DNA Microarray evaluation for Salmonella serotyping

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INTRODUCTION

Salmonella serotyping is an important tool for classification of strains, identification of sources of contamination, and epidemiologic investigations. In addition, regulations require monitoring of certain serovars. Traditional serotyping is based on the Kaufmann-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-

3-PCR procedures

The DNA extract supematant are added to a freshly prepared proprietary mix containing ligation probes and thermostable DNA ligase. The samples are hybridized in a Molecycler PCR instrument (Biorad, La Jolla, CA) during 3m in 95°C followed by 24 cycles of 0.5 min at 95°C and 5 min at 65°C, by a final denaturation at 98°C for 2 min. Next, a second proprietary exornuclease mix was added. The sample was denatured for 45 min at 70°C and for 10 min at 95°C to remove non-

4- Hybridization

The PCR product were then subjected to DNA microarray 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-

5-Reading and serotype determination

DNA hybridization results are read on a single channel ATRO3 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 confirmed Salmonella serotypes associations. Presentation of the final result on an Array Tube is shown in Fig.2

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 as non-agglutinable species at the slide level. The monophasic Typhimurium strains could not always be differentiated from Typhi strains (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 serovar close to the antigenic formula. Moreover, the genovars detected for the non-agglutinable strains (rough) correspond perfectly to the expected serotype deduced by the PFGE pattern after Xbal macro-restriction (Fig. 3).

> Salmonella spp. recognition

The PTS method was able to recognize all the subspecies of S. enterica and the serovar 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).