Meat Juice Multiserology – Developing a protein microarray for simultaneously detecting antibodies against zoonoses and production diseases in pigs


(1) Institute for Food Quality and Food Safety, University of Veterinary Medicine, Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany
(2) Field Station for Epidemiology, University of Veterinary Medicine Hannover, Buescheler Str. 9, D-49456 Bakum, Germany
(3) Institute of Virology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany
(4) QIAGEN Leipzig GmbH, Leipzig, Deutscher Platz 5b, D-04103 Leipzig, Germany
(5) Alere Technologies Gmbh, Jena, Loebstedter Str. 103, D-07749 Jena, Germany

*corresponding author: diana.meemken@tiho-hannover.de

Abstract
In the European Union serological and/or bacteriological monitoring results on zoonoses are to be taken into account for the risk assessment of slaughter pig herds in the framework of the risk-based meat inspection. Furthermore, the European food safety strategy pursues the additional goal to increase herd health and animal welfare. To meet the two goals identifying and controlling the mainly latent zoonoses as well as production diseases, the challenge was to develop and validate a cost-efficient diagnostic tool for simultaneously testing meat juice samples or blood serum samples for antibodies against various pathogens. The following antigens were chosen for detecting the corresponding antibodies by means of a protein microarray: a) zoonotic pathogens (Salmonella spp., Toxoplasma gondii, Trichinella spiralis, Yersinia enterocolitica, hepatitis E virus) and b) production disease causing pathogens (influenza A virus, Mycoplasma hyopneumoniae, PRRSV, Actinobacillus pleuropneumoniae). After validation by reference to the single-ELISA test results seven out of the nine chosen pathogens could be serologically detected by the developed microarray with test accuracy values between 0.71 and 1.

Introduction
The new European food safety concept for products of animal origin pursues three main and equally important goals: optimizing food safety, animal health as well as animal welfare by means of process control along the entire food chain (Anonymous, 2002). The need for this legislative enhancement is on the one hand due to the fact, that the traditional ante- and post-mortem meat inspection methods (inspecting, palpating and incising) alone are not able to control subclinical or asymptomatic zoonotic diseases (Hathaway and Richards, 1993 and EFSA, 2011). On the other hand there is a growing social demand for food from healthy herds with a high level of animal welfare quality. The Regulation (EC) No. 853/2004 defines as minimum nine criteria for the so-called food chain information (Anonymous, 2004). Most parts of the food chain information are already available, because they have to be documented for other purposes at the farm or the abattoir or they are easy to attain. But other parts of the food chain information are not answerable without additional diagnostic tests. Although national serological salmonella monitoring systems are established in some European countries like Denmark, The Netherlands and Germany, information about other important zoonotic pathogens in pigs is missing. In the „Scientific opinion on the public health hazards to be covered by inspection of meat“ from 2011 the European Food Safety Authority (EFSA) ranked the following zoonotic pathogens as the most important public health hazards due to the consumption of pork: Salmonella spp., Trichinella spp., Yersinia enterocolitica and Toxoplasma gondii. The EFSA underlines the importance of serological monitoring programmes for subclinical zoonoses in pigs (EFSA, 2011). The concept of the meat juice multi-serology (Meemken and Blaha, 2011) is to use meat juice samples from the salmonella monitoring programmes and to extent the analysis to other zoonotic and animal health relevant pathogens.
Material and Methods

For developing a swine specific microarray various recombinant or native antigens were acquired with relevance for zoonoses or for production diseases. Except for the hepatitis E virus antigen, which was provided by the Institute of Virology, University of Veterinary Medicine Hannover, Germany, all other zoonotic antigens were provided by QIAGEN Leipzig, Germany, who applies the same antigens in their commercial single-ELISA tests. The antigens with relevance for production diseases were partly provided by third party or were self-made and, thus, have not been used so far in commercial single-ELISA tests. Assisted by Alere Technologies, Jena, Germany a “swine-specific protein microarray” was produced. The chosen antigens were spotted in different concentrations on a glass platform located at the bottom of a microarray tube (Fig. 1). On the surface of the glass platform which has a size of 4 mm² up to 200 antigens can be fixed.

Positive and negative controls as well as antigens of the following zoonotic and production disease were spotted on the microarray: *Salmonella* spp., *Toxoplasma gondii*, *Trichinella spiralis*, *Yersinia enterocolitica*, hepatitis E virus, influenza A Virus, *Mycoplasma hyopneumoniae*, Porcine reproductive and respiratory syndrome disease (PRRSV) and *Actinobacillus pleuropneumoniae* (App). The concentration of specific antibodies in a sample is visualized by the coloration intensity of the spots after processing and is calculable via a computer software between zero (no specific antibody in the sample) and one (high concentration of antibodies in the sample). During the validation phase different dilutions of meat juice samples and blood serum samples, different test substrates and test conjugates as well as different concentrations of the chosen antigens were tested. The results of the microarrays were compared to the respective single-ELISA test results. For each spot, i.e. antigen in a specific concentration, a cut-off was determined for optimal sensitivity, specificity and test accuracy via a Receiver Operating Characteristic curve analysis (ROC curve analysis).

Results

The test duration of a microarray test procedure for establishing nine serological results accounts for 1.5 h and is comparable to the test duration of any of the corresponding single-ELISA tests. If meat juice samples are used as specimen, a preparation of the meat juice sample is required, i.e. that meat juice samples are to be diluted tenfold lesser than blood serum samples. Due to this preparation the test procedure for both specimens are equal. After validation of the microarray and determination of cut-off values per spot, specificity, sensitivity and test accuracy could be calculated for each antigen used in the microarray. The highest level of test accuracy values are measured for *Trichinella* spp. (1.0), *Toxoplasma gondii* (0.99), hepatitis E virus (0.95), *Yersinia enterocolitica* (0.94), *Salmonella* spp. (0.92), PRRSV (0.91) and influenza A virus (0.71) with values between 0.7 and 1. Lowest test accuracy values were measured for those spots using the self-made antigens of *Actinobacillus pleuropneumoniae* (0.61) and *Mycoplasma hyopneumoniae* (0.54) with values between 0.5 and 0.6.

In Fig. 2 a completely processed microarray is shown as example.
Discussion

The major challenges during the development of a swine specific protein microarray for simultaneously detecting antibodies against zoonoses and production diseases were a) acquiring efficient antigens for the antibody detection, b) preparing meat juice samples and blood serum samples suitable for testing via microarray, and c) applying the same conjugates and substrates as well as the same time sequences for each step of the procedure for nine different antigens due to the simultaneous approach in one reaction tube.

Regarding the suitability of the acquired antigens, all such antigens used in single-ELISA tests (Trichinella spp., Toxoplasma gondii, hepatitis E virus, Yersinia enterocolitica, Salmonella spp.) showed the highest sensitivity and specificity. This was due to the prior implemented standardisation of the single-ELISA tests regarding substrates, conjugates and procedures by the manufacturer. The self-made antigens of Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae showed the lowest sensitivity and specificity and are to be revised in further research projects.

Although most ELISA tests for production diseases are licensed for blood serum only, it could be shown by Meemken and Blaha (2011) that a tenfold lesser dilution of the meat juice sample compared to blood serum leads to comparable serological results for production diseases. This finding is in accordance with Nielsen et al. (1998) and Molina et al. (2008), who investigated a tenfold lesser Salmonella spp. antibody concentration and PRRSV antibody concentration in meat juice compared to the corresponding blood sera.

Measuring antibodies against zoonotic and animal disease pathogens in meat juice or blood serum samples and designing serological herd profiles is a meaningful tool for risk-categorizations of pig herds for the risk-based meat inspection and could be useful as basis for herd health management initiatives. By developing miniaturized and simultaneous test systems like the developed protein microarray the cost effectiveness can be considerably increased.

Furthermore, identifying herd specific serological differences on a continuous basis will help to understand the underlying management factors determining the infectious status of pig herds especially the latent infections and will become a valuable benchmarking tool for targeted intervention measures to improve food safety and herd health.

To design a serology based herd profile at least 60 meat juice samples per herd (to detect at least 5% intra-herd seroprevalence) should be taken at slaughter. If a serological monitoring programme like the German or the Danish salmonella monitoring programme is established, the already taken meat juice samples can easily be used for detecting antibodies against additional pathogens, too.
The composition of the test targets can be adapted to actual needs such as emerging risks, and special herd health threats.

**Conclusion**

The developed protein microarray is a valuable diagnostic tool to analyze antibodies against different zoonotic and pig disease pathogens at once in a meat juice or blood serum sample. Used in the framework of a serological monitoring program the resulting serological herd profiles can be used by farmers and veterinarians for targeted improvement measures as well as by food business operators and official veterinarians for risk-based decisions. The logistics of an existing meat juice or blood serum based salmonella monitoring programme could be utilised for the multi-serology concept to increase the feasibility and cost-effectiveness. If included in a surveillance system by continuously taking random samples per herd meat juice multi-serology via protein microarray could become a powerful diagnostic tool for improving food safety and pig herd health.

**Funding**

This work was financially supported by the German Federal Ministry of Food, Agriculture and Consumer protection (BMELV) through the Federal Office for Agriculture and Food (BLE), grant number 28011HS013

**References**


EFSA, 2011, Scientific Opinion on the public health hazards to be covered by inspection of meat (swine), EFSA J., 9: 2351.
