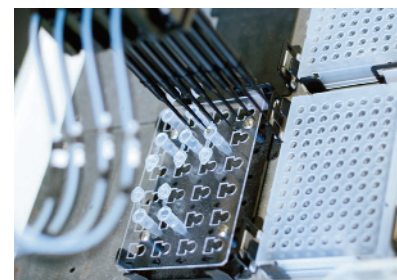
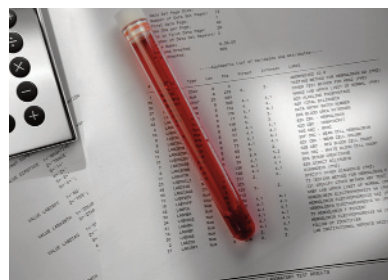
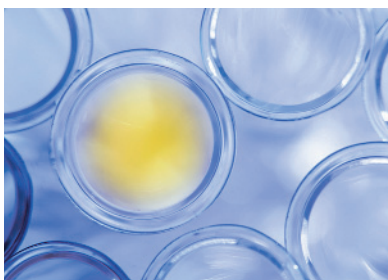




# SAL PLEX™ KIT PS



DATA FILE  
SALMONELLA TEST FOR PORCINE SERUM



THE NEW STANDARD IN ASSAYS

# DATA FILE SAL PLEX™ KIT PS

## SALMONELLA TEST FOR PORCINE SERUM

### PREDICTIVE VALUE

A positive/negative predictive value indicates the proportion of animals with positive/negative test results who have been correctly diagnosed. In a predictive value, the prevalence of a pathogen or infection in a population is inherently discounted.

For the Dutch situation with a prevalence of 8.5% (highest subprevalence for serogroup B) the following predictive values were calculated: at a cut-off value of 60 as well as at 100, the negative predictive value is 96%. These are excellent test characteristics if the delivery of an absolutely *Salmonella*-free herd is been aimed for.

### SENSITIVITY AND SPECIFICITY

The diagnostic sensitivity (DSe) and specificity (DSp) are important parameters to describe an assay. These parameters are usually obtained by a comparison of test results. In this case, between the RnA Sal Plex™ and an assay, considered to be a field standard. In this instance we have collected more than 1,040 serum samples from Dutch abattoirs to assess the DSe and DSp of the Sal Plex™ kit PS (Tables 1-3). **All, i.e. 100%, of the EFSA reported *Salmonella* isolated serovars can be detected with the Sal Plex™ kit PS.**

As the standard method, selective culturing of *Salmonella* from the mesenteric lymph nodes was applied. To provide an alternative test, a commercially available ELISA was employed to detect anti-*Salmonella* serum antibodies, similar to the indirect analysis strategy of the Sal Plex™ kit PS.

The DSe for the Sal Plex™ kit PS was calculated for serogroup B at 72% and the DSp was found to be at 70% for a cut-off of 60, whereas these values were 75% and 62%, respectively, for the ELISA kit (at cut-off 10), bearing in mind that the Sal Plex™ kit provided serogroup information as well.

The DSe and DSp depends on the cut-off value used (see Table 1 and Figure 1). The corresponding data for a commercially available ELISA-test kit are summarized as well, calculated for serogroup B.

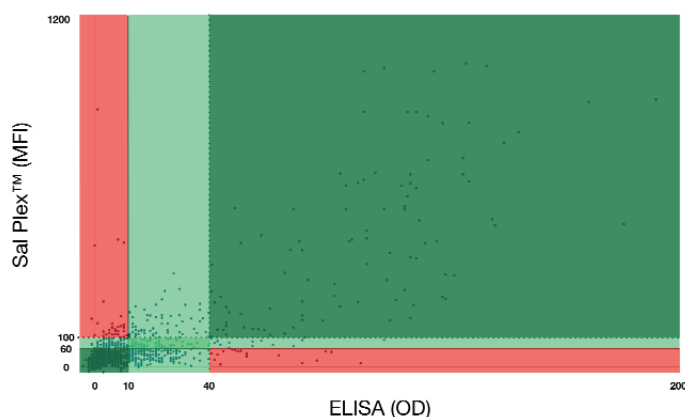


Figure 1 Correlation of ELISA and Sal Plex™ results for serogroup B. Cut-off values at 10 (vertical curve) and 40 (vertical dashed curve) for ELISA and the corresponding values at 60 (horizontal curve) and 100 (horizontal dashed curve) for Sal Plex™ are shown. The green colored boxes indicate the matches, whereas the red colored boxes indicate the mismatches.

If a sample scores for all serogroups then this is a very strong indication of an occasionally occurring non-specific antibody response (e.g. originating from a humoral response against a non-*Salmonella* Enterobacteriaceae infection). Consequently, the chance of confirmation actions, on the basis of false-positive results will decrease and so will the associated costs.

**Positive results may be prone for confirmation by selective bacterial culturing. The power of the unique serogroup-fingerprinting of the Sal Plex™ lies in it's ability to discriminate specific signals from non-specific signals, which is not possible in the typical ELISA assay.**

Table 1. Sensitivity and specificity for the B serogroup (similar for C<sub>1</sub>, C<sub>2</sub>, D and E serogroups) when determined at the indicated cut-off values.

SENSITIVITY (%)	SAL PLEX™ KIT PS (B) SPECIFICITY	CUT-OFF VALUE
97	5	3
90	19	17
81	46	37
71	76	72
60	86	101
50	93	158

### PERFORMANCE

The Sal Plex™ was tested in 1,040 pig field serum samples (only serogroup B data) and compared against lymph node bacterial cultures. The so-called grey-zone (comparable to the 10-40 range in the ELISA) for the Sal Plex™ can be considered as the range of values between 90% DSe and 90% DSp: samples that have a correlating MFI (Mean Fluorescence Intensity) read-out can be considered as suspect. The intermediate range for serogroup B at 90% is between 11 and 125.

Table 2. Performance data of ELISA and Sal Plex™ PS in the case of the Dutch *Salmonella* prevalence in slaughtered pigs (8.5% as determined by analysis of bacterial isolates from lymph nodes). DSe, diagnostic sensitivity; DSp, diagnostic specificity; -PV, negative predictive value.

PARAMETER	SAL PLEX™	ELISA	SAL PLEX™	ELISA
CUT-OFF	60	10	100	40
DSe (%)	72	75	60	38
DSp (%)	70	62	85	90
-PV	96	96	96	94

Table 3. Performance data of ELISA and Sal Plex™ PS in the case of the Spanish *Salmonella* prevalence in slaughtered pigs (29% as determined by analysis of bacterial isolates from lymph nodes).

PARAMETER	SAL PLEX	ELISA	SAL PLEX	ELISA
CUT-OFF	60	10	100	40
DSe (%)	72	75	60	38
DSp (%)	70	62	85	90
-PV	86	86	84	78



- NOTE 1: For the calculation of -PV in Table 3 (prevalence in Spain) it was assumed that DSe and DS<sub>p</sub>, as calculated from the Dutch pork samples, applies.
- NOTE 2: All performance data are based on a Dutch field sample series.
- NOTE 3: Prevalence of *Salmonella* in slaughter pigs in UK in 2007 was 21.2% (Figure 2; EFSA, 2009).
- NOTE 4: Prevalence of *Salmonella* in slaughter pigs in DK in 2007 was 7.7% (Figure 2, EFSA, 2009).
- NOTE 5: The average signal-to-noise ratio (S/N) for serogroup B at cut-off value 60 is 25.  
This indicates a comfortable resolution and 'distance' between analytical and diagnostic sensitivities. Therefore, it will be easier to discriminate the positive from the negative samples.

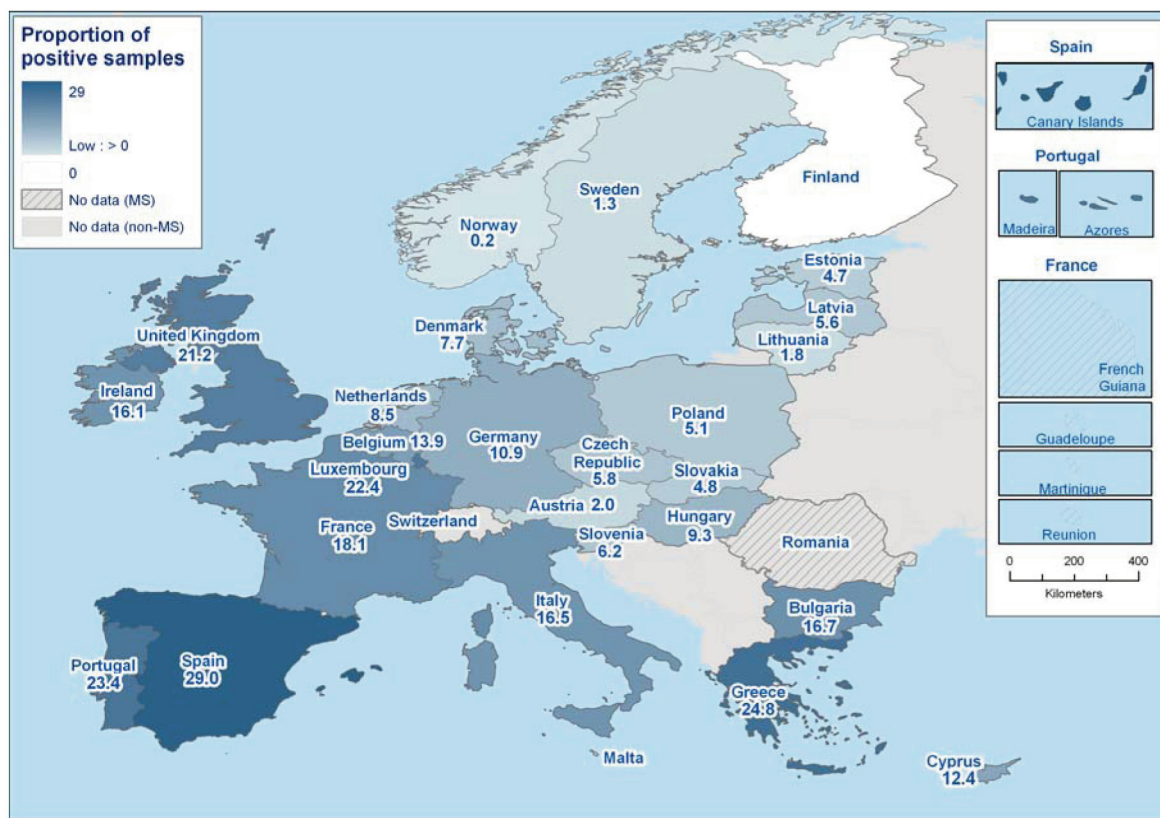


Figure 2. Prevalences of *Salmonella* in pigs (lymph node bacterial cultures) in Europe 2007 (EFSA Journal 2009, 223 pag. 86, Fig. SA 25).

### PRECISION\*

	Sal Plex™ PS
intraplate variation:	5%
interplate variation:	3%
interday variation:	4%

\* Precision calculated on serogroup B

The precision of the Sal Plex™ outperforms that of ELISA assays, for which the relative standard deviation is approximately 10%. A high relative standard deviation can give rise to false-positive results and in particular if the final result is close to the decision limit of the assay. On the other hand, a smaller CV, such as found for the Sal Plex™, can give less false positive results and thus will reduce the costs incurred in re-analysis of the sample and in confirmation analysis by for example selective bacterial culturing methods.



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