DNA Microarray evaluation for *Salmonella* serotyping

A. Brisabois[1], S. Fremy[1], F. Moury[1], M. Marault[1], R.van Santen[2], P.Vos[3],J.M Fabre[4]

[1] *Unité Caractérisation et Epidémiologie Bactérienne, AFSSA - Lerqap, 23 avenue du Général De Gaulle, 94700 Maisons-alfort – France*

[2] *DSM Nutritional products, Postbox 1163, 6160 BD Geleen, The Netherlands,*

[3] *Check-Points bv, Binnenhaven 5, 6709 PD Wageningen, The Netherlands*

[4] *Phylum, Europarc III, rue de la Découverte BP17530, 31675 Labège cedex, France*

Tel +33 (1) 49772826, Fax +33 (1) 49774666, email a.brisabois@afssa.fr

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, for the molecular serotyping of *Salmonella*. A set of genetic markers has been selected with the purpose of yielding unique microarray hybridization profiles to recognize the *Salmonella* genus and to identify *S.enterica* subsp.*enterica* serovars. 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 to evaluate the performance of the PTS on a large diversity of strains routinely collected at the Salmonella network and to define the specificity of the test on a variety of *Salmonella* and non *Salmonella* isolates.

MATERIALS & METHODS

➢ **- Bacterial strains:** 172 *Salmonella* strains belonging to 60 different serotypes were tested. The selection comprised 20 of the most frequently encountered serotypes, including the ones mentioned in European and US regulation (Table 1). An additional panel of 19 isolates representing partial or no agglutinable strains were also tested.

➢ *Salmonella* species: In total, 17 strains belonging to the 5 other *Salmonella* subspecies and to the *S. bongori* species were tested. A variety of 26 non *Salmonella* species was also analysed

➢ The PTS protocol was applied after culturing the *Salmonella* strains on XLD, TSA or Drigalski media.

Regulation serotypes	Additional serotypes for the Top 20
Typhimurium	Agona
Virchow	Anatum
Infantis	Derby
Hadar	Dublin
Enteritidis	Indiana
Herdelberg	Kentucky
Montevideo	Kottbus
Newport	Mbandaka
S 1 1,4, [5], 12:i:-	Paratyphi B
	Saintpaul
	Senftenberg

Table 1 *: Top 20 serotypes tested*

➢ **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 subsp.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).

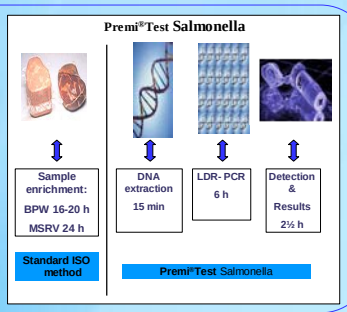

Figure 1

• 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.

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

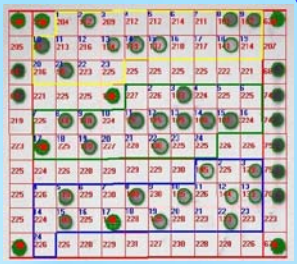

Figure 2 *: Lay out Salmonella array*

RESULTS

➢ **Molecular serotyping assignment**

From the 158 strains belonging to serovar known to be detected with PTS, 92% gave a molecular serotype or genovar code identical with the slide agglutination results. Ten strains were identified only at the *Salmonella spp.* level.The monophasic Typhimurium strains could not always be differentiated from Typhimurium (Table 2).

Most of other isolates were detected as "*Salmonella spp*" and 11/15 strains with incomplete antigenic formulae were assigned to a genovar corresponding to a serotype very close to the antigenic formulae. Moreover, the genovars detected for the non agglutinable strains (rough) correspond perfectly to the expected serotype deduced by the PFGE pattern after *Xba*I macro-restriction (Fig. 3).

	Strains tested		PTS results			
	Nb of serotypes	Nb of strains	Correct genovar detection	« *Salmonella*» detection	Discordant serovar	Agreement %
Top 20	20	94	90	4	0	96%
PTS list	31	64	55	6	3	86%
Total	51	158	145	10	3	92%

Table 2: **PTS Results and agreement with the serotyping method**

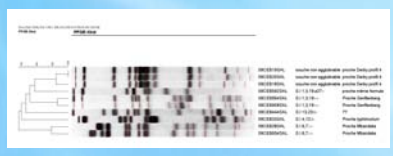

Figure 3: **PFGE profiles on some partial-or non-agglutinable isolates**

➢ ***Salmonella spp*. recognition**

The PTS method was able to recognize all the subspecies of *S.enterica* and the *bongori species* also was well detected.

Finally, 34 strains belonging to 26 non *Salmonella* species were tested. The PTS correctly identified 34 strains as non *Salmonella* giving specificity and sensitivity results of 100% for *Salmonella* detection (Table 3)

		PTS results	
		« *Salmonella* »	« No *Salmonella* »
Strains tested	*Salmonella spp*	198	0
	Non *Salmonella species*	0	34

Table 3: Synthesis of PTS Salmonella detection

REFERENCES

1-Andreoli P., Thijssen J., Anthony R., Vos P., de Levita W. 2004 Fast method for detecting micro-organisms in food samples. European Patent 1633887.

2-van Eijk,M.J., Broekhof,J.L., van der Poel,H.J., Hopgers,R.C., Schneiders,H., Kamerbeek,J., Verstege,E., van Aart,J.W.,Geerlings,H., Buntjer,J.B., van Oeveren,A.J., Vos,P.2004. SNPWave: a flexible multiplexed SNP genotyping technology. Nucleic Acids Research 32,e47..

3-Wattiau, P., Weijers, T., Andreoli P., Schliker,C., Maas,M.E., Verbruggen A.J., Heck,M.E.O.C., Wannet,W.J., Imberechts, H., Vos,P. Evaluation of the Premi-Test Salmonella, a commercial low-density DNA microarray system for routine identification and typing of Salmonella enterica, In.J.Food.Microbiol. 2008, doi:10.1016j.ijfoodmicro.2008.01.006

4-Wattiau P., Van Hessche M., Schlicker C., Vander Veken H., Imberechts H. Comparison of classical serotyping and PremiTest assay for routine identification of common enterica serovars. Journal of Clinical Microbiology, 2008 p 4037-4040.

CONCLUSION

The evaluation study of the PremiTest Salmonella method clearly shows good agreement with the classical slide agglutination method for Salmonella serotyping. Hence, it should be regarded as a valuable alternative method for the laboratories interested in performing routine identification and serotyping of *Salmonella* strains as mentionned in other belgian studies as well.(3,4). The method offers the pratical advantages to reduce the delay of result in only one day (8 hours) instead 3 to 4 days, and to be easily perform without complex devices requirement. Moreover, three serotyping results can be obtained in one single AT, auto-agglutinable isolates can be identied. Nevertheless some steps are critical and serovar only differ in one spot, consequently dual results could be detected. Finally, these results are promising and recognition by an approval organization will be the next step.