	Salmonella Panama (Genovar 15933) or Napoli (Genovar 15917)	OK
S. newport	Salmonella Newport (Genovar 15023.)	OK
S. ouakam	Salmonella Minnesota (Genovar 5669)	wrong serotype
S. panama	Salmonella Panama (Genovar 14909.)	OK
S. panama	Salmonella Panama (Genovar 14909.)	OK
S. paratyphi B var. Java	Salmonella Paratyphi B v Java (Genovar 13892)	OK
S. stanley	Salmonella Stanley (Genovar 14112)	OK
S. typhimurium "NT"	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 104	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 12	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 120	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 193	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT 44	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT U302	Salmonella Typhimurium (Genovar 10909.A)	OK
S. typhimurium DT120	Salmonella Typhimurium (Genovar 10909.A)	OK

Table 2: Data from testing MDR S. Typhimurium DT104

	Correct	Fail
S. Typhimurium DT104 MDR (n = 8)	8	0
S. Typhimurium, other (n = 8)	8	0
Other MDR Salmonella (n = 4)	4	0

When testing pure cultures, 58 out of 59 strains gave the expected result using the Premi® Test method. Also 8 multidrug resistant *S. Typhimurium* DT104 were correctly identified as "MDR *S. Typhimurium* DT104", while 8 other *S. Typhimurium* (amongst them 3 multidrug resistant strains belonging to other phage-types and 1 DT104 not being multidrug resistant) were correctly identified as "*S. Typhimurium*" only. 4 other serotypes also being multidrug resistant were correctly identified as the serotype in question (*S. Albany*, *S. Newport*, *S. Stanley* and *S. Infantis*)