

Combined serology and antibiotic residue detection in a Luminex assay

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Abstract

Serum of slaughter pigs is being used for routine testing of antibodies against pathogens and can also be used for detection of antibiotic residues. Since the assay formats differ (direct detection of antibodies vs. inhibition assays for residues), these tests are performed in parallel, not in a multiplex set-up. For this study, a protocol for testing both antibodies and residues in one sample was investigated using the dedicated multiplex xMAP platform of Luminex. The results suggest that direct detection of antibodies combined with an inhibition assay for antibiotic residues is possible. Although the required sensitivities are not yet met, the results are promising and further optimizations to successfully combine the two detection methods in one multiplex assay are ongoing.

Introduction

Slaughter pigs are tested for several types of hazards, such as zoonotic pathogens like Salmonella or Trichinella and antibiotic residues. Pathogen detection can be done by the detection of serum antibodies with immunoassays like ELISA. For the detection of antibiotic residues, a microbial inhibition test in renal pelvis fluid (pre-urine) is being used. As a faster alternative, these (small) residues can also be detected in serum with an immunoassay, i.e. an inhibition test. The purpose of the research described here is to combine these different assay principles (Fig. 1) in one protocol using the bead-based multiplex technology of Luminex.

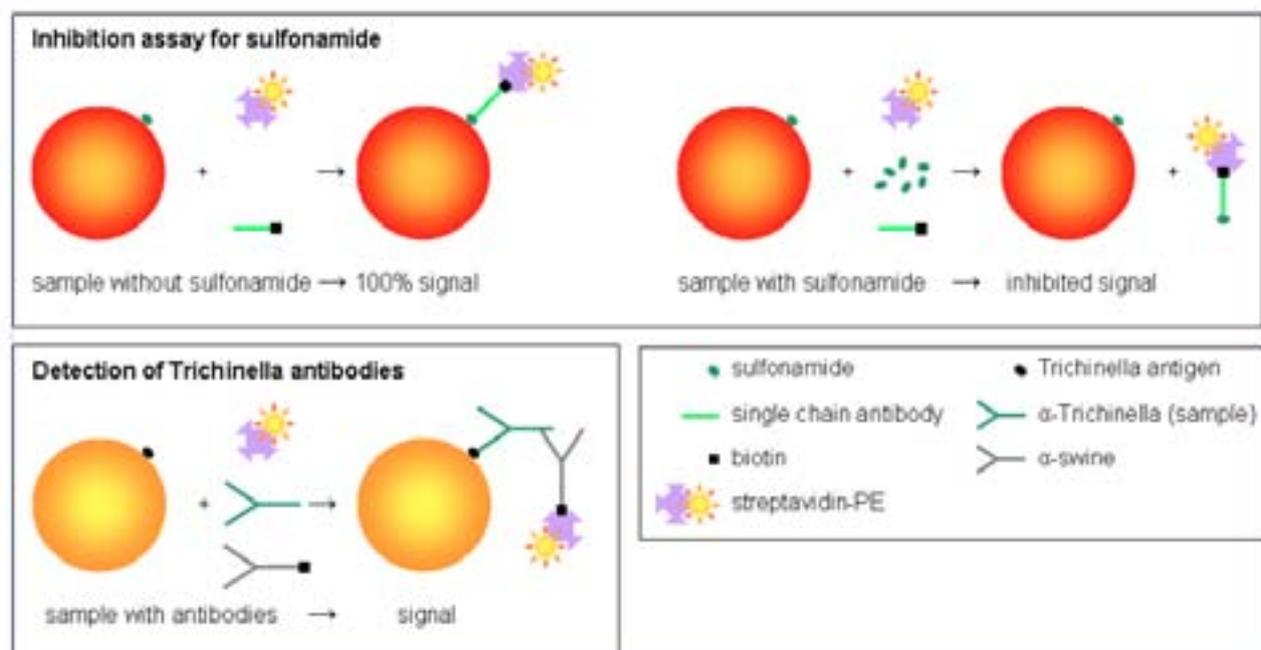


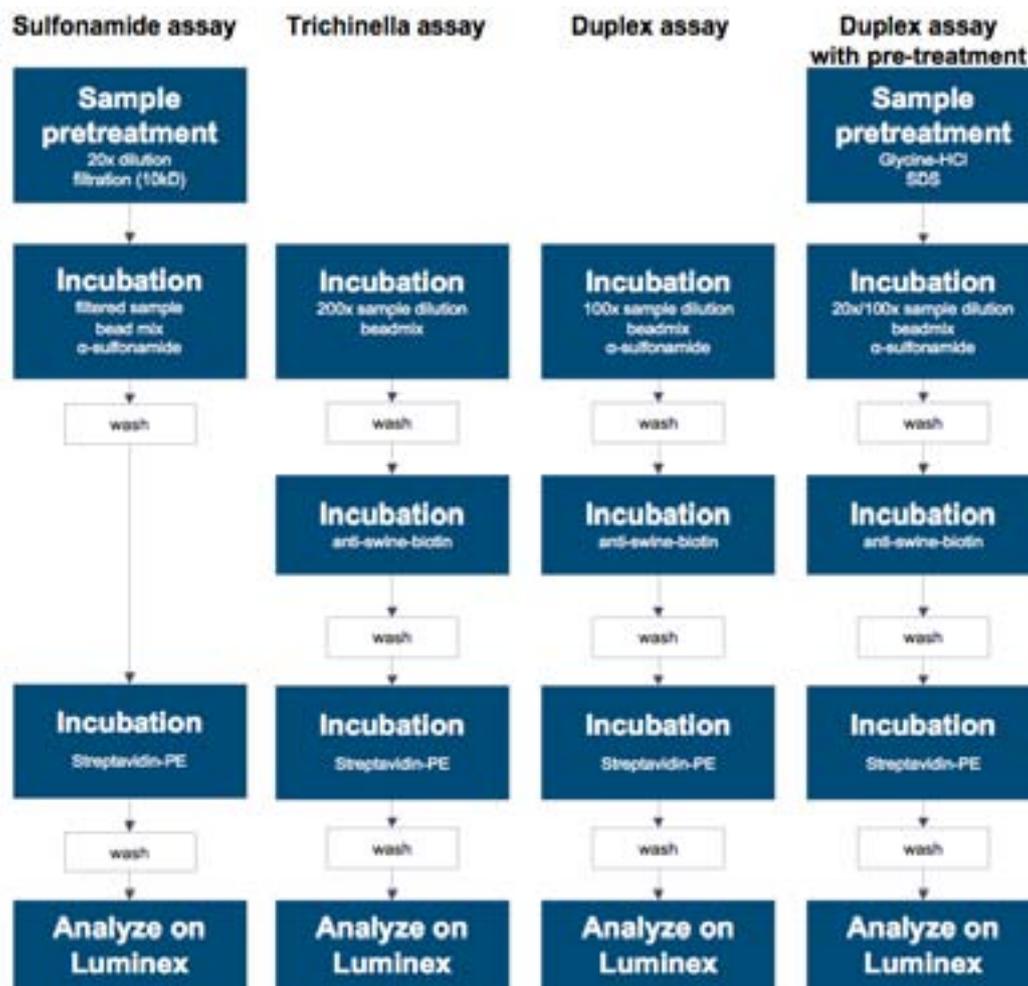
Figure 1 Assay principles. For each type of assay Luminex beads are depicted that are either conjugated with sulfonamide (top) or Trichinella antigen (bottom). All ingredients required for the respective assays are described in the legend. Abbreviations: PE: phycoerythrin.

Material and Methods

A previously developed *Trichinella* Luminex assay (1) was chosen as representative serological assay, while the detection of sulfonamides was chosen as a representative inhibition assay, with sulfa-methoxazole as model compound (2). For setting up the protocols, well-described swine serum samples with and without the addition of sulfamethoxazole were used. Sera were spiked with 100ppb and 20ppb, respectively the maximum residue limit (MRL) and the minimum detection limit.

Results

To combine the serological assay with the residue inhibition assay, the effects of various conditions were investigated. Differences between the two assays were the number of incubation steps, the required serum dilutions and the sample pre-treatment (Fig. 2). As the standard protocols of the two assays require different serum dilutions, an effort was made to reduce the background in the serology assay and to increase the sensitivity of the inhibition assay by evaluating the effects of sample pre-treatment and sample dilution.



In the sulfonamide assay, a 10 kDa filtration step is performed after sample dilution in order to remove proteins and other large compounds. Since this includes removal of antibodies, this is not appropriate for the *Trichinella* assay. Therefore, this step was omitted in the combined duplex assay format that was established. When spiked swine serum samples were tested in this duplex assay, the sulfonamide assay did not detect the required MRL (Fig. 3). When unfiltered pre-treated samples (acid glycine and SDS)(3) were diluted 20x (optimal for sulfonamide), both assays showed modest differences between positive and negative samples. When these pre-treated samples were diluted 100x, the *Trichinella* assay was re-established and the signal of the sulfonamide assay increased, albeit with a high standard deviation.

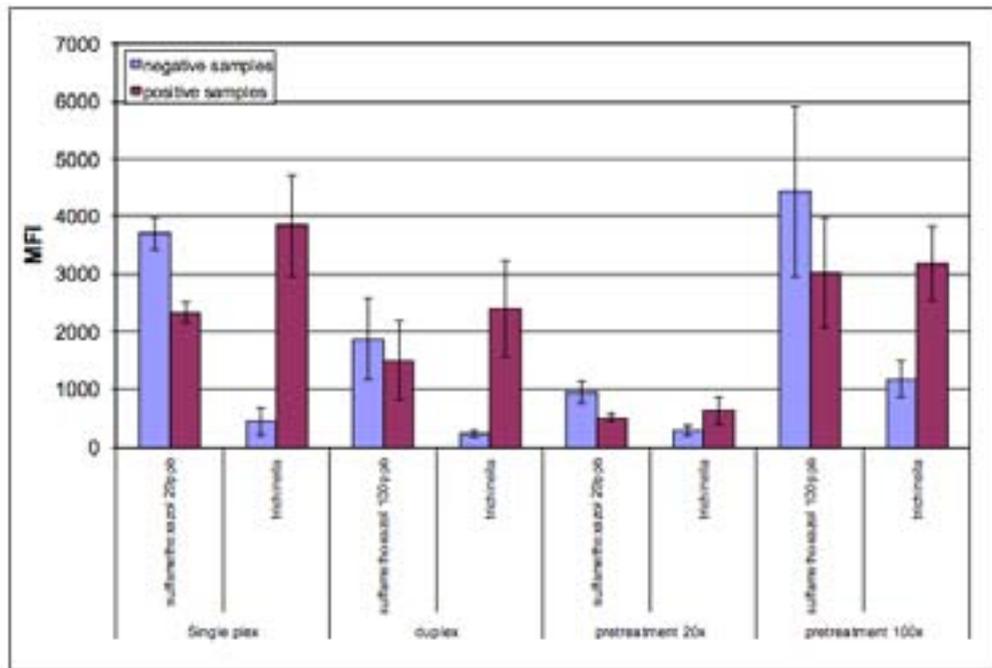


Figure 3 Results of combined assays. Mean results of the 20 swine sera with and without sulfamethoxazole are presented.

It is clear that filtering and only modest serum dilution are crucial for the sulfonamide assay, whereas a higher sample dilution is important for the *Trichinella* assay and filtration is absolute impossible as it depletes the serum of antibodies. In order to solve these problems an alternative 'split-and-pool' method was designed. In this alternative procedure, serum is split in two and treated according to the requirements of the respective assay. These treated samples are then pooled in a microplate well. In detail: after making a 10x dilution the sample is split in two. One half is filtered to remove anything larger than 10kDa. The other half is further diluted to a 100x dilution. The filtered 10x diluted sample is pooled with the 100x diluted sample, resulting in a combined sample with 20x diluted residues and 200x diluted antibodies (Fig 4). Early results with this split-and-pool procedure show that both sulfonamide residues (i.e. sulfa-chloro-pyridazine) and *Trichinella* antibodies can be detected in this combined assay format.

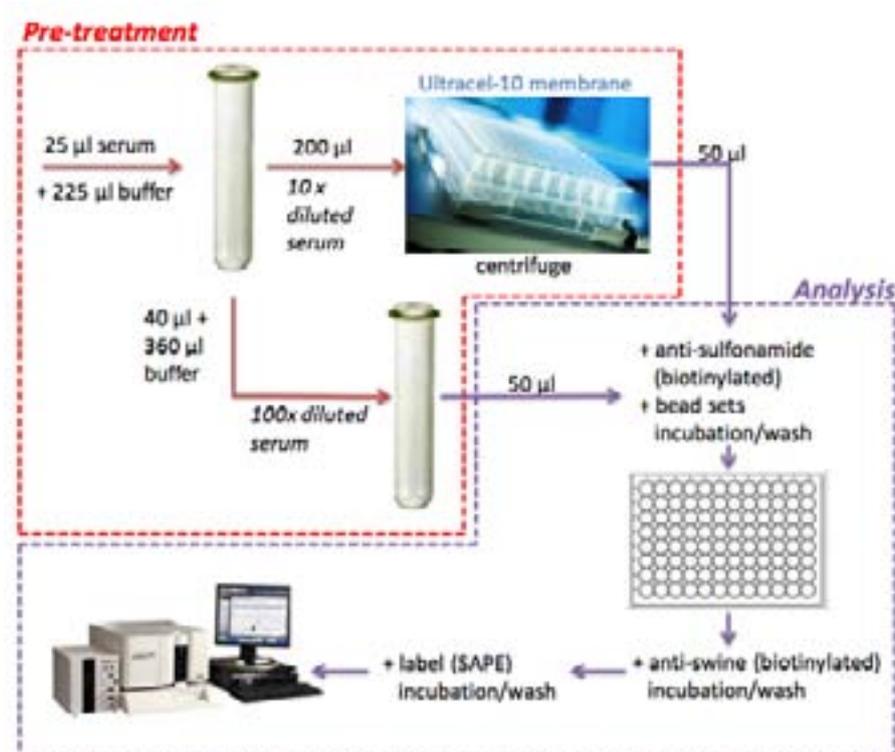


Figure 4 Split-and-pool assay. Schematic representation of the proposed split-and-pool method to detect residues and anti-bodies in a Luminex assay.

Discussion and conclusion

The results demonstrate that it is possible to combine a serological assay and an inhibition assay in one Luminex protocol. Since the used representative assays are optimized for their respective goals, the resulting duplex assay needs to be further optimized for variables like bead production, buffer composition and labelling. The split-and-pool protocol is promising but needs further investigation and optimization. This type of assay could find its use in cost-reducing monitoring systems in a production chain where several pathogens and small compounds like antibiotic residues are being monitored.

References

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Acknowledgments

This research was financed by the Dutch Ministry of Economic Affairs, Agriculture and Innovation (Food Safety - Veterinary Drugs WOT-02-003; Food Safet