

## A Short Commentary on “Serotype Determination of Salmonella by xTAG Assay”

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### Introduction

Currently, no protocols or commercial kits are available to determine the serotypes of Salmonella by using Luminex MagPix<sup>®</sup> reader system. xMAP<sup>®</sup> Salmonella Serotyping Assay (SSA) kit was manufactured by Luminex corporation, in which antigen-specific oligonucleotide probe-coupled microspheres were used. However, the microspheres used in the SSA kit are non-magnetic and the kit could not be applied on Luminex MAGPIX<sup>®</sup> reader system, on which magnetic microspheres are required. Moreover, since the process of coupling antigen-specific oligonucleotide probes to microspheres is complicated and the preparation of bead arrays acquires considerable efforts, the xMAP assays reported before are not easy to be shared by other laboratories. Thus, for laboratories facilitated with Luminex MAGPIX<sup>®</sup> rather than Luminex<sup>®</sup> 100/200<sup>™</sup>, no protocols or commercial kits are available for high-throughput to serotype determination of Salmonella.

Instead of using antigen-specific oligonucleotide probe-coupled microspheres in the SSA kit, xTAG technology and Magplex-TAG<sup>™</sup> microspheres were used in our study [1]. It could be used to detect 10 serogroups, 31 H antigens and Vi antigen of Salmonella, covering most of the prevalent serovars. Only primers rather than both primers and probes were needed in the xTAG assay. The 5' end of each reverse primer was modified with TAG sequence, which is complementary with the anti-TAG sequences pre-coupled on Magplex-TAG<sup>™</sup> microspheres. The PCR products of different O serogroups, H antigens

and Vi antigen of Salmonella could be identified accurately by hybridizing with the mixture of various of MagPlex-xTAG microsphere sets. Each microsphere set was pre-coupled with its unique anti-TAG sequences by Luminex Corporation.

Because of the difference between xMAP and xTAG technology in principle and procedure, the primers needed for serotyping Salmonella are quite different. All the primers used in the xTAG assay, which served the technical core of the assay, were different from those of xMAP assays, and were all designed originally by our group.

Compared to the SSA kit, the advantages of this xTAG assay are: First, the magnetic beads make it applicable to both Luminex<sup>®</sup> 100/200<sup>™</sup> and MAGPIX<sup>®</sup> reader systems. Second, asymmetric PCR and denaturation of PCR products before hybridization are not needed in the assay. Third, this xTAG assay is easily shared by other laboratories. With the use of Magplex-TAG<sup>™</sup> microspheres, which are pre-coupled with unique 24-base DNA sequences (anti-TAGs), the process of coupling antigen-specific oligonucleotide probes to beads is circumvented. Only biotin-labeled forward primers and TAG sequence-modified reverse primers, which can be synthesized by biotech corporations, are needed in the assay. Thus, this xTAG assay can be conveniently utilized by other laboratories. In conclusion, the xTAG assay could serve as an efficient alternative or complementary approach for traditional Salmonella serotyping methods.

### References

1. Zheng Z, Zheng W, Wang H, Pan J, Pu X (2017) Serotype determination of Salmonella by xTAG assay. J Microbiol Methods 141: 101-107.