

The Effect of the Culture Medium on the Performance of the Premi®Test Salmonella: A Multiplex Molecular Serotyping Test using a DNA Microarray System

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INTRODUCTION

Salmonella serotyping is an important tool for classification of strains, identification of sources of contamination and epidemiological purposes. In addition, regulations require monitoring of certain serotypes. Traditional serotyping is based on the Kauffmann-White antigen-antibody scheme. Application of this method is limited by the high costs, deviations in quality of sera, time-consumption and presence of non-typable isolates. Therefore, Check-Points BV and DSM have developed a general fast functional bacterial typing system based on DNA chips from ClonDiag, and the first product is the molecular serotyping of *Salmonella*. This new procedure, Premi®Test Salmonella (PTS) can be performed directly on animal, food or environmental samples after the enrichment and isolation steps. The aim of this study was therefore to evaluate the potential effect of various culture media for *Salmonella* isolation on the PTS result.

MATERIALS & METHODS

► **- Strains:** Two representative isolates of eight serotypes regulated in Europe and the US were tested: Typhimurium, Enteritidis, Hadar, Virchow, Infantis, Heidelberg, Newport and Montevideo.

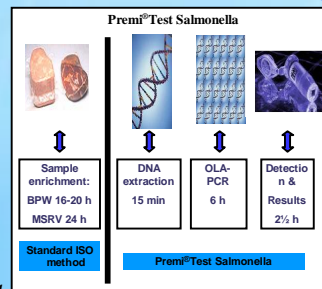
Nine isolation or selective culture media were selected from either selective plating media used in the Standard reference *Salmonella* detection methods (XLD, BGA, Hektoen, MSR/V, XLT4, Rapid[®]Salmonella (BioRad), SMS agar (AES)) or culture media routinely used in reference laboratories for serotyping (TSA and Drigalski).

The PTS protocol was applied after culturing the *Salmonella* strains on these different culture media.

► PTS method :

• **Principe :** The Premi®Test Salmonella system uses a methodology called multiplex ligation detection reaction to generate a collection of circular DNA molecules that are subsequently PCR amplified by means of a single pair of amplimers (1,2). The PCR products are next sorted by hybridization to a low-density DNA microarray. Positive hybridization is detected using a biotin label incorporated in one of the PCR primers. A set of genetic markers has been selected with the purpose of yielding unique microarray hybridization profiles to identify and discriminate *S. enterica* serovars. The test allows single-tube processing, which simplifies the technical work associated with strain typing and can be applied directly after the enrichment and isolation steps of the Standard ISO method for *Salmonella* detection (Fig.1).

Figure 1



• Different steps :

1- Sampling

Small amount of cells from a single colony of a fresh agar plate is taken and resuspended in lysis Buffer.



2- DNA extraction

The resuspended cells are transferred into a heating block and incubated at 99°C for 15 min and then spinned down.



3- PCR procedures

The DNA extract supernatant are added to a freshly prepared proprietary mix containing ligation probes and thermostable DNA ligase. The sample was heated in a MyCycler PCR instrument (Biorad, La Jolla, CA) during 3min at 95°C followed by 24 cycles of 0.5 min at 95°C and 5 min at 65°C followed by a final denaturation at 98°C for 2 min. Next, a second proprietary exonuclease mix was added. The sample was incubated for 45 min at 37°C and subsequently for 10 min at 95°C to remove non-ligated LDR probes. Next, a third proprietary mix containing PCR primers, deoxynucleoside triphosphates and thermostable polymerase was added. The sample was heated during 10 min at 95°C followed by 30 cycles of 0.5 min at 95°C, 0.5 min at 55°C, 1 min at 72°C, and a final denaturation step of 2 min at 98°C.



4- Hybridization

The PCR product were then subjected to DNA hybridization in a customized 1,5ml Array Tube (ClonDiag, Jena, Germany) containing microarray spotted with DNA oligonucleotides complementary to a set of unique sequences. Thanks to the use of 3 independent probe sets, each ArrayTube® could detect 3 independent amplification reactions at once.

Hybridization reaction was detected by a biotin labelled system with horseradish peroxidase-conjugated streptavidin. After washing steps, final detection is performed at room temperature with a peroxidase substrate.



5- Reading and serotype determination

DNA hybridization results are read on a single channel ATR03 reader connected to a standard computer. The data are processed by a customized software supplied by Check-Points. The presence/absence profile of the different spots is translated into a unique number (genovar score). The software then searches the database for the serotype associated with this profile. This database is regularly updated with new genovar - *Salmonella* serotypes associations. Presentation of the final result on an Array Tube is shown in Fig.2

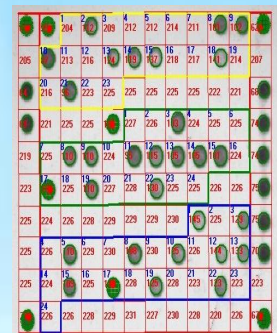


Figure 2 : Lay out Salmonella array



RESULTS

A total of 126 results were obtained out of which 113 tests gave the expected serotype, giving an agreement of 90 % between the both methods (Figure 3). Sometimes, one of the two selected isolates didn't give a result since its specific genovar code was not yet present in the PTS data-base system. That was the case for one "Hadar" and one "Newport" strain and their results were excluded from the results.

When the serotype was not detected by the PTS method, the results were expressed as "*Salmonella* spp" or "Assay not OK". In the first case, identification of the serotype was not possible as the genovar score was not yet associated with a serotype. The deviations from known genovar scores were small in these cases. The second result indicates some trouble during the handling. Overall, no significant effect of the medium was identified whatever the serotype. Agreement values for each medium ranged from 100 % for BGA and Rapid[®]Salmonella to 78,5% for SMS medium.

The repeatability of the assay could be estimated regardless of the culture medium. It ranges from 100% for Hadar, Virchow, Infantis serotypes to 66% to Newport.

Figure 3 : PTS serotypes detection according to the culture media

	Typhimurium	Enteritidis	Hadar	Virchow	Infantis	Heidelberg	Newport	Montevideo	Total
XLD	2/2	1/2	1/1	2/2	2/2	2/2	1/1	1/2	12/14
GVB	2/2	2/2	1/1	2/2	2/2	2/2	1/1	1/2	14/14
Hektoen	2/2	2/2	1/1	2/2	2/2	1/2 (1)	1/1	1/2 (2)	12/14
Drigalski	2/2	2/2	1/1	2/2	2/2	2/2	0/1 (1)	1/2 (1)	12/14
MRSV	2/2	2/2	1/1	2/2	2/2	2/2	1/1	1/2 (2)	13/14
XLT4	2/2	2/2	1/1	2/2	2/2	2/2	1/1	1/2 (2)	13/14
TSA	2/2	2/2	1/1	2/2	2/2	1/2	0/1 (1)	2/2	12/14
Rapid [®] Salmonella	2/2	2/2	1/1	2/2	2/2	2/2	1/1	2/2	14/14
SMS	1/2 (1)	1/2 (2)	1/1	2/2	2/2	2/2	0/1 (2)	2/2	11/14
Total	94% (17/18)	89% (16/18)	100% (5/5)	100% (18/18)	100% (18/18)	89% (16/18)	66% (6/9)	72% (13/18)	90% (113/126)

(1) Result = Assay Not OK - (2) Result = Salmonella genovar score

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CONCLUSION

This study was a preliminary assay using a PTS prototype. The results indicate that the *Salmonella* serotype detection with the PTS system is not depending on the culture media used for colonies.

These results are promising and will be improved in the future with a new PTS version. This will include a harmonized isolation protocol and an updated database consisting of more genovar codes. This will further improve the repeatability and extend the serotype designation.