Testing the Proficiency of the German Test Kit “SALMOTYPE® - ELISA” to Identify Salmonella Antibodies in Porcine sera and meat juices in USA Diagnostic Laboratories

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Introduction

Antibodies against various Salmonella serovars can be quantitatively detected in serum and meat juice of naturally infected pigs via enzyme immunoassay method (ELISA), and be used for monitoring the Salmonella status of swine herds over time. Ongoing testing of random samples from swine herds via ELISA technique provides, on a herd basis, sufficient information for a "Salmonella categorization" of these herds by determining the in-herd prevalence of anti-salmonella antibodies.

With the "SALMOTYPE®-ELISA", a test kit based on the so called Danish mix ELISA test (1), licensed and commercially available in Germany, has been used on a routine basis for the current German, governmentally supervised, but voluntary program "Reduction of the Introduction of Salmonella into the Food Chain via Slaughter Hogs" since June 1998 (2, 3).

The goal of the presented proficiency test that was carried out in 7 different U.S. laboratories* (import permit No. 5151/1999 by USDA of April 7, 1999) is to test the applicability of this ELISA test in the USA.

Material and Methods

This assay is designed to measure the quantity of antibodies to Salmonella in swine meat juice or in swine serum. Salmonella mix-antigen (1) is coated on 96-well plates. Upon incubation of the test sample in the coated well, antibodies specific to Salmonella O-antigens 1,4,5,6,7 and 12 forms a complex with the coated Salmonella antigen. Unbound material is washed away and conjugate is added which binds to any attached anti-pig antibody in the wells. After washing away unbound conjugate from the wells, enzyme substrate is added. Subsequent colour development from conjugate-bound enzyme is directly related to the amount of antibody to Salmonella present in the test sample.

The "SALMOTYPE®-ELISA" detects more than 90% of the most common Salmonella serovars found in pigs.

In the presented proficiency test, 7 U.S. laboratories participated using 4 test samples from experimentally infected pigs, the negative control sera are derived from a salmonella-negative swine herd of a university experiment station, which is monitored for several pathogens including Salmonella on a regular basis.

In one test kit, the detection of the antibody titers of the control sera and the 4 test sample sera was carried out threefold per 96-well plate. The test sera were lyophilized and pre-diluted. There was (arbitrarily) no personal instruction for any of the participants - the tests were conducted in all seven laboratories only following a written instruction.

As for the proficiency test conducted in Germany and Austria (4), the following limits for acceptance of the test' accuracy were defined: deviation from the desired value ≤ 10%, and correlation coefficient ≥ 0.95 with a final value -10 to +10 (figure 1).

Results

| Statistics: | Sample 1 | Sample 2 | Sample 3 | Sample 4 | R | mean value 
|-------------|----------|----------|----------|----------|---|----------------
| desired value, Ab-Titer [%] | 37,3     | 64,6     | 13,4     | 0,5      |   |                  |
| mean value, Ab-Titer [%]    | 36,9     | 64,6     | 13,2     | 0,1      | 0,9788 | 38,2                |
| standard deviation          | 3,7      | 3,0      | 0,6      | 1,1      | 0,014 | 2,4                |
| variation coefficient [%]   | 9,9      | 4,6      | 4,8      | 1602     | 1,4 | 6,4                |

Figures 1 and 2 show the results of the individual laboratories in comparison to the maximum and minimum values of deviation from the desired value (%) that were allowed to accept the test result in the framework of the proficiency test and the distribution of the antibody titers of each test samples determined in the seven laboratories.
Figure 1. Comparison of the proficiency test results of the seven participating labs.

![Graph showing deviation from desired value for Laboratories 1-7 (calculated from the correlation and the % deviation from the desired value)]

Figure 2. Distribution of the antibody titers [%] determined by all seven participants in the proficiency test.

![Graph showing antibody titer distribution for proficiency samples 1-4]

Discussion

The calculation of a regression straight line from the OD-values and the concentrations of the control sera is necessary to compensate for inevitable inconsistencies in the control sera. The concentrations for the test samples are then determined from the OD-values of the samples and the established regression formula.

The variation coefficients of the test results from the seven participating laboratories (inter-assay variation) for the test samples 1-3 are extremely low (4.6 - 9.9%, average 6.4%).

The conclusions are:

- The commercial, “ready for use” test kit SALMOTYPE®-ELISA is easy to use and produces highly consistent results in different laboratories, even if only a written instruction is provided.
- The test results from different laboratories can be directly compared to each other, since the test conditions are highly standardized.
- The presented test results from the USA proficiency test are highly consistent with those of the proficiency test in Germany and Austria (4, 5, 6, 7).

The results suggest that the test kit SALMOTYPE®-ELISA could be used for any research on the Salmonella in herd prevalence, especially if different herds, regions and production systems are to be compared to each other. There is also a potential for the test to be used for monitoring systems in pork production systems that are interested in categorizing their herds for any pre-harvest food safety and quality assurance programs.

References


