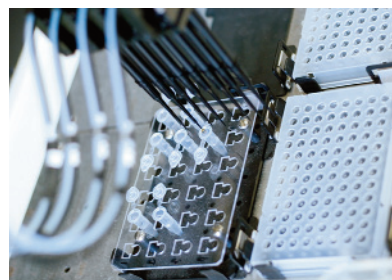
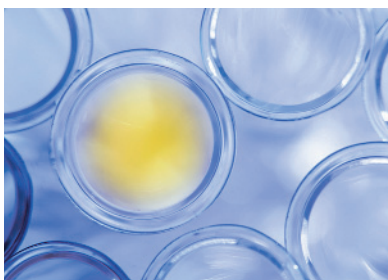




SAL PLEX™ KIT PS



PRODUCT INFORMATION FILE
SALMONELLA TEST FOR PORCINE SERUM



THE NEW STANDARD IN ASSAYS

PRODUCT INFORMATION FILE SAL PLEX™ KIT PS

SALMONELLA TEST FOR PORCINE SERUM

SALMONELLOSIS

Salmonellosis is an infection with *Salmonella* bacteria. Human infection with *Salmonella* may result in diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In most cases, the illness lasts 3 to 7 days and affected persons usually recover completely without treatment. Young children, the elderly, and immunocompromised people are most likely to have severe illness [2]. Following the White/Kauffman/Le Minor scheme, approximately 1,500 serovars are listed for the species *S. enterica*. So far strains isolated from humans are distributed in 194 serovars. Fifteen serovars represent 91% of all human isolates with typhimurium and enteritidis the most common serovars (81% of all isolates) [3,6].

HUMAN SALMONELLOSIS

In 2007, a total of 155,500 cases of human salmonellosis were reported in the EU. Overall, the incidence in the EU was 31 per 100,000 population [6]. Because many mild cases are not diagnosed or reported, the actual number of infections may be 4 to 30 times higher or even more. In Denmark, The Netherlands and Germany, it was estimated that 10-15%, 14-19% and 18-23%, respectively, of salmonellosis cases in humans were attributable to pork [4]. The estimated contribution of laboratory confirmed salmonellosis from different reservoirs to (data collected in The Netherlands, 2006) were 36% for laying hens, 21% for pigs, 14% for chicken, 13% for cattle, 16% for travel and other. *Salmonella* infections usually result from a combination of contaminated foods, poor kitchen hygiene and inadequate cooking. Salmonellosis is considered a major public health hazard with a mortality rate up to 0.25% (1 per 400) of affected patients.

SALMONELLA PREVALENCE IN ANIMALS

Salmonella bacteria are usually contracted by ingesting raw or undercooked animal products, such as eggs, poultry, meat, milk and dairy products. In fresh eggs, at processing and at retail level, *Salmonella* is found in the EU at levels below 3%. The dominant serovar is *S. enteritidis* in poultry and *S. typhimurium* in pigs (Table 1) [1,6].

Clinical salmonellosis in pigs is of minor importance for the animal itself but will result in shedding of the bacteria at a high level in the faeces. Pigs with sub-clinical infections only excrete bacteria intermittently and at low levels [1,8].

Table 1. *Salmonella* prevalences in the EU

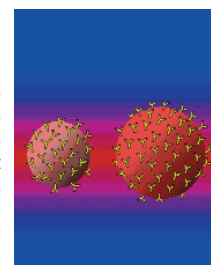
SOURCE	SALMONELLA POS
Fresh eggs	< 3%
Fresh broiler meat - at slaughter	6% - 9%
- at processing	< 22%
Fresh turkey meat	0% - 11%
Turkey meat products	< 5%
Fresh duck meat	15% - 39%
Fresh geese meat	< 10%
Pigs at slaughter	0% - 9%
Pigs at processing	0% - 18%

SALMONELLA MONITORING

Successful implementation of 'feed-to-food' *Salmonella* control programs in Denmark, Germany, United Kingdom, Netherlands and Belgium [5] has created an expectation for other pork-producing countries and regions to investigate *Salmonella enterica* spp. in their pig populations and to evaluate the feasibility of potential control options. Knowledge of prevailing serogroups of *Salmonella* is important because all EFSA (European Food Safety Authority) reported isolates from pigs belong to one of the O-serogroups B (*S. typhimurium*, *S. brandenburg*), C₁ (*S. infantis*, *S. livingstone*), or D (*S. panama*). About 60 to 70% of all isolates are represented by *S. typhimurium*, serogroup B. Serogroups B, C₁ or D accounted for 89-95% of isolates in The Netherlands and Denmark [1,8]. Other serovars that can be found are C₂ (*S. goldcoast*) and E (*S. anatum*, *S. london*).

MULTIPLEX SALMONELLA ASSAY

The RnAssays® product-line is based on a system of multi-analyte Plex assay testing which quantifies multiple microbial and non-microbial contaminations simultaneously, in a single sample and uses minimal animal-derived sample volumes. It represents a significant advantage in effectiveness and productivity over a standard ELISA assay. The Sal Plex™ kit PS is the new multiplex assay developed by RnA for *Salmonella* serogroup testing and measures anti-*Salmonella* antibodies in pig serum (PS).



Microspheres

The technology uses an immunoassay with capturing antigens fixed on distinct sized microspheres designated to bind antibodies. Discrimination of individual microspheres is made possible through different internal fluorescent intensities, which provides multiple analytic results per sample. With flow cytometry not only are fluorescent intensities of microspheres detected, but also small size variations can be distinguished. This provides a solution offering up to 3 different bead sizes, allowing the simultaneous detection of 36 different analytes per size of bead.

The combined technology of microspheres and a patented unique surface modification of these microspheres performed by RnA enables one to detect an infection by *Salmonella* serovars from serogroups B, C₁, C₂, D and E. As a result of this breakthrough RnA have developed unique time- and cost-saving diagnostic assays for safety and quality control. Specific antibody-antigen binding is detected following incubation with fluorescently tagged anti-immunoglobulin antibodies, analyzing on a concurrent basis, passing microspheres and tagged antibodies. Only when fluorescences of both microsphere and antibody are detected simultaneously, is there a response recorded for that specific microsphere interaction and binding anti-*Salmonella* antibody interaction.

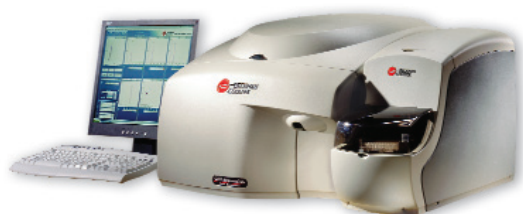
In the Sal Plex™ kit PS a sample is diluted and filtrated followed by incubation with specific antigen coated microspheres for serogroups B, C₁, C₂, D and E and reporter molecules at ambient temperature (total incubation time: 45 min). Captured primary, matrix-derived anti-*Salmonella* antibodies are detected by cytometric flow analysis (the reference sera are supplied in the kit and will mark the interpretation of Sal Plex™ kit PS analysis).



MATERIALS

The Sal Plex™ kit PS analyses are carried out with the Beckman Coulter (BC) Cell Lab Quanta™ SC MPL (Basic Plex™), an advanced, cost-effective, flow cytometer with multi-platform loader that provides microtitre plate and microtube analysis. A convenient software interface allows fingertip access for collecting and analyzing sample data.

The unit can be expanded with a Biomek NX automation workstation with shaker- and vacuum-unit robotic liquid handler (Silver Plex™), or ultimately with a Biomek Robotic Transport device (BRT) and the Cytomat Microplate Hotel Storage system (Gold Plex™). The results of the test cannot be warranted if not carried out on the validated BC equipment.



BC Quanta SC MPL
Basic Plex™ flow cytometer



BC Biomek NX
Silver Plex™ Automation workstation



BC Cytomat
Gold Plex™ Microplate Hotel Storage system

The benefits of Sal Plex™ kit PS

Sensitive: 100% of the EFSA (European Food Safety Authority) reported *Salmonella* isolated serovars (more than present ELISA tests).

Specific: serogroup-typing (B, C₁, C₂, D and E).

Flexible: Single or multiple analyte analysis.

Precise: Serogroup profiling reveals non-*Salmonella* cross reacting antibodies. The low CV's (Coefficient of Variation) give less false-positive results, in particular, close to the decision limit. In both cases, the number of false-positives are decreased, which can reduce the costs of confirmational analysis and of falsely condemned carcasses.

Fast: less than 1 hour for a single sample and at high throughput approx. 90 samples/hr, equaling 450 data points per hour with a Sal Plex™ kit PS.

Economical benefit: labour and overall time-saving. There is the option of leasing out the hardware.

Versatile: Combining the detection of *Salmonella* along with other microbiological and non-microbiological contaminants, such as *Trichinella spiralis* and sulphonamides, will be possible according to the needs of the user.

Table 2. Diagnostic Sensitivity (DSe) and Specificity (DSp) of the Sal Plex™ kit PS for *Salmonella* serogroup B, compared with lymph node culture. The corresponding data for a frequently applied commercially available ELISA-test kit are summarized as well.

	SAL PLEX™ PS (B)		ELISA	
CUT-OFF	60	100	10	40
DSe	72	60	75	38
DSp	70	85	62	90

SAL PLEX™ KIT PS DATA

PRECISION

The repeatability of the Sal Plex™ kit PS was evaluated by running positive pig serum 18 times on 4 repeating plates over 3 days. Five serogroups were tested for. The average interday, interplate, and intraplate variation was 5%, 3% and 4%, respectively, for all tested serogroups.

SENSITIVITY AND SPECIFICITY

The diagnostic sensitivity (DSe) and specificity (DSp) for serogroup B were determined by comparing the results of mesenterial lymph node culture with the results of the Sal Plex™ kit PS on corresponding sera, collected from 1,040 pigs originating from multiple farms in various Dutch regions. At a recommended cut-off value of 60, these samples with an unknown infection background, revealed a relative sensitivity and specificity of 72% and 70%, respectively (Table 2).

RnA ASSAYS®

RnA is the inventor of the RnAssays® product line comprising of assays developed at the Utrecht University, and owns global patents for the innovative covalent coupling of antigenic and receptor structures to solid surfaces, such as those of well-defined microspheres, sensor-surfaces or sensor-tips. RnA is specialized in the development and application of time- and cost-saving diagnostic assays for safety and quality control in the primary and secondary food production sectors with the focus on foods of animal origin [7]. Following the Sal Plex™ kit PS, RnA is developing series of easy-to-use kits to determine the presence of agents, such as PRRS virus (Porcine Reproductive and Respiratory Syndrome), *Trichinella spiralis*, *Toxoplasma gondii*, and sulphonamides. RnAssays® kits meet current and future legislation requiring food products, free from specific diseases and contaminants. The kits are suitable for modern routine laboratories needing high-throughput and short hands-on times, and are cost effective.

RnA received the World Poultry Award 2006 for its innovative Plex™ technology and was awarded the international Eureka status in November 2007.

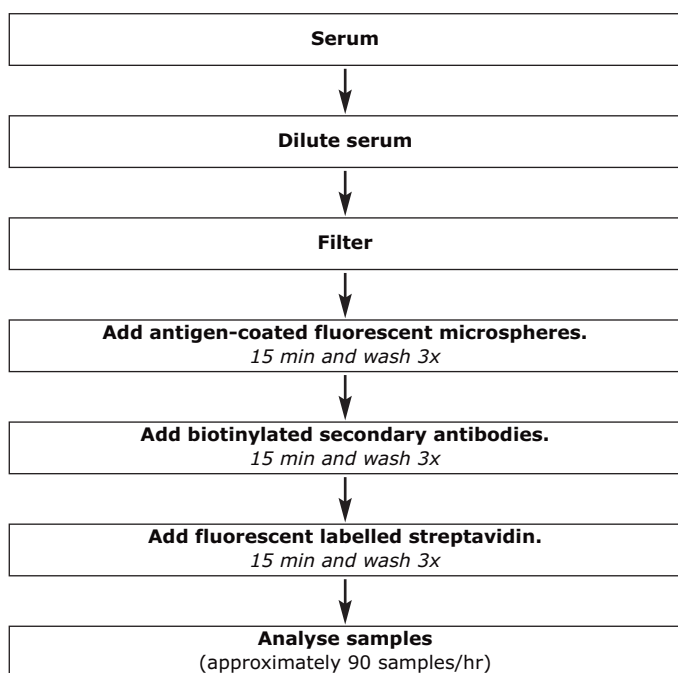


REFERENCES

1. Baggesen DL, Wegener HC, Bager F, Stege H, Christensen J. Herd prevalence of *Salmonella enterica* infections in Danish slaughter pigs determined by microbiological testing. *Prev Vet Med* 1996; 26: 201-13.
2. Centers for Disease Control and Prevention. *Salmonella* Infection (salmonellosis) and Animals, www.cdc.gov.
3. Grimont PAD, Grimont F, Bouvet Ph. Taxonomy of the genus *Salmonella*. In: *Salmonella* in domestic animals. Wray C and Wray A. Eds. CABI Publishing Oxon, New York. 2000: 1-19.
4. Hald T, Wegener HC. Quantitative assessment of the sources of human salmonellosis attributable to pork, Proceedings of the Third International Symposium on the Epidemiology and Control of *Salmonella* in Pork. Washington DC, USA 1999: 200-5.
5. Nielsen B. Pork safety - a world overview. Proceedings of the 17th Congress of the International Pig Veterinary Society Ames, IA, USA. 2002: 121-35.
6. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, *EFSA J* 2009; 223.
7. Thomas ME et al. Detection of egg yolk antibodies reflecting *Salmonella enteritidis* infections using a surface Plasmon resonance biosensor. *J Immunol Methods* 2006; 315: 68-74.
8. Van der Wolf PJ et al. *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. *Vet Microbiol* 1999; 67: 263-75.



Sal Plex™ kit PS reagents and methods



ORDER CODES

- 76025.000: 1000+ assays, incl. 22 filter plates
- 76025.001: 2000+ assays, excl. filter plates

CONTENT SAL PLEX™ KIT PS

- 22 filter plates
- Positive and negative reference sera
- Bead mixture
- Secondary antibodies and fluorescent tags
- Necessary buffers and other solutions

REQUIRED AUXILLARY MATERIAL

(not provided within the kit)

- Microsphere analyzer: Quanta SC MPL
Beckman Coulter, Cat No: 774476
- Microwell plate shaker
Heidorf Titramax 100, Cat No: P/N 544-11200-00
- Vacuum system for microwell filter plates
Multiscreen HTS, vacuum manifold, Millipore, Cat. No: MSVMHTS002
Multiwell plate vacuum manifold, Pall Corp, Cat. No: 5017
Vacuum pump
- 0.2 µm filters to filter aqueous solutions
Bottle top filter 500 ml, 45 mm, Omnilabo, Cat. No: 595-4520
- Microwell plates
U96 PP-0.5 ml, NUNC, Cat. No: 267385
- Vortex mixer
- Measuring cylinders
- Pipettes
- Serum samples
- High-quality water

NORMALIZATION AND CALIBRATION

- Normalization and calibration of the assay is carried out using the positive and negative reference sera provided.
- Decision limits for each serogroup depend on local circumstances in terms of incidence of serovars and requested sensitivity in relation to specificity. This criteria should be evaluated and decided upon by the end-user.

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RnA BV

Yalelaan 2, Nieuw Gildestein
3584 CM Utrecht, The Netherlands
☎ +31 (0)30 253 53 62
info@RnAssays.com
www.RnAssays.com