1st National ring trial on detection of antibodies to trichinella in pigs

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Abstract
Regulation (EC) 2075/2005 ensures official inspection of food of animal origin with specific rules on official controls for Trichinella in meat. Regarding certification of Trichinella-free farms, this regulation recommends serological methods for monitoring. The aim of the ring trial was to evaluate a new ELISA regarding test accuracy and practical usage. The participants of the ring trial tested 22 sera prepared by the German National Reference Laboratory for Trichinellosis and additionally 22 field samples from their own sample collection using the commercial ELISA kit. This ELISA demonstrated a very good diagnostic sensitivity and robustness in the ring trial.

Introduction
Trichinellosis is a food-borne disease caused by nematodes of the genus Trichinella, which occurs seldom in Germany. Humans are infected by consuming raw or insufficiently heated meat or raw sausages containing parasitic larvae.
According to the Regulation (EC) 2075/2005 different methods of artificial digestion have been approved for the detection of Trichinella in fresh meat. Serological tests such as the ELISA are useful for monitoring purposes and may be implemented in surveillance programs for farms or regions with a negligible risk of infection with Trichinella. ELISAs for the diagnosis of infection with Trichinella in swine are well established (Gamble et al. 1983, Nöckler et al. 1995).
To evaluate a new ELISA a ring trial was organised by the German National Reference Laboratory for Trichinellosis at the BfR with 21 participants in September 2009.

Material and Methods
During the ring trial, 21 laboratories from 11 states of Germany tested 22 serum samples from experimentally infected pigs, as well as 212 serum, 33 plasma and 169 meat juice samples from hogs and 26 sera from wild boars, respectively, from their routine submission. The samples were tested using the BfR in-house ELISA and the PIGTYPE® Trichinella Ab ELISA (Labor Diagnostik GmbH Leipzig; officially approved in Germany in 2009). The number of correct, false positive and false negative results per laboratory was compared to the sample status obtained by the German National Reference Laboratory for Trichinellosis. Furthermore the repeatability of the assay was analysed for the OD-values obtained by the ring trial participants by calculation of the variation coefficient. Additionally z-scores were calculated to determine the deviation of the laboratory mean from the overall mean and the result variance of the labs was compared to the mean variation by Mandel´s k (DIN ISO 5725-2:2002-12 [2002], ISO 13528:2005 [2005]). At last the Pearson correlation coefficient was determined in comparison to the results determined at BfR to evaluate the reproducibility of the test results in different laboratories.

Results
14 of 21 participants reported all results for the reference samples as expected. Incorrect result calculation and testing performed by two different lab technicians were identified as one major cause of laboratories reporting false positive or false negative results. All tested field samples but four wild boar samples scored negative. The ELISA-kit demonstrated very good diagnostic sensitivity, specificity and robustness. None of the laboratories experienced major problems implementing the assay in their daily testing routine, but thorough reading and following the manufacturer´s instructions is crucial for good test results.

Discussion

The main objective of the ring trial comprising 21 laboratories was to evaluate the PIGTYPE® Trichinella Ab ELISA regarding test accuracy and practical usage. Overall the ELISA results for laboratories demonstrate a good sensitivity and specificity of ELISA with 98.93% and 95.39%, respectively. Most of the borderline sera were identified as positive indicating that the diagnostic sensitivity of the evaluated ELISA was higher than the in-house ELISA. Variation coefficients were used to assess the repeatability of the ELISA. As only 6.8 % of the sera showed a variation coefficient above 30 %, the repeatability of the ELISA was good for the participating laboratories (OIE [2009]). Z-scores and Mandel's k were calculated to analyse the variability of the test results in more detail and also demonstrated that the test results of both tests were reproduced by most laboratories.
Taken together the PIGTYPE® Trichinella Ab showed a stable performance in both repeatability and reproducibility in this ring trial. The close correlation for S/P ratios between participants and the reference laboratory also demonstrate a good performance of the ELISA.

Conclusion

In conclusion monitoring of pigs determined for human consumption for Trichinella by serological examination using ELISA seems to be a suitable tool, since the method is well established and standardized.

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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.

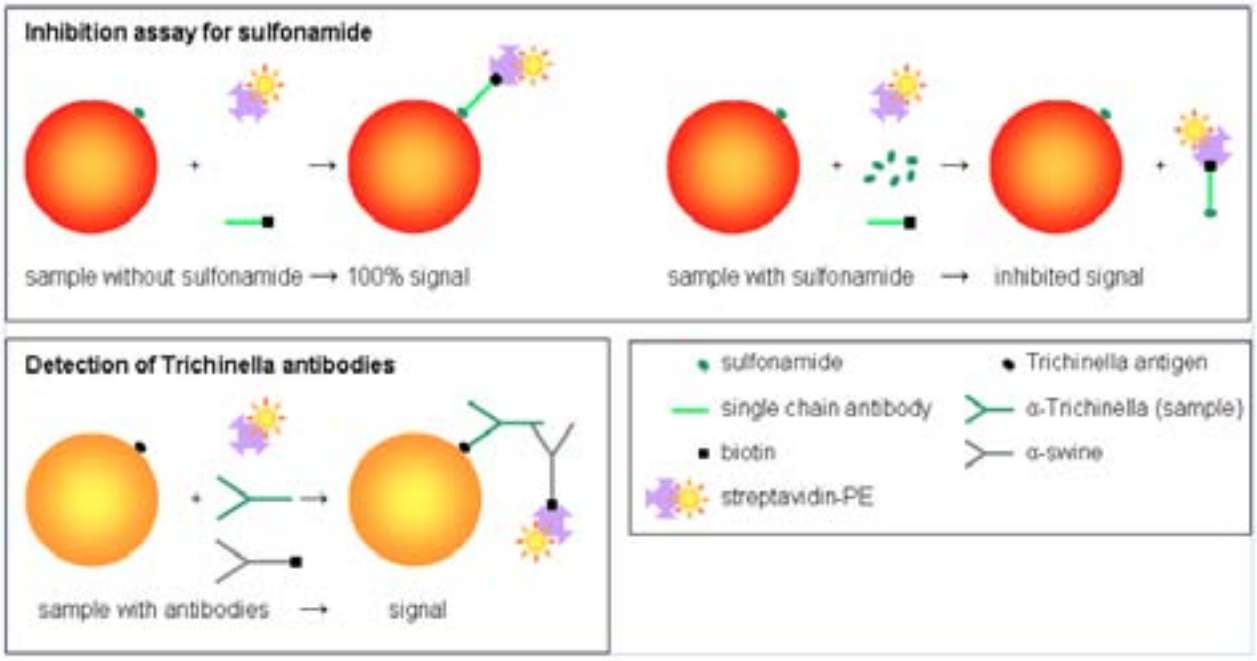


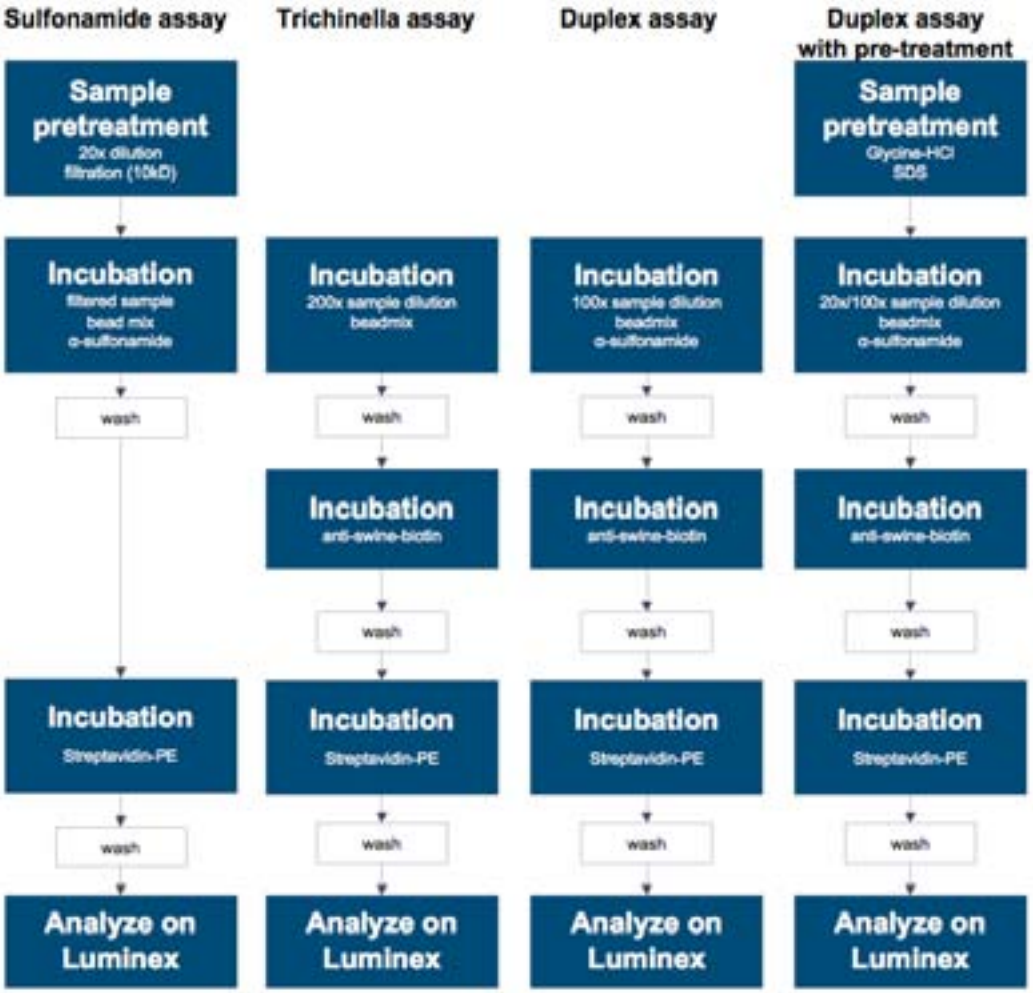
Figure 1 Assay principles. For each type of assay Luminex beads are depicted that are either conjugated with sulfonamide (top) or Trichinella antigen (bottom). All ingredients required for the respective assays are described in the legend. Abbreviations: PE: phycoerythrin.

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.

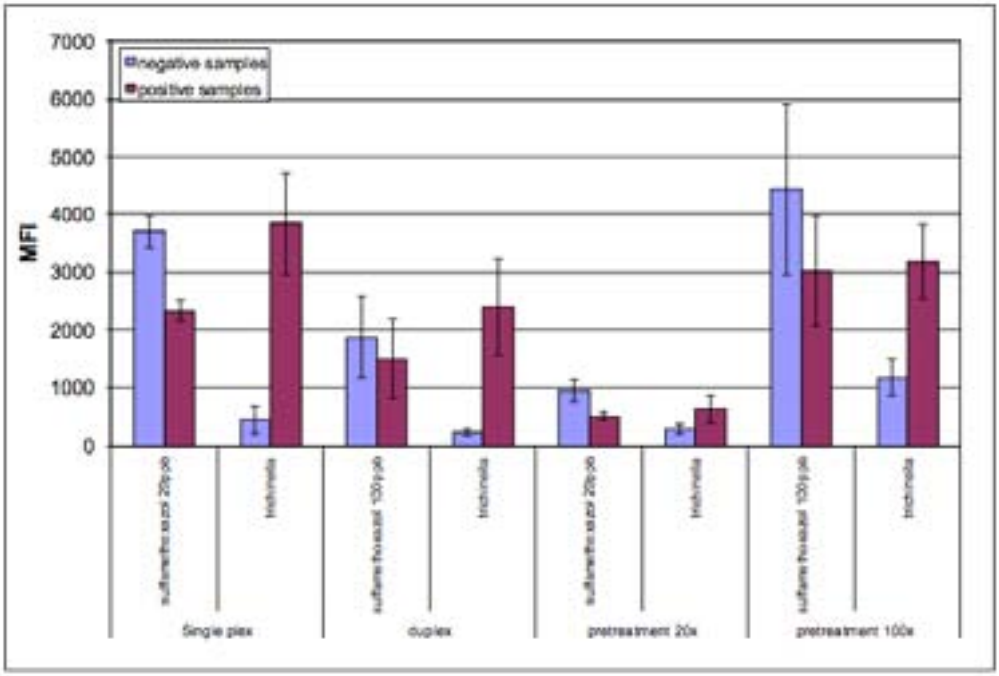


Figure 3 Results of combined assays. Mean results of the 20 swine sera with and without sulfamethoxazole are presented.

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.

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