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## REFINING THE LPS-ANTIGEN IN SALMONELLA ANTIBODY ELISA FOR POULTRY ENHANCED SPECIFICITY WITHOUT IMPAIRING SENSITIVITY

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### INTRODUCTION:

In the Danish serological surveillance for Salmonella in poultry (serum and egg yolk) a mix-ELISA is used, based on *S. typhimurium* and *S. enteritidis* antigens (Feld et al., 2000). When we evaluated results of the test retrospectively, over the years an unacceptably large fraction of seropositive findings could not be confirmed by the subsequent confirmatory bacteriological sampling in the herd. Therefore we tried to enhance specificity of the ELISA, without losing sensitivity, by refining the antigens used.

### MATERIALS AND METHODS:

New LPS antigen preparations were made based on the antigens currently used in the ELISA (Feld et al. 2000). Lyophilized antigen was resuspended in running buffer with 0,25% deoxycholate and gel filtered on a HiPrep 26/60 Sephacryl S-100 HR column (Sigma Aldrich) (Klausen et al. 2007, Wiuff et al. 2002). The product was collected in fractions and characterized by SDS-PAGE (Fig.1). Fractions containing high molecular LPS were selected for the new antigen preparation (Fig.1). They were pooled, dialyzed and then lyophilized. This antigen was resuspended in MilliQ water for validation in the ELISA. Coating concentrations of the new antigens were adjusted, for the two serotypes separately, to give the same values for the internal control sera in the test as did the old antigens. For the validation a total of 365 samples (serum and egg yolk) were collected in the laboratory during 2010-2012. The samples originated from the Danish Salmonella surveillance and represented submissions where one or more samples had been classified as non-herd confirmed reactors i.e. serologically positive samples that could not be confirmed at the following bacteriological sampling in the herd. Both ELISA positive and ELISA negative samples were represented in this material. As positive gold standards for the validation we used samples from well described Salmonella seropositive poultry (Fig.2).

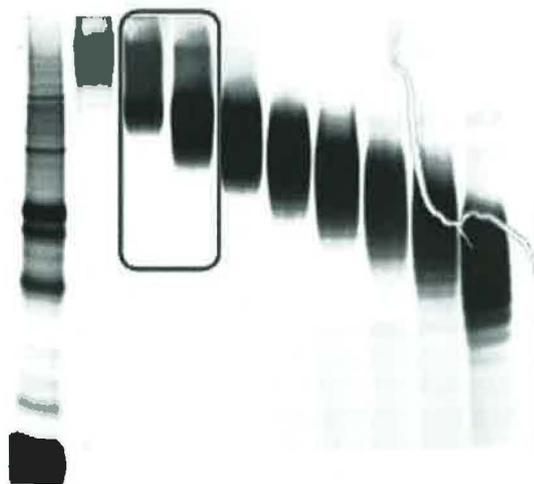


Fig. 1: Product from *S. typhimurium* LPS gel-filtration. Red frame indicates the fractions included in the new ELISA antigen. Outermost left: Marker 'Mark 12'.

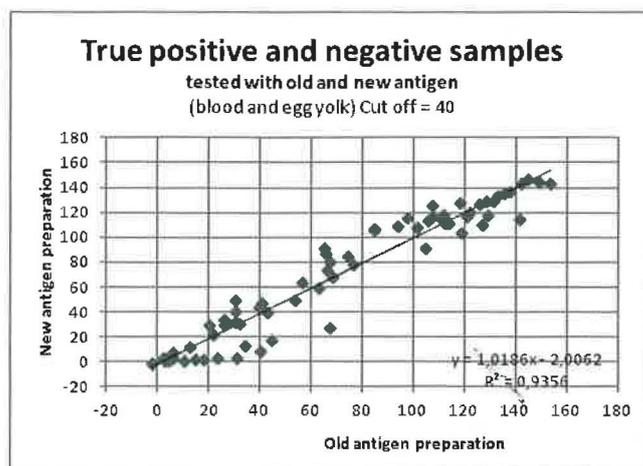


Fig. 2: Samples from poultry with bacteriologically confirmed salmonella infection (or vaccination) and from non-infected animals. The new antigen preparation detected salmonella specific antibodies in known positive samples just as well as the old antigen, showing that sensitivity is unaltered.

### RESULTS:

When testing the 365 samples from non-herd confirmed reactors 58 samples were found positive with both antigens and 220 samples were found negative with both antigens. No samples that were negative with the old antigen were found positive with the new antigen. 87 samples that were positive with the old antigen were found negative with the new antigen indicating that specificity was improved. Sensitivity was unaltered when testing the gold standard samples (Fig.2). Finally we tested 240 of our daily diagnostic samples, which primarily consist of negative samples, with the old and new antigen in parallel and got the same results with both antigens.

### DISCUSSION AND CONCLUSIONS:

This new method for preparing Salmonella antigen for our mix-ELISA does with benefit replace the antigen preparation that we have used until 2013. Without decreasing the sensitivity of the test, many positive reagents, that could not be verified bacteriologically, have been removed.

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