Proficiency Test of Four *Salmonella* Antibody ELISA-Tests for their Harmonization

Th. Blaha¹, J. Ehlers², U. Methner³, W. Leyk⁴, K. Rohn⁵, and L. Kreienbrock⁵

¹Field Station for Epidemiology, School of Veterinary Medicine Hanover, Buescheler Str. 9, D-49456 Bakum, Germany. Tel.: (+49) 4446 9599110, Fax: (+49) 4446 9599112, e-mail: thomas.blaqa@tiho-bakum.de ²Animal Health Service, Chamber of Agriculture for Lower Saxony, Oldenburg, Germany, e-mail: lehlers@lwk-we.de, ³Federal Institute of Viral Animal Diseases, Department of Bacterial Diseases, Jena, Germany, e-mail: umethner@bfav-jena.de, ⁴Institute of Animal Health, Chamber of Agriculture for Rhineland-Westphalia, Muenster, Germany, e-mail: wolfgang.ley@lwk-nrw.de, ⁵Institute of Biometrics and Epidemiology, School of Veterinary Medicine Hanover, Germany, e-mail: lothar.kreienbrock@tiho-hannover.de

**Keywords:** ring test, test congruence, test sensitivity, herd categorization

**Summary:** The paper describes the necessity to only use tests for national salmonella monitoring and reduction programmes that are “harmonized”, i.e. that produce at least at herd level the same results. Four in Germany licensed tests were audited in a proficiency test by four independent and neutral laboratories. The test was designed rather to harmonize the tests, if necessary, than to evaluate the single tests. The methods used to provide a high credibility for the proficiency test’s outcome are explained. The conclusion of the ring test is that three of the four tests can be used for the salmonella monitoring programme in Germany as long as not single results are compared to each other, but the results of sets of sera are used for the herd categorization for their risk level of introducing *Salmonella* spec. into the food chain.

**Introduction:** In Germany, a national “Salmonella Monitoring and Reduction Programme” (Blaha, 2003) has been implemented, which started in 2002 has been mandatory since April 1, 2003, for those farmers and slaughter plants that participate in the German newly developed quality management system for food, the so-called “QS-System” (Anonymous, 2003). In the framework of its implementation, the following problem emerged: when the programme started, 4 Salmonella-antibody ELISA tests had been licensed by the veterinary authority of Germany. This means that it is the right of anybody participating in the programme to use any of the 4 licensed tests. However, when the tests were being licensed some time ago, there was no national programme and, thus, no need for making sure that testing sets of random samples of a herd tested with all four tests should produce the same results. Due to this fact, the producers of the four tests defined their test specific algorithms for identifying a certain OD% value independently of each other. The result is that the perception that the tests differ in their sensitivity. It could be predicted that an ongoing discussion about which results are right and which are wrong would be unpreventable, since farmers will start to claim that their herd could be negative if only another test had been used. There were two possibilities to handle this: a) the QS-System had to decide to accept only one of the tests, or b) the tests had to be “harmonized”. Soon there was a consensus not to hinder the free competition between different test providers, i.e. a proficiency test had to be organized to objectively measure to which degree the tests really differ from each other.

**Material and Methods:**

**Test design:** Based on the experience of the first international ring trial of ELISAs for antibody detection in swine (van der Heijden, 2001), the test was planned and conducted under the leadership of the Field Station for Epidemiology of the School of Veterinary Medicine of Hanover, which also served as one of the four independent test laboratories that have no conflict of interest (the three other labs were: the Department of Bacterial Animal Diseases of the Federal Institute of Viral Animal Diseases in Jena, and the Diagnostic Department of the Chamber of Agriculture of Lower Saxony in Oldenburg.
and the Institute of Animal Health of the Chamber of Rhineland-Westphalia in Muenster). Since the test was designed and planned in full agreement of the test producers, they were asked to test the set of test sera, but only with their own test. In other words, the four neutral laboratories tested the set of test sera with all four tests, the producers tested the set of test sera only with their own test. The sera were randomly numbered and the laboratories and the tests were anonymized. All test participants were provided with a standardized Excel-table to make the data handling for the statistical analysis of the test results easier.

**Test sera:** Out of several thousand sera, the test results from one of the four ELISA tests of which were already known, 400 sera (200 meat juices, 200 blood sera) were selected. To make sure that the majority of the test sera were sera with expected OD% values “around” the target cut-off value of 40 OD%, the following range of values was chosen for the set of test sera for both the meat juice and the blood sera: 20% of the test sera with very low expected OD% values (0% or very close to 0%), 20% of the test sera with very high expected OD% values (above 100%), and 60% of the test sera with an expected OD% value around the cut-off value of 40%.

These sera were split into 8 aliquots and send to the four test laboratories asking them for “running the tests following the instructions as given by the test producers”- the four test producers just ran their own test without any particular instruction.

**Data handling:** 1) The four data sets of the producers were combined to one data set with all four tests so that, additionally to the four data sets of the neutral test labs (with four test results for every serum), there was a fifth data set with all four test results for every serum: the “producers’ data set”). This resulted in 5 data sets with results from 5 test participants having tested the same set of sera with 4 ELISA tests. 2) Although all test participants had gotten the same table to fill in, the data still had to be cleaned and tested for their plausibility, since some data were rounded up, others with a comma and some with dots; some high values were capped at a certain value, others were not etc.

**Statistical analysis:** Any statistical calculation was done using the SAS version 8.1e. 1) The calculation of mean and median values was performed traditionally. 2) The evaluation of correlations (two tests each) was estimated by scatter blots. 3) Box blots were constructed following the classical proposal of TUKEY (box range is from the 25 to the 75% quantile). 4) The variance analysis was performed (ANOVA) both on the original and the logarithmic scale. The comparative sensitivity analysis for the four tests was conducted by omitting the laboratories one by one and the ELISA tests one by one. 5) Finally, a descriptive compliance analysis was conducted by calculating the degree of congruence between the labs and the tests at varying cut-off values resulting in a variety of surface plots.

**Results:** All statistical testing of the four tests showed that the variance between the four tests was significantly higher than the variance between the five test laboratories. None of the tests correlated with one of the others completely, but one was clearly out of the range of the three other tests. The producer of this test agreed to retract it from the market until harmonized with the other three tests, although this test, as proven in the dossier for the licensing procedure, reliably recognizes sera with low and sera with high concentrations of Salmonella antibodies. The remaining tests were statistically tested again using the above-mentioned methods. This time, there was still a slight higher variance between the tests than between the laboratories (ring test participants), but this difference was not statistically significant.

**Conclusion:** These results suggest that the three remaining tests (produced by Labordiagnostik Leipzig, IDEXX, and Boehringer/Ingelheim) could be used alternatively for the Salmonella monitoring programme in the framework of the German “QS-System”. However, this conclusion does not apply to the comparison of “positive/negative” results of single sera, since those sera with an antibody concentration around the cut-off value can to up to 20% be identified differently as positive or negative if different tests are used. However, if the result of sets of 60 (= minimum random sample per herd) are used for categorizing the corresponding herd as “low”, “medium” or “high” risk herds, the inter-test variance of the tests is then definitively lower than the inter-laboratory variance of single
test results. In other words, although single sera, which have Salmonella antibody concentrations around the cut-off point, can be recognized as positive with one test and negative with the other test or vice versa, the three tests can be used, since less than 10% of the sera tested over years in Germany, have Salmonella antibody concentrations around the cut-off point (around 70% have very low titers, and about 15% have quite high titers, which are always correctly identified by all three tests as negative or positive). This means that the risk that a set of sera is categorized differently by using another test out of the three tests is so low that it can be neglected in the light of the overall goal of the German Salmonella Monitoring and Reduction Programme: Identifying via an ongoing semi-quantitative estimation the herds with the relatively highest risk of introducing zoonotic Salmonella spec. into the food production chain to be able to implement measures for a) reducing the cross-contamination of Salmonella spec. in the slaughter plant and b) reducing the salmonella load of swine herds identified as high risk herds. Finding the herds with the relatively highest salmonella risk, at which cut-off point ever, will be performed by all three tests at a comparative herd sensitivity level.

References:
Anonymous (2003): The German Quality Assurance Programme “QS” (= Quality and Safety) for the food production chain from feed to retail. www.q-s.de

ABBREVIATED IDENTIFICATION SCHEME FOR ESCHERICHIA COLI IN SWINE FECES

ANDREW MACK and JULIE FUNK*

Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, 1900 Coffey Road, Columbus, OH 43210, Ph: 614-247-6635, Fax: 614-292-4142, Email: mack.104@osu.edu *Presenting author

Summary: Antimicrobial susceptibility profiles of Escherichia coli (EC) are often used to monitor the effect of antimicrobial use regimens on the antimicrobial resistance (AR) reservoir in animal species. Epidemiological studies of AR may involve the identification of thousands of bacterial isolates, so complete biochemical identification of EC can be prohibitively expensive and time consuming. In this study an abbreviated biochemical scheme using colony phenotype and the indole test results in a sensitivity and specificity of 91.7% and 100% respectively for identification of EC as compared to a commercial biochemical identification kit. This abbreviated scheme results in over US$500 savings per 100 candidate EC isolates identified. These savings have significant benefits to the economics of conducting epidemiologic investigations of AR.

Keywords: Indole, Antimicrobial Resistance, API 20E, Sensitivity, Specificity

Introduction: The gram negative fecal flora represents a reservoir of antimicrobial resistance (AR) genes transferable to foodborne pathogens. Escherichia coli (EC) are often used to define the reservoir of AR. Epidemiological studies may involve testing thousands of isolates, so complete biochemical identification of EC can be prohibitively expensive. The goal of this project was to develop an inexpensive and effective method of identification of EC. Previous investigators (Hyatt et al, 2002) determined that 99.4% of colonies isolated from cattle feces that had typical EC phenotype on MacConkey agar and were positive for indole production were EC. According to the Manual of