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Comparability of different ELISAs on the detection of *Salmonella* spp. antibodies in meat juice and serum

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CRL-*Salmonella*
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This investigation has been performed by order and for the account of the European Commission, Directorate-General for Health and Consumer Protection (DG-Sanco), the Dutch Food and Consumer Product Safety Authority (VWA) and the Community Reference Laboratory for *Salmonella*, within the framework of RIVM project V/330604/07/CS

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Abstract

Comparability of different ELISAs on the detection of *Salmonella* spp. antibodies in meat juice and serum

At the request of the European Commission, it has been investigated whether faster methods for detecting *Salmonella* in slaughter pigs can be used. This appears not to be the case. Routinely *Salmonella* is detected in the lymph nodes of pigs using the prescribed culture method. The alternative methods can detect the presence of antibodies against *Salmonella* in the meat juice of thawed pork meat and blood (serum) in pigs.

The Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*), situated at the RIVM, tested the quality of these methods in cooperation with the Animal Health Service (GD Deventer). This study which was commissioned by the European Union was carried out from October 2006 to October 2007.

The study consisted of two parts. For the first part, ten member states had to send sixty meat juice samples to the CRL-*Salmonella*. The member states had tested these samples using their own method of testing. The GD tested all these meat juice samples using one and the same method on behalf of the CRL-*Salmonella*. The results of nine of the ten member states differed from the results of the GD. The methods used by the different member states are therefore not comparable where the testing of meat juice samples is concerned.

For the second part of the study, the member states received serum samples from pigs that were either infected or not infected with *Salmonella*. These samples were tested for the presence of antibodies against *Salmonella*. All member states showed good results regarding this. Theoretically, these methods could therefore be used. However, specific expertise is needed both to take blood from pigs and with its further handling. This expertise is not yet present in all slaughterhouses in Europe.

Key words:
Salmonella, ELISA, detection, antibodies

Rapport in het kort

Vergelijkbaarheid van verschillende ELISAs voor de detectie van antilichamen tegen *Salmonella* spp. in vleesdrip en serum

De Europese Commissie heeft laten onderzoeken of snellere methoden om een *Salmonella* besmetting bij slachtvarkens op te sporen, geschikt zijn. Dit blijkt niet het geval. Normaal gesproken worden de *Salmonella* bacteriën met een voorgeschreven kweekmethode uit de lymfeklieren van varkens geïsoleerd. De alternatieve methoden analyseren de aanwezigheid van antilichamen tegen *Salmonella* in het vocht van ontdooid vlees (vleesdrip) of het bloed (serum) van varkens.

Het Communautair Referentie Laboratorium voor *Salmonella* (CRL-*Salmonella*), gevestigd op het RIVM, heeft in samenwerking met de Gezondheidsdienst voor Dieren (GD, Deventer) de kwaliteit van deze methoden getest. De studie vond plaats tussen oktober 2006 en oktober 2007 in opdracht van de Europese Unie.

Het onderzoek bestond uit twee onderdelen. Bij het eerste onderdeel stuurden tien lidstaten zestig vleesdripmonsters naar het CRL. De lidstaten hadden deze monsters met hun eigen methode onderzocht. Voor het CRL onderzocht de GD al deze vleesdripmonsters met één methode. Negen van de tien lidstaten vonden andere resultaten dat de GD. De methoden van de lidstaten zijn daardoor niet vergelijkbaar om vleesdrip te testen.

Bij het tweede onderdeel kregen de lidstaten serummonsters van varkens met en zonder *Salmonella*-infectie toegestuurd. Deze monsters werden getest op de aanwezigheid van antilichamen tegen *Salmonella*. Alle lidstaten hebben goede resultaten behaald. In theorie zou deze methode dus gebruikt kunnen worden, maar voor het afnemen van bloed bij varkens tijdens de slachtfase en het opwerken van het bloed is specifieke kennis nodig die niet in alle slachthuizen aanwezig is.

Trefwoorden:
Salmonella, ELISA, detectie, antilichamen

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Acknowledgements

We thank Peter van de Wolf from the GD Deventer for the fruitful discussions and critically reading the manuscript.

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Contents

List of abbreviations

S.E.M.	Standard error of the mean
OD%	Relative optical density percentage
S/P ratio	Sample value related to positive control value
ELISA	Enzyme-linked Immunosorbent Assay
ROC	Receiver Operating Characteristic plot
CRL-*Salmonella*	Community Reference Laboratory for *Salmonella*
NRL-*Salmonella*	National Reference Laboratory for *Salmonella*

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Summary

From 1 October 2006 to 1 October 2007 a Community-wide baseline survey on the prevalence of *Salmonella* in slaughter pigs was carried out in the Member States' (2006/668/EC). New in this study, when compared to former baseline studies, was the possibility to use a serological method in addition to the bacteriological method for the detection of *Salmonella* spp. antibodies in pigs sampled in the slaughterhouse. Ten National Reference Laboratories (NRLs) for *Salmonella* agreed to perform serology based on meat juice samples, while one NRL in addition used blood samples. As no standard method existed for serology, the NRLs were allowed to use their own methods. In order to enable comparison of serological results, CRL-*Salmonella* organised both a duplicate analysis study based on field samples as well as an interlaboratory comparison study using reference sera.

For the duplicate analyses study the participating NRLs had to send a selection of 60 meat juice samples from the baseline study to the CRL-*Salmonella* where these samples were tested with one 'reference'method, the HerdCheck Swine Salmonella ELISA from IDEXX.
Four NRLs used the Salmotype PigScreen ELISA (Labor Diagnostik Leipzig), three NRLs used the HerdCheck Swine Salmonella ELISA (IDEXX), one laboratory used the VetSign Porcine Salmonella ELISA (Guildhay) and two NRLs used an in-house ELISA. Different cut-off values were used by different NRLs. The NRLs which used the Salmotype Pigscreen ELISA all used the same cut-off values (- = OD% <10, ± = OD% >10 and <20, + = OD% >20). The NRL which used the VetSign Porcine Salmonella ELISA used cut-off values based on the S/P ratio (- = S/P ratio <0.10, ± = S/P ratio >0.10 and <0.25, + = S/P ratio >0.25). The NRLs which used the HerdCheck Swine ELISA all used different cut-off values (OD% >10, OD% >15 and OD% >20) and expressed their results only in – or +. The two NRLs that used an in-house ELISA also expressed their results as – or +, both used different cut-off values (OD% >20 and OD% >40).

Comparing the results from the CRL with those of the NRLs using a dependent t-test and a more complicated bivariate mixture fitting, statistically differences were found for most of the comparisons. For 5 NRLs the average OD% was statistically higher than that of the CRL, for 2 NRLs the average OD% was statistically lower, for 1 NRL the average S/P ratio was statistically lower than that of the CRL and for 2 NRLs no statistical differences were found. Four of the 5 NRLs which found higher OD% than the CRL used the same ELISA (Salmotype PigScreen), the other NRL used an in-house ELISA. The 2 NRLs which found lower OD% than the CRL used the HerdCheck Swine Salmonella ELISA and the NRL which found lower S/P ratios than the CRL used the VetSign Porcine Salmonella ELISA. The two NRLs which found no statistical different OD percentages from the CRL, used the HerdCheck Swine ELISA and the in-house ELISA. From the results of this study it was concluded that, it is difficult to compare quantitative serological results of meat juice samples from different NRLs and different ELISAs. Even when the same ELISA (HerdCheck Swine Salmonella) was used by different laboratories, statistical differences were found.

At the end of the baseline study (September 2007) an interlaboratory comparison study on serological methods was organised by the CRL-*Salmonella.* In this study the same NRLs-*Salmonella* have participated as the ones participating in the method comparison study. The NRLs received a set of 'standard' sera to test with their own method. A total number of 40 sera had to be tested. Two sera were obtained from *Salmonella*-free pigs and two samples were obtained after inoculation of pigs with *Yersinia enterocolitica* O3-/O9-, which can possibly result in cross-reaction. All other 32 samples were obtained after experimental inoculation of pigs with different *Salmonella* strains (*S.* Typhimurium,

S. Brandenburg, *S.* Panama, *S.* Goldcoast and *S.* Livingstone). Each NRL was asked to interpret their results by using the cut-off value that was routinely used in the baseline study.
A quantitative comparison of *Salmonella*-ELISAs was performed by computing Receiver Operating Characteristic (ROC) plots. The area below this curve is proportional to the diagnostic accuracy of a test. For all NRLs the ROC-area was very high, which indicates that all tests are able to detect the true status of the samples, however at different cut-off values.

The results from the 3 NRLs using the HerdCheck from IDEXX are comparable between the laboratories. On average labcode 8 showed the lowest OD% values and labcode 4 the highest, however this difference was mainly found for sera yielding high OD% values. Three of the 4 NRLs using the Salmotype ELISA showed comparable results for the different sera. However, the OD% values for labcode 10 were higher than for the other 3 NRLs (labcode 1, 2 and 6) for almost all sera. The results for the other 3 NRLs were almost identical, indicating a high interlaboratory reproducibility, especially in the more relevant low OD% range.

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1 Introduction

From 1 October 2006 – 1 October 2007 a Community-wide baseline survey on the prevalence of *Salmonella* in slaughter pigs was carried out in the Member States' (2006/668/EC). New in this study, when compared to former baseline studies, was the possibility to use a serological method in addition to the bacteriological method for the detection of *Salmonella* spp. antibodies in pigs sampled in the slaughterhouse. Ten NRLs-*Salmonella* agreed to perform serology based on meat juice samples, while one NRL used blood samples in addition. As no standard method existed for serology, the NRLs were allowed to use their own methods. In an attempt to compare the different methods, the following activities have been organised by the CRL-*Salmonella:*

1. A duplicate analysis study in which the participating NRLs-*Salmonella* have sent, during the one year baseline study, a selection of in total 60 meat juice samples to the CRL-*Salmonella*, where the samples were analysed with a single serological method;
2. An interlaboratory comparison study, performed at the end of the baseline study. In this study the same NRLs-*Salmonella* have participated as the ones participating in the method comparison study, using the methods they applied during the baseline study. For this the NRLs received a set of 'standard' sera to test with their own method.

The results of both the baseline study and the interlaboratory comparison study are described in this report.

2 Participants

Country	City	Institute
Cyprus	Nicosia	Cyprus Veterinary Services Animal Health Laboratory, Bacteriology Serology Section
Denmark	Copenhagen	National Veterinary Insitute Department of Veterinary Diagnostics and Research
France	Ploufragan	Agence Française de Sécurité Sanitaire des Aliments (AFSSA) Laboratoire d'Etudes et de Recherches Avicoles et Porcines (LERAP)
Germany	Berlin	Federal Institute for Risk Assessment Molekulare Diagnostik und Genetik
Ireland	Kildare	Central Veterinary Laboratory Department of Agriculture & Food, Bacteriology
Lithuania	Vilnius	National Veterinary Laboratory of Lithuania Department of serology
Netherlands	Bilthoven	National Institute for Public Health and the Environment Laboratory for Zoonoses and Environmental Microbiology
Slovenia	Ljubljana	Institute for health care of pigs Laboratory for pig diseases
Sweden	Uppsala	National Veterinary Institute (NVI) Department of Bacteriology
United Kingdom	Suffolk	Veterinary Laboratories Agency (VLA)

3 Materials and Methods

3.1 General

All NRLs used their own serological method and cut off value for both the method comparison study and the interlaboratory comparison study. The NRLs were assigned a laboratory code 1-10 by CRL-*Salmonella.* The protocol of the 60 meat juice samples for the baseline study was sent by email to the NRLs in week 47 of 2006 and is given in Annex 1. The protocol and test report for the interlaboratory comparison study are given in Annex 2 and 3, respectively. Four weeks before the start of the interlaboratory comparison study the NRLs received the protocol and test report by e-mail.

3.2 Duplicate analysis study

3.2.1 Selection of the samples

For the baseline study the 10 NRLs-*Salmonella* had to collect muscle samples for serology on meat juice from the same selected pigs from which lymph nodes were collected for bacteriological examination. These meat juice samples had to be stored frozen (-20 °C) for two years. During the one year baseline study (1 October 2006 – 1 October 2007) the NRLs selected a total of 60 meat juice samples. Every 3 months, the selected meat juice samples were sent to the CRL-*Salmonella.*

To obtain a high range of different concentrations of antibodies against *Salmonella* the NRLs were requested to select the meat juice samples as much as possible following the criteria as indicated in Table 1. In this table the sample results are indicated in OD%, which refers to a set of standard sera, defined according to the Danish Mix ELISA system (Mousing et al., 1997; Nielsen et al., 1995; Nielsen et al., 1996). The NRLs-*Salmonella* were asked to send at least 120 µl of meat juice per sample to the CRL-*Salmonella*. The samples had to be sent in leak proof screw cap tubes and should be cooled during transport (with a courier service).

Table 1 Selection criteria of the meat juice samples

Number of samples	OD%
10	0 – 10
10	10 – 20
10	20 – 30
10	30 – 40
10	40 – 50
10	>50

3.2.2 Reporting to the CRL-*Salmonella*

All participating NRLs received an Excel file, which they were requested to use to report their data. On the first sheet of this Excel file the NRLs could report some general information about the serological method they used. In the second sheet the results per meat juice sample could be reported. Per meat juice sample the NRLs had to report the actual OD, the S/P ratio, the OD% and whether the sample was considered positive (+), doubtful (+/-) or negative (-) for *Salmonella*. Also the bacteriological results from the same animals (lymph nodes and, if available, carcass swabs) were reported in this second sheet. An example of the Excel file is given in Annex 4.

3.2.3 Analyses of the samples by the CRL-*Salmonella*

Each NRL had to give each meat juice sample a unique code, as follows: country abbreviation followed by a number (1-60). For example for the Netherlands: NL-1, NL-2, …., NL-60. All 10 NRLs sent the meat juice samples to the CRL-*Salmonella*, where they were collected and stored at -20˚C. Every three months the collected meat juice samples were put in micronic tubes for transport to the Animal Health Service (GD) in Deventer, the Netherlands. The GD blindly analysed all meat juice samples using the HerdCheck Swine *Salmonella* ELISA from IDEXX. The remaining meat juice samples were returned to the CRL-*Salmonella,* where they were stored at -20 ˚C. After analyses the GD reported the results to the CRL-*Salmonella.*

3.2.4 Statistical analyses

A dependent t-test was used to compare the results of the 60 meat juice samples from the NRLs with those from the CRL. In addition to the dependent t-test a bivariate mixture fitting was used.

3.3 Interlaboratory comparison study

3.3.1 Sera used for serological detection of *Salmonella*

A set of sera was prepared by the Animal Health Service (GD, Deventer, the Netherlands), by inoculation of pigs with different strains of *Salmonella.* The sera were freeze dried and transported to the CRL-*Salmonella* in July 2007. At the CRL-*Salmonella* the sera were stored at -20 ˚C until they were distributed to the NRLs. The NRLs had to test a total number of 40 sera (numbered S-1 till S-40). Two sera were obtained from *Salmonella*-free pigs and two samples were obtained from pigs inoculated with *Yersinia enterocolitica* O3-/O9-, which can possibly result in cross-reaction with the *Salmonella* ELISA. All other 32 samples were obtained after experimental inoculation of pigs with different *Salmonella* strains. Part of these samples was collected as part of the EU collaborative research project SALINPORK (FAIR1 CT95-0400) (Lo Fo Wong and Hald, 2000; Van der Heijden, 2001). In Table 2 the complete set of sera is shown.

Table 2 Description of the 40 sera used for the serological detection of Salmonella

Number	Group	Description	Number	Group	Description
S-1	C2	*S*. Goldcoast	S-21	C2	*S*. Goldcoast
S-2	-	*Y. enterocolitica* O3-O9-	S-22	B	*S*. Typhimurium
S-3	-	negative	S-23	B	*S*. Typhimurium
S-4	C1	*S*. Livingstone	S-24	B	*S*. Typhimurium
S-5	B	*S*. Typhimurium	S-25	B	*S*. Typhimurium
S-6	B	*S*. Brandenburg	S-26	B	*S*. Brandenburg
S-7	C2	*S*. Goldcoast	S-27	B	*S*. Typhimurium
S-8	-	negative	S-28	B	*S*. Typhimurium
S-9	-	*Y. enterocolitica* O3-O9-	S-29	B	*S*. Typhimurium
S-10	C1	*S*. Livingstone	S-30	B	*S*. Typhimurium
S-11	B	*S*. Typhimurium	S-31	B	*S*. Typhimurium
S-12	B	*S*. Typhimurium	S-32	C2	*S*. Goldcoast
S-13	B	*S*. Typhimurium	S-33	B	*S*. Brandenburg
S-14	B	*S*. Typhimurium	S-34	B	*S*. Brandenburg
S-15	B	*S*. Typhimurium	S-35	B	*S*. Typhimurium
S-16	B	*S*. Typhimurium	S-36	B	*S*. Typhimurium
S-17	B	*S*. Typhimurium	S-37	D	*S*. Panama
S-18	B	*S*. Typhimurium	S-38	B	*S*. Typhimurium
S-19	B	*S*. Typhimurium	S-39	B	*S*. Typhimurium
S-20	D	*S*. Panama	S-40	B	*S*. Typhimurium

3.3.2 Transport

All samples were packed and transported as UN3373 Biological Substance, Category B and transported by door-to-door courier service.

3.3.3 Statistical analysis

A quantitative comparison of *Salmonella*-ELISAs was performed by computing Receiver Operating Characteristic (ROC) plots (Zweig and Campbell, 1993; Stegeman et al., 1996). The expected result of the samples was used as the true status of those samples. For each individual ELISA a graph was constructed by plotting the sensitivity against the specificity at cut-off's for the whole range of the test. The area below this curve is proportional to the diagnostic accuracy of a test. The ROC-area varies between 0.5 for a random test and 1 for a perfect test.

4 Questionnaire

A questionnaire was incorporated in the test report of the interlaboratory comparison study (See Annex 3). In this part of the report the questions and answers of this questionnaire are summarised.

4.1 General Questions

Question 1: What was the date of receipt of the parcels at the laboratory?

All NRLs received their packages in the same week as it was sent (week 37 of 2007). The average transport time was 1.3 days.

Question 2: Was your parcel damaged at arrival?

All packages were received in perfect state and no damage occurred during transport.

4.2 Questions regarding the serological method used

Question 3: What is the name of the serological kit used?
Question 4: Who is the manufacturer of the used serological kit?
Question 5: What was the batch number of the serological kit used?

Table 3 Name serological kit, manufacturer and batch number per laboratory

Labcode	Name kit	Manufacturer	Batch number
1	Salmotype PigScreen	Labor Diagnostik Leipzig	1200S72
2	Salmotype PigScreen	Labor Diagnostik Leipzig	900S71
3	Herdcheck Swine Salmonella	IDEXX	44100-7074
4	Herdcheck Swine Salmonella	IDEXX	44130-P171
5	VETSIGN Porcine Salmonella	Guildhay	VP022-24307
6	Salmotype PigScreen	Labor Diagnostik Leipzig	900S71
7	In-House assay	-	-
8	Herdcheck Swine Salmonella	IDEXX	44100-7074
9	Mix-ELISA	In-House	-
10	Salmotype PigScreen	Labor Diagnostik Leipzig	600S67

Question 6: What type of ELISA is used?

All NRLs used an indirect ELISA

Question 7: What types of antigens are used?

All NRLs used LPS antigens

Question 8: Which combination of antigens is used?

Table 4 Manufacturer and antigen combinations of the used ELISAs

ELISA	Manufacturer	Combination of antigens
SALMOTYPE PigScreen	Labor Diagnostik Leipzig	O-1, 4, 5, 6, 7 and 12
Herdcheck Swine Salmonella	IDEXX	B, C1 and D
VETSIGN Porcine Salmonella	Guildhay	B and C1
Mix-ELISA	In-House	B and C1

Question 9: What data are used routinely?
Question 10: What cut-off value is normally used?

Table 5 Data and cut-off values routinely used by each laboratory

Labcode	Data	Cut-off values used		
		-	±	+
1	OD%	<10	>10 and <20	>20
2	OD%	<10	>10 and <20	>20
3	OD%	<10	>10 and <20	>20
4	OD%	<10		>10
5	S/P ratio	<0.10	>0.10 and <0.25	>0.25
6	OD%	<10	>10 and <20	>20
7	OD%	<40		>40
8	OD%	<15		>15
9	OD%	<20		>20
10	OD%	<10	>10 and <20	>20

5 Results

5.1 Duplicate analysis study

5.1.1 Serological result per NRL

To obtain a high range of different concentrations of antibodies against *Salmonella* the NRLs were requested to select the meat juice samples following the criteria as indicated in Table 1. In Table 6 it is shown how many samples in each OD% category was received from each NRL.
The OD-percentages of the NRL with labcode 5 are not known and therefore not included in this table. The NRL with labcode 5 reported their results in S/P ratios. This NRL sent 14 meat juice samples with an S/P ratio <0.1, 12 samples with an S/P ratio between 0.1 and 0.2, six samples with an S/P ratio between 0.2 and 0.3, four samples with an S/P ratio between 0.3 and 0.4, one sample with an S/P ratio between 0.4 and 0.5 and 23 samples with an S/P ratio >0.5.

All NRLs have sent in approximately 60 samples. One NRL (labcode 2) was able to meet the criteria completely; the other NRLs have little discrepancies. The main reason for this is that in most of the countries fewer samples are found in the 'middle categories' (OD% 30-40 and OD% 40-50). The majority of the pigs either have a low antibody titre against *Salmonella* (OD% <30) or have a high antibody titre (OD% >50 %).

Table 6 number of samples in the different categories sent in by the NRLs

OD%	Laboratory codes								
	1	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**
<10	11	10	9	11	24	13	10	11	10
10-20	9	10	18	13	22	10	11	10	10
20-30	11	10	13	12	9	10	10	9	10
30-40	3	10	4	6	1	9	10	7	7
40-50	5	10	5	5	1	8	9	3	10
>50	21	10	11	13	3	10	10	20	10
total	60	60	60	60	60	60	60	60	57

In Figures 1 to 10 the results found by each NRL and by the CRL are shown for all 60 meat juice samples per NRL. For each NRL the results of the different meat juice samples are sorted from the lowest OD% to the highest OD%. The OD% obtained by the CRL for the same samples is also shown in the figure. Furthermore the cut off value normally used by the NRL is indicated in each figure. Large differences are found between the results of the CRL and the results of the different NRLs, even for the NRLs which used the same ELISA as the CRL.

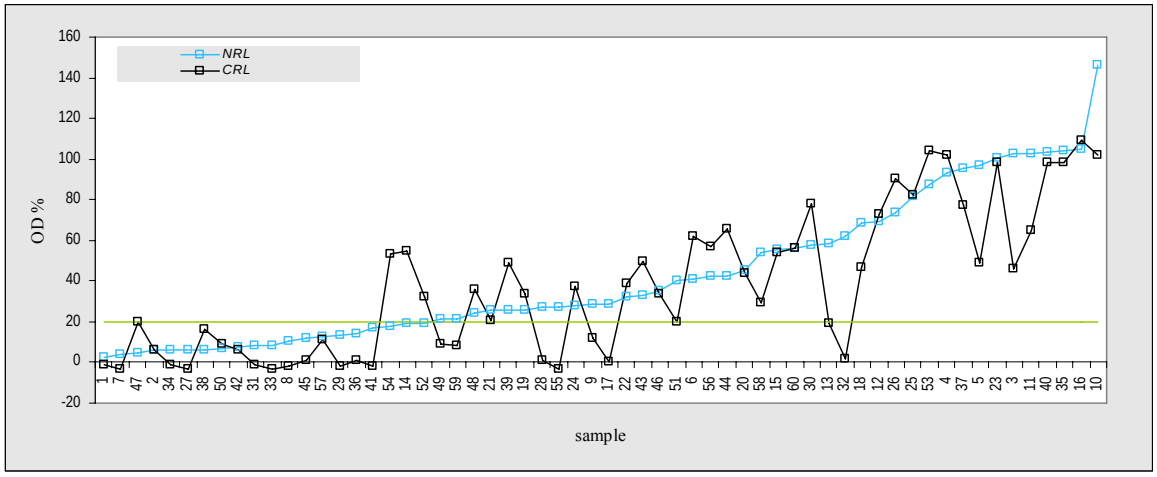

Figure 1. Results of the NRL-*Salmonella* with labcode 1. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL are shown in black. The green line is the cut off value normally used by the NRL. Laboratory 1 used the Salmotype PigScreen of Labor Diagnostik Leipzig and the CRL used the HerdCheck Swine Salmonella of IDEXX.

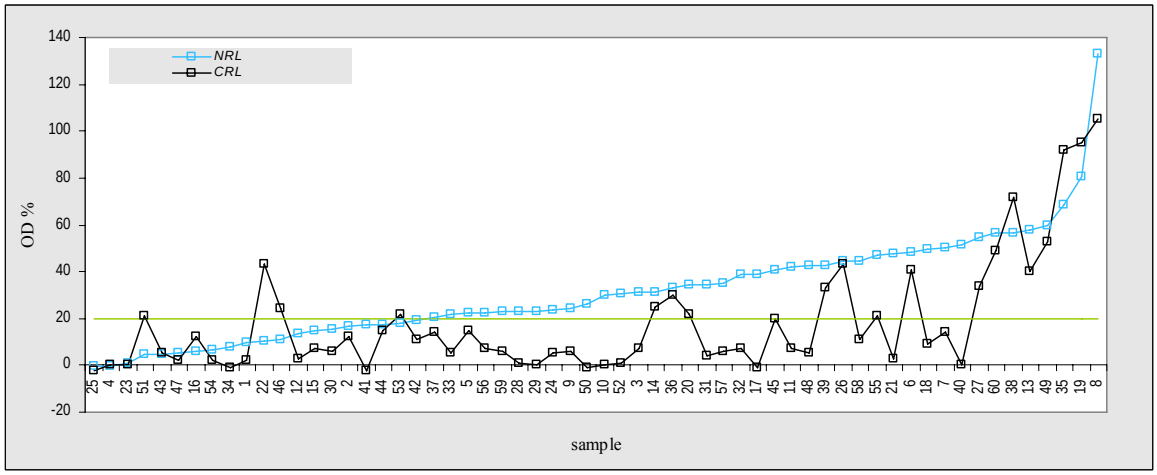

Figure 2. Results of the NRL-*Salmonella* with labcode 2. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 2 used the Salmotype PigScreen of Labor Diagnostik Leipzig and the CRL used the HerdCheck Swine Salmonella of IDEXX.

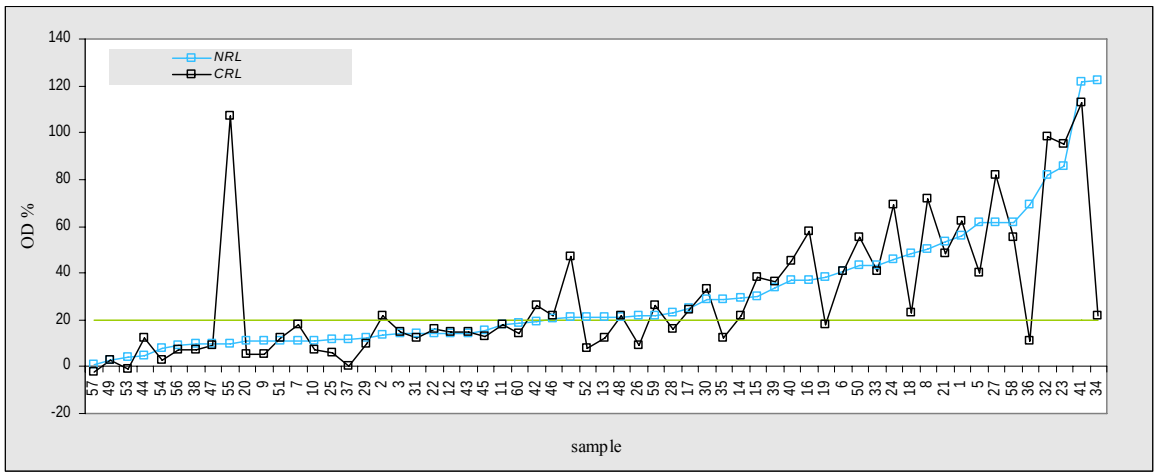

Figure 3. Results of the NRL-*Salmonella* with labcode 3. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Both laboratory 3 and the CRL used the HerdCheck Swine Salmonella of IDEXX.

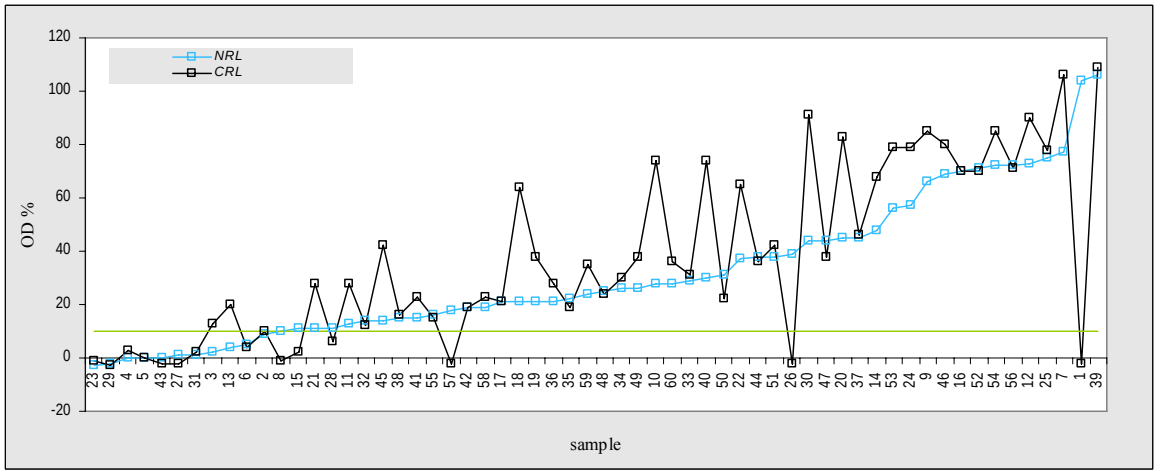

Figure 4. Results of the NRL-*Salmonella* with labcode 4. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Both laboratory 4 and the CRL used the HerdCheck Swine Salmonella of IDEXX.

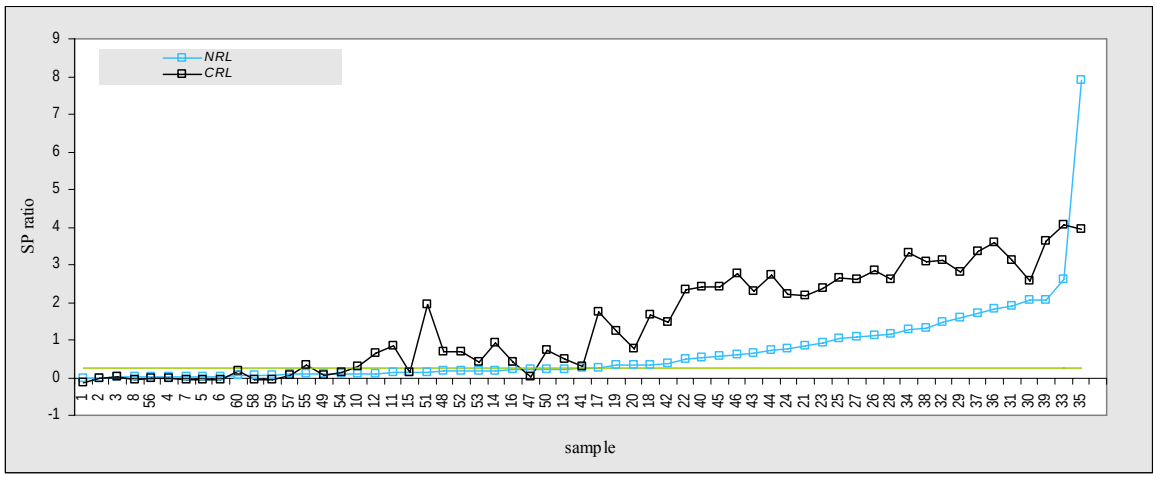

Figure 5. Results of the NRL-*Salmonella* with labcode 5. The samples are sorted by S/P ratio determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 5 used the VetSign Porcine Salmonella of Guildhay and the CRL used the HerdCheck Swine Salmonella of IDEXX.

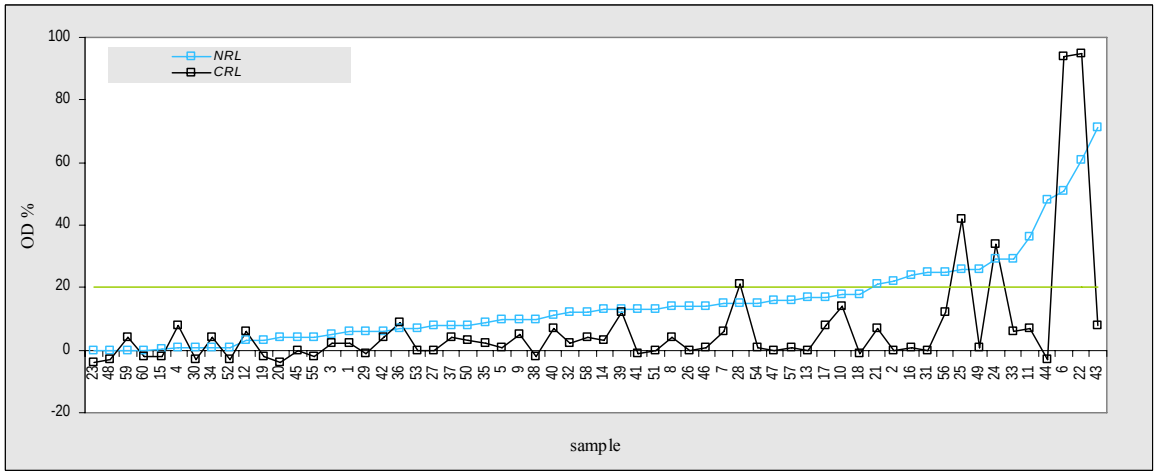

Figure 6. Results of the NRL-*Salmonella* with labcode 6. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 6 used the Salmotype PigScreen of Labor Diagnostik Leipzig and the CRL used the HerdCheck Swine Salmonella of IDEXX.

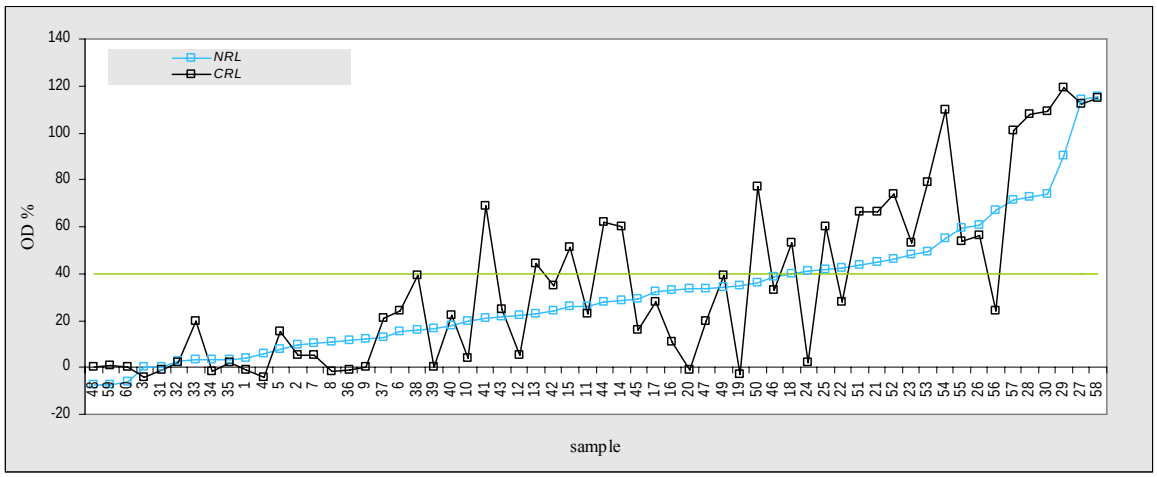

Figure 7. Results of the NRL-*Salmonella* with labcode 7. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 7 used an in-house ELISA and the CRL used the HerdCheck Swine Salmonella of IDEXX.

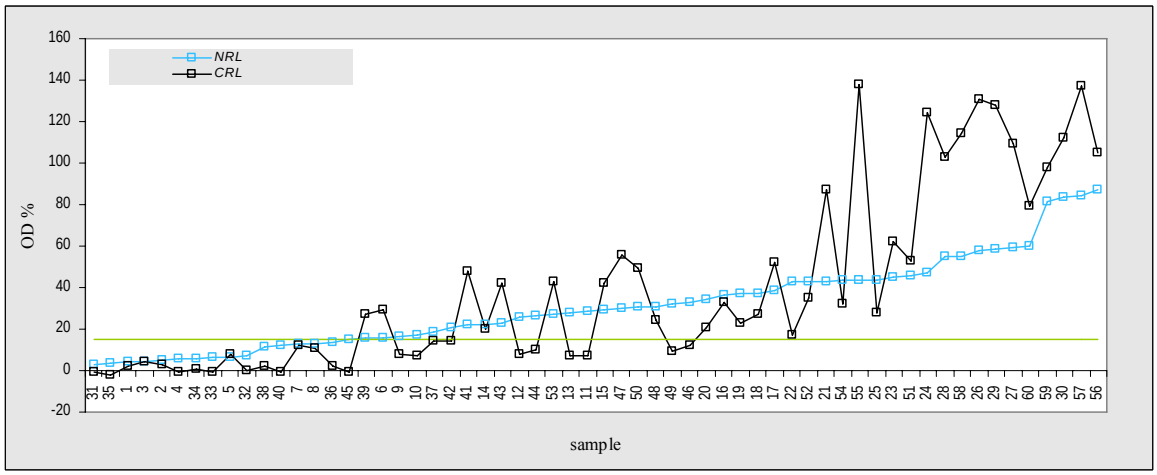

Figure 8. Results of the NRL-*Salmonella* with labcode 8. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Both Laboratory 8 and the CRL used the HerdCheck Swine Salmonella of IDEXX.

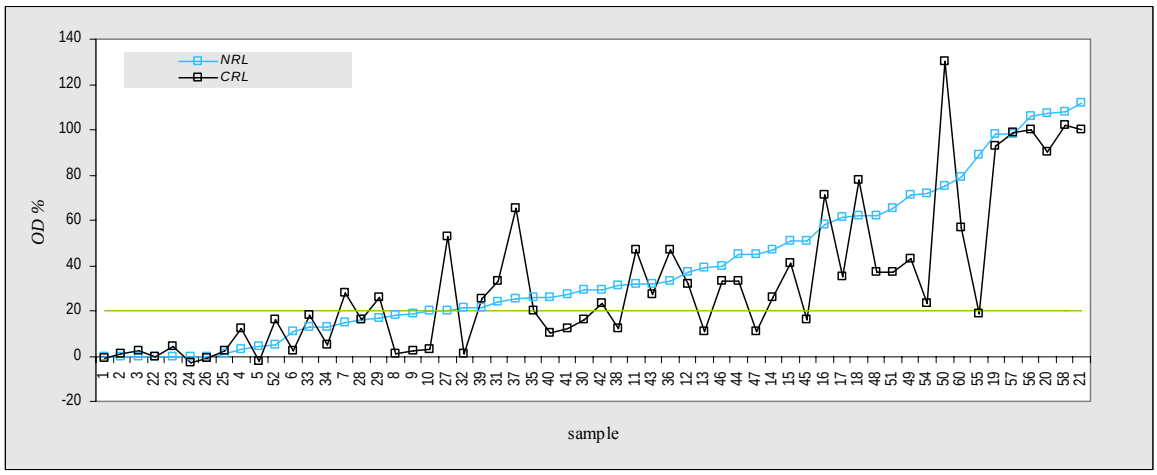

Figure 9. Results of the NRL-*Salmonella* with labcode 9. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 9 used an in-house ELISA and the CRL used the HerdCheck Swine Salmonella of IDEXX.

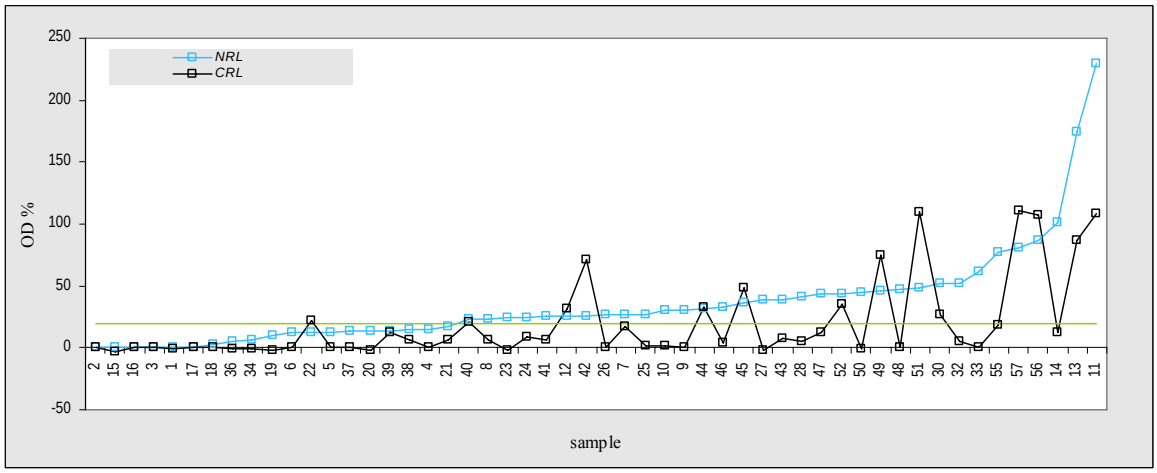

Figure 10. Results of the NRL-*Salmonella* with labcode 10. The samples are sorted by OD% determined by the NRL (blue line), the results of the CRL is shown in black. The green line is the cut off value normally used by the NRL. Laboratory 10 used the Salmotype PigScreen of Labor Diagnostik Leipzig and the CRL used the HerdCheck Swine Salmonella of IDEXX.

In Tables 7 to 16 the results for each NRL are compared to that of the CRL. The cut-off value that is used by each NRL is indicated in Table 5. The CRL-*Salmonella* used a cut off value of OD% >10.

Table 7 Serological results of the NRL with labcode 1 compared to those of the CRL

Results NRL labcode 1	**Results CRL** +	-	**Total**
+	34	6	40
±	4	5	9
-	2	9	11
Total	40	20	60

Table 8 Serological results of the NRL with labcode 2 compared to those of the CRL

Results NRL labcode 2	**Results CRL** +	-	**Total**
+	20	20	40
±	6	4	10
-	2	8	10
Total	28	32	60

Table 9 Serological results of the NRL with labcode 3 compared to those of the CRL

Results NRL labcode 3	**Results CRL** +	-	**Total**
+	44	8	52
-	2	6	8
Total	46	14	60

Table 10 Serological results of the NRL with labcode 4 compared to those of the CRL

Results NRL labcode 4	**Results CRL** +	-	**Total**
+	43	6	49
-	3	8	11
Total	46	14	60

Table 11 Serological results of the NRL with labcode 5 compared to those of the CRL

Results NRL labcode 5	**Results CRL** +	-	**Total**
+	30	0	30
±	11	3	14
-	1	14	15
Total	42	17	59

Table 12 Serological results of the NRL with labcode 6 compared to those of the CRL

Results NRL labcode 6	Results CRL +	Results CRL -	Total
+	5	9	14
±	3	19	22
-	0	24	24
Total	8	52	60

Table 13 Serological results of the NRL with labcode 7 compared to those of the CRL

Results NRL labcode 7	Results CRL +	Results CRL -	Total
+	17	1	18
-	22	20	42
Total	39	21	60

Table 14 Serological results of the NRL with labcode 8 compared to those of the CRL

Results NRL labcode 8	Results CRL +	Results CRL -	Total
+	38	7	45
-	2	13	15
Total	40	20	60

Table 15 Serological results of the NRL with labcode 9 compared to those of the CRL

Results NRL labcode 9	Results CRL +	Results CRL -	Total
+	36	1	37
-	7	14	21
Total	43	15	58

Table 16 Serological results of the NRL with labcode 10 compared to those of the CRL

Results NRL labcode 10	Results CRL +	Results CRL -	Total
+	17	16	33
±	2	8	10
-	0	9	9
Total	19	33	52

Using a dependent t-test the results of the 60 meat juice samples from the CRL were compared to those of the different NRLs. In Table 17 the average OD% of all 60 samples are shown for both NRL and CRL together with the results of the paired t-test. For 5 NRLs (labcodes 1, 2, 6, 9 and 10) the average OD% is significantly higher than the results of the CRL. Four of these NRLs (labcodes 1, 2, 6 and 10) used the Salmotype PigScreen ELISA from Labor Diagnostik Leipzig. The other NRL used an in-house ELISA.

For 2 NRLs (labcodes 4 and 8) the average OD% is statistically lower than that of the CRL and for 1 NLR (labcode 5) the S/P ratio is significantly lower than the S/P ratio of the CRL. Two of these NRLs used the HerdCheck Swine Salmonella ELISA from IDEXX and one used the VetSign Porcine Salmonella ELISA from Guildhay.

For 2 NRLs (labcodes 3 and 7) no statistical difference was found between the OD% of the NRL and the CRL. One of these NRLs used the HerdCheck Swine Salmonella ELISA from IDEXX and the other used an in-house ELISA.

Table 17 Dependent T-test

Labode	**ELISA**	**Average OD%**		**p value dependent t-test**
		NRL	CRL	
1	Salmotype PigScreen	42.84	37.43	0.047
2	Salmotype PigScreen	31.41	18.13	1.31E-07
3	HerdCheck Swine	30.12	29.15	0.74
4	HerdCheck Swine	31.70	37.60	0.039
5*	VetSign Porcine	0.692	1.433	3.4E-07
6	Salmotype PigScreen	14.87	6.87	0.0001
7	In-house	31.06	35.47	0.11
8	HerdCheck Swine	31.44	39.37	0.029
9	In-house	38.09	31.71	0.018
10	Salmotype PigScreen	36.08	19.46	0.0004

** For the NRL with labcode 5 the average S/P ration is given for both NRL and CRL, since the OD% are unknown. Results were significant if $p < 0.05$.*

The data were also analysed with a more complicated bivariate mixture fitting. By using this analysis the results of all meat juice samples from each NRL were analysed presuming that the data represent a mixture of two components: sera of positive pigs and sera of negative pigs. Both positive and negative populations can be described with a distribution of the probability of OD%. In this model it is assumed that both components are normally distributed, that the average OD% of the negative sera is lower than the average OD% of the positive sera and that the lowest OD% of the positive sera can not be lower than the lowest OD% of the negative sera. Using this binary mixture model the maximum likelihood fit can be estimated.

For all NRLs both NRL and CRL data were clearly divided in positive and negative components and comparability of the results between the NRL and the CRL could be tested.
For this, the following null hypothesis was used: the average, standard deviation and ratio of positive and negative sera (prevalence) are different for the NRL and the CRL. Alternative hypotheses (e.g. all parameters are the same for both NRL and CRL) were tested against this null hypothesis.

For all but one NRL (labcode 3) the null hypothesis could not be rejected which indicates that the results of the NRLs are different from the CRL results.
In conclusion, the results from both statistical analyses indicate that it is difficult to compare the results of the CRL with the results of the different NRLs, even when the same serological method is used.

5.1.2 Serological results compared to bacteriological results

In the EU baseline survey serology could be used in addition to bacteriology on lymph nodes (compulsory) and carcass swabs (optionally) of the same pigs. Eight of the 10 participating NRLs in this study performed bacteriology on carcass swabs. In Tables 18 to 27 the bacteriological results found by the different NRLs are compared to results they had found with their ELISA method. For both lymph nodes and carcass swabs Annex D of ISO 6579: 2000 was the prescribed bacteriological method (Anonymous, 2007). In general no correlation could be found between the serological results and the bacteriological results for the 60 pigs tested per NRL.

Six NRLs found *Salmonella* positive lymph nodes while the serology was negative. In two cases the *Salmonellas* found were *Salmonella* that could not be detected with the ELISA, *S.* London (group E) and *S.* Manhattan (group C2), in three cases no serotyping was performed on the positive lymph nodes, in all other cases the *Salmonellas* detected belonged to group B or C (e.g. *S.* Typhimurium, *S.* Infantis, *S.* Livingstone, *S.* Derby, *S.* Bredeney). These could be recent infections since these *Salmonella* can be detected with the ELISAs used.

Table 18 Labcode 1: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	1	39	40
±	3	6	9
-	0	11	11
total	4	56	60

serology result	**carcass swabs** +	-	total
+	3	37	40
±	1	8	9
-	0	11	11
total	4	56	60

serological method: Salmotype PigScreen of Labor Diagnostik Leipzig
serology results: + = OD% >20, ± = OD% >10 and < 20, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 19 Labcode 2: Bacteriological results of lymph nodes compared to the serological results

serology result	**lymph nodes** +	-	total
+	8	32	40
±	2	8	10
-	0	10	10
total	10	50	60

serological method: Salmotype PigScreen of Labor Diagnostik Leipzig
serology results: + = OD% >20, ± = OD% >10 and < 20, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 20 Labcode 3: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	5	47	52
-	0	8	8
total	5	55	60

serology result	**carcass swabs** +	-	total
+	0	52	52
-	0	8	8
total	0	60	60

serological method: HerdCheck Swine Salmonella of IDEXX
serology results: + = OD% >10, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 21 Labcode 4: Bacteriological results of lymph nodes compared to the serological results

serology result	**lymph nodes** +	-	total
+	17	32	49
-	2	9	11
total	19	41	60

serological method: HerdCheck Swine Salmonella of IDEXX
serology results: + = OD% >10, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 22 Labcode 5: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	14	16	30
±	3	12	15
-	2	13	15
total	19	41	60

serology result	**carcass swabs** +	-	total
+	5	25	30
±	4	11	15
-	2	13	15
total	11	49	60

serological method: VetSign Porcine Salmonella of Guildhay
serology results: + = S/P ratio >0.20, ± = S/P ratio >0.10 and <0.20, - = S/P ratio <0.10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 23 Labcode 6: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	1	13	14
±	1	21	22
-	4	20	24
total	6	54	60

serology result	**carcass swabs** +	-	total
+	0	14	14
±	0	22	22
-	0	24	24
total	0	60	60

serological method: Salmotype PigScreen of Labor Diagnostik Leipzig
serology results: + = OD% >20, ± = OD% >10 and < 20, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 24 Labcode 7: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	8	10	18
-	8	34	42
total	16	44	60

serology result	**carcass swabs** +	-	total
+	1	17	18
-	8	34	42
total	9	51	60

serological method: in-house mix-ELISA
serologyresults: + = OD% >40, - = OD% <40
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 25 Labcode 8: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	25	20	45
-	8	7	15
total	33	27	60

serology result	**carcass swabs** +	-	total
+	4	5	9
-	0	7	7
total	4	12	16

serological method: HerdCheck Swine Salmonella of IDEXX
serology results: + = OD% >15, - = OD% <15
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 26 Labcode 9: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	12	20	32
-	0	17	17
total	12	37	49

serology result	**carcass swabs** +	-	total
+	0	31	31
-	0	17	17
total	0	48	48

serological method: in-house mix-ELISA
serology results: + = OD% >20, - = OD% <20
bacteriology results: + = Salmonella detected, - = Salmonella not detected

Table 27 Labcode 10: Bacteriological results of lymph nodes and carcass swabs compared to the serological results

serology result	**lymph nodes** +	-	total
+	5	28	33
±	0	10	10
-	3	7	10
total	8	45	53

serology result	**carcass swabs** +	-	total
+	0	33	33
±	0	10	10
-	0	10	10
total	0	53	53

serological method: Salmotype PigScreen of Labor Diagnostik Leipzig
serology results: + = OD% >20, ± = OD% >10 and <20, - = OD% <10
bacteriology results: + = Salmonella detected, - = Salmonella not detected

5.2 Interlaboratory comparison study

All NRLs, but one, reported their results as relative optical densities (OD%). The NRL with labcode 5 reported their results in S/P ratio (sample value related to positive control value).

5.2.1 Results per NRL

Each NRL was asked to interpret their results using a cut-off value used routinely. Five NRLs (labcode 1, 2, 3, 6 and 10) interpreted the results negative if the OD% value was <10 and positive if the OD% value was >20, intermediate results were presented as doubtful (±). The NRL with labcode 4 used a cut-off value of OD% >10, the NRL with labcode 8 used a cut-off value of OD% >15, the NRL with labcode 9 used a cut-off value of OD% >20 and the NRL with labcode 7 used a cut-off value of OD% >40. The NRL with labcode 5 used cut-off values based on S/P ratios; negative if S/P ratio <0.1, positive if S/P ratio >0.25 and intermediate results as doubtful (see Table 5).

The quantative results (OD%) and the qualitative results of the specificity serum panel; the negative sera, the *Y. enterocolitica* sera and *S.* Goldcoast sera are shown in Table 28 and 29. No reaction is expected with the negative sera and the *Y. enterocolitica* sera. Also for the *S.* Goldcoast no reaction is expected since the ELISAs used contain only antisera against group B, C_1 and D and *S.* Goldcoast belongs to group C_2. However *Salmonella* from serogroup C_2 have the O-6 antigen in common with serogroup C_1, therefore cross reaction can occur as was the case with the NRL with labcode 3, who found a positive result for S-7 containing *S.* Goldcoast serum. The laboratory with labcode 10 found one *Y. enterocolitica* serum positive and both negative sera, one *Y. enterocolitica* serum and four *S.* Goldcoast sera doubtful.

Table 28 Quantitative results of the specificity serum pane (given as OD%)

		participant									
No.	**Description**	**1**	**2**	**3**	**4**	**5***	**6**	**7**	**8**	**9**	**10**
S-3	negative	1	0	2	8	0.018	0	-1	9	-6	12
S-8	negative	1	1	3	4	0.018	0	4	7	-5	12
S-2	*Y. enterocolitica O3-O9-*	2	3	7	7	0.044	1	2	6	-4	25
S-9	*Y. enterocolitica O3-O9-*	1	0	2	5	0.032	0	-4	5	-8	12
S-1	*S.* Goldcoast	0	0	3	5	0.009	0	-3	12	-8	10
S-7	*S.* Goldcoast	0	0	22	9	0.055	0	-3	9	-6	10
S-21	*S.* Goldcoast	0	0	6	6	0.030	0	-5	8	-8	11
S-32	*S.* Goldcoast	0	0	1	0	0.009	0	-6	-1	-9	13

** results are given as S/P ratio*

Table 29 Qualitative results of the specificity serum panel

			participant									
No.	**Description**	**exp**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S-3	negative	-	-	-	-	-	-	-	-	-	-	±
S-8	negative	-	-	-	-	-	-	-	-	-	-	±
S-2	Y. enterocolitica O3-O9-	-	-	-	-	-	-	-	-	-	-	+
S-9	Y. enterocolitica O3-O9-	-	-	-	-	-	-	-	-	-	-	±
S-1	*S.* Goldcoast	-	-	-	-	-	-	-	-	-	-	-
S-7	*S.* Goldcoast	-	-	-	+	-	-	-	-	-	-	±
S-21	*S.* Goldcoast	-	-	-	-	-	-	-	-	-	-	±
S-32	*S.* Goldcoast	-	-	-	-	-	-	-	-	-	-	±

In Tables 30 and 31 respectively, the quantitative and qualitative results per NRL are shown for the sera of *S.* Brandenburg, *S.* Typhimurium, *S.* Livingstone and *S.* Panama. Two *S.* Typhimurium sera (S-16 and S-19) were found positive by all NRLs. Two other *S.* Typhimurium sera were found positive or doubtful by all NRLs. Two *S.* Brandenburg (S-33 and S-34) and 4 *S.* Typhimurium sera (S-5, S-15, S-25 and S-35) were found positive by 9 of the 10 NRLs. However one *S.* Panama serum (S-20) was found positive by only 1 out of 10 NRLs and 2 *S.* Typhimurium sera (S-11 and S-39) were found positive by only 2 out of 10 NRLs. All other sera showed intermediate results.

The most positive results were found by labcode 10 (31 positives and 1 doubtful) and the least positive results were found by labcode 7 (8 positives). The reason that the NRL with labcode 7 found the least positive results is due to the fact that this NRL is the only NRL which used a cut-off value of OD% >40, all other NRLs used lower cut-off values. The reason why laboratory 10 found so many positive results is not clear.

In Tables 32 to the results are shown 41 per NRL compared to the expected results for the set of sera. The cut off values used per NRL are given in Table 5.

Table 30 Quantitative results found in the serum samples (given as OD%)

		participant									
No.	**Description**	**1**	**2**	**3**	**4**	**5***	**6**	**7**	**8**	**9**	**10**
S-6	*S*. Brandenburg	0	1	11	18	0.044	0	0	20	-5	17
S-26	*S*. Brandenburg	21	16	21	27	0.100	22	1	15	9	71
S-33	*S*. Brandenburg	73	72	110	117	0.588	67	63	88	88	118
S-34	*S*. Brandenburg	68	51	79	86	0.475	59	43	62	76	100
S-5	*S*. Typhimurium	78	97	109	126	1.349	81	93	81	77	133
S-11	*S*. Typhimurium	4	3	3	3	0.071	3	-1	6	-3	45
S-12	*S*. Typhimurium	39	42	56	82	0.303	36	34	43	34	118
S-13	*S*. Typhimurium	70	83	107	121	1.101	74	83	79	76	119
S-14	*S*. Typhimurium	22	18	16	24	0.175	22	19	14	13	75
S-15	*S*. Typhimurium	49	38	62	87	0.357	46	47	59	40	105
S-16	*S*. Typhimurium	88	89	109	125	1.178	72	95	78	74	136
S-17	*S*. Typhimurium	9	8	8	19	0.060	10	4	12	5	59
S-18	*S*. Typhimurium	52	42	73	90	0.335	51	53	47	47	104
S-19	*S*. Typhimurium	59	53	56	77	0.507	52	57	45	35	104
S-22	*S*. Typhimurium	39	33	72	87	0.360	34	24	53	14	88
S-23	*S*. Typhimurium	21	20	65	81	0.217	20	15	53	10	109
S-24	*S*. Typhimurium	25	22	35	47	0.194	23	20	33	12	87
S-25	*S*. Typhimurium	48	37	84	97	0.292	47	37	64	36	102
S-27	*S*. Typhimurium	23	20	34	39	0.169	23	17	32	15	78
S-28	*S*. Typhimurium	42	41	53	61	0.302	33	23	49	43	115
S-29	*S*. Typhimurium	48	48	46	62	0.316	46	33	43	60	99
S-30	*S*. Typhimurium	3	3	13	14	0.065	3	0	11	-3	27
S-31	*S*. Typhimurium	40	43	49	62	0.266	40	31	44	32	107
S-35	*S*. Typhimurium	48	35	35	46	0.254	40	30	26	35	97
S-36	*S*. Typhimurium	13	11	14	15	0.060	13	10	13	6	65
S-38	*S*. Typhimurium	15	13	28	30	0.116	12	13	25	11	67
S-39	*S*. Typhimurium	1	0	7	7	0.040	0	-2	7	-4	25
S-40	*S*. Typhimurium	30	20	31	42	0.147	26	16	34	20	79
S-4	*S*. Livingstone	4	4	7	23	0.077	3	3	19	-2	37
S-10	*S*. Livingstone	54	47	29	56	0.411	52	12	36	28	108
S-20	*S*. Panama	1	0	4	6	0.032	0	-4	4	-8	21
S-37	*S*. Panama	53	43	101	103	0.209	45	35	84	54	99

** results reported in S/P ratio*

Table 31 Qualitative results of experimental serum samples

			participant									
No.	**Description**	**exp**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S-6	*S*. Brandenburg	+	-	-	±	+	-	-	-	±	-	±
S-26	*S*. Brandenburg	+	+	±	+	+	-	+	-	±	-	+
S-33	*S*. Brandenburg	+	+	+	+	+	+	+	+	+	+	+
S-34	*S*. Brandenburg	+	+	+	+	+	+	+	+	+	+	+
S-5	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-11	*S*. Typhimurium	+	-	-	-	-	-	-	-	-	-	+
S-12	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-13	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-14	*S*. Typhimurium	+	+	±	±	+	±	+	-	-	-	+
S-15	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-16	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-17	*S*. Typhimurium	+	-	-	-	+	-	±	-	-	-	+
S-18	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-19	*S*. Typhimurium	+	+	+	+	+	+	+	+	+	+	+
S-22	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	-	+
S-23	*S*. Typhimurium	+	+	+	+	+	±	±	-	+	-	+
S-24	*S*. Typhimurium	+	+	+	+	+	±	+	-	±	-	+
S-25	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-27	*S*. Typhimurium	+	+	±	+	+	±	+	-	±	-	+
S-28	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-29	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-30	*S*. Typhimurium	+	-	-	±	+	-	-	-	-	-	+
S-31	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-35	*S*. Typhimurium	+	+	+	+	+	+	+	-	+	+	+
S-36	*S*. Typhimurium	+	±	±	±	+	-	±	-	-	-	+
S-38	*S*. Typhimurium	+	±	±	+	+	±	±	-	±	-	+
S-39	*S*. Typhimurium	+	-	-	-	-	-	-	-	-	-	+
S-40	*S*. Typhimurium	+	+	±	+	+	±	+	-	±	+	+
S-4	*S*. Livingstone	+	-	-	-	+	-	-	-	±	-	+
S-10	*S*. Livingstone	+	+	+	+	+	+	+	-	±	+	+
S-20	*S*. Panama	+	-	-	-	-	-	-	-	-	-	+
S-37	*S*. Panama	+	+	+	+	+	±	+	-	+	+	+

Table 32 Result of Labcode 1

Results NRL labcode 1	expected results +	-	Total
+	23	0	23
±	2	0	2
-	7	8	15
Total	32	8	40

Table 33 Results of Labcode 2:

Results NRL labcode 2	expected results +	-	Total
+	19	0	19
±	6	0	6
-	7	8	15
Total	32	8	40

Table 34 Results of Labcode 3

Results NRL labcode 3	expected results +	-	Total
+	23	1	24
±	4	0	4
-	5	7	12
Total	32	8	40

Table 35 Results of Labcode 4

Results NRL labcode 4	expected results +	-	Total
+	29	0	29
-	3	8	11
Total	32	8	40

Table 36 Results of Labcode 5

Results NRL labcode 5	expected results +	-	Total
+	16	0	16
±	7	0	7
-	9	8	17
Total	32	8	40

Table 37 Results of Labcode 6

Results NRL labcode 6	expected results +	-	Total
+	22	0	22
±	4	0	4
-	6	8	14
Total	32	8	40

Table 38 Results of Labcode 7

Results NRL labcode 7	expected results +	-	Total
+	8	0	8
-	24	8	32
Total	32	8	40

Table 39 Results of Labcode 8

Results NRL labcode 8	expected results +	-	Total
+	17	0	1
±	8	0	8
-	7	8	15
Total	32	8	40

Table 40 Results of Labcode 9

Results NRL labcode 9	expected results +	-	Total
+	17	0	17
-	15	8	23
Total	32	8	40

Table 41 Results of Labcode 10

Results NRL labcode 10	expected results +	-	Total
+	31	1	32
±	1	6	7
-	0	1	1
Total	32	8	40

A quantitative comparison of *Salmonella*-ELISAs was performed by computing Receiver Operating Characteristic (ROC) plots. The expected result of the samples was used as the true status of those samples. For each individual ELISA a graph was constructed by plotting the sensitivity against the specificity at cut-off's for the whole range of the test. The area below this curve is proportional to the diagnostic accuracy of a test. The ROC-area varies between 0.5 for a random test and 1 for a perfect test. In Figures 11 to 20 the ROC curves are shown for each NRL. The ROC-areas for all NRLs are shown in Table 42. All ROC-areas are very high, which indicates that all the tests are able to detect the true status of the samples, however at different cut-off values.

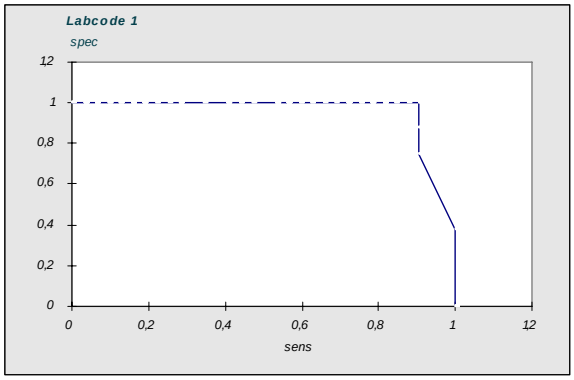

Figure 11 ROC curve Labcode 1

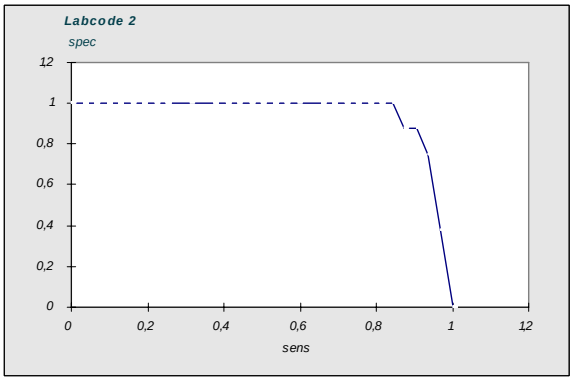

Figure 12 ROC curve Labcode 2

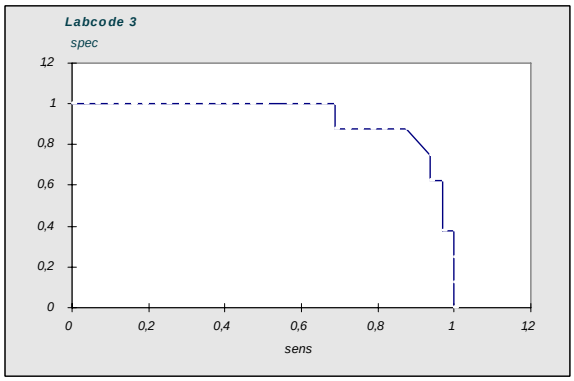

Figure 13 ROC curve Labcode 3

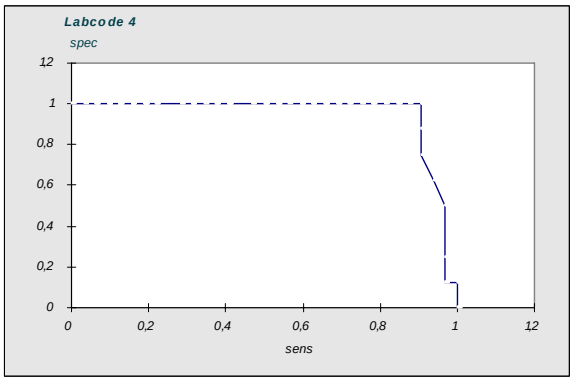

Figure 14 ROC curve Labcode 4

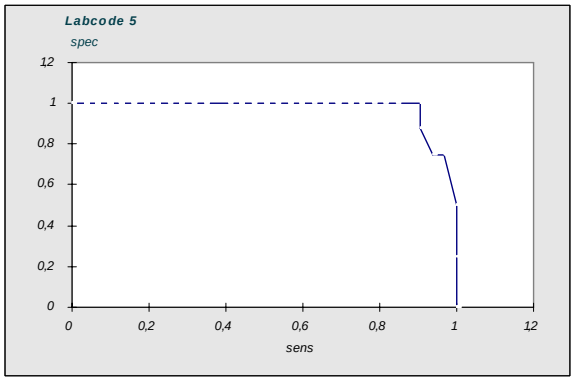

Figure 15 ROC curve Labcode 5

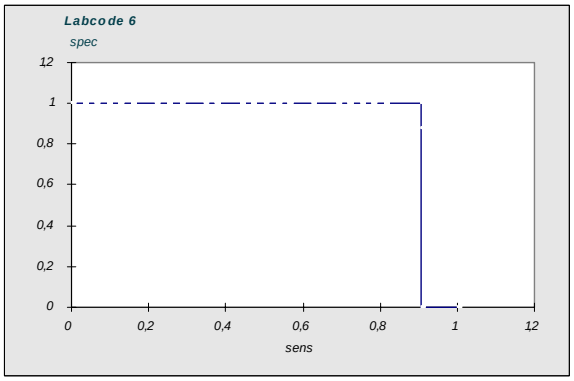

Figure 16 ROC curve Labcode 6

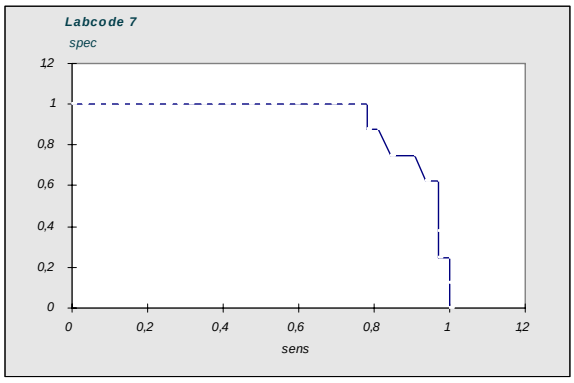

Figure 17 ROC curve Labcode 7

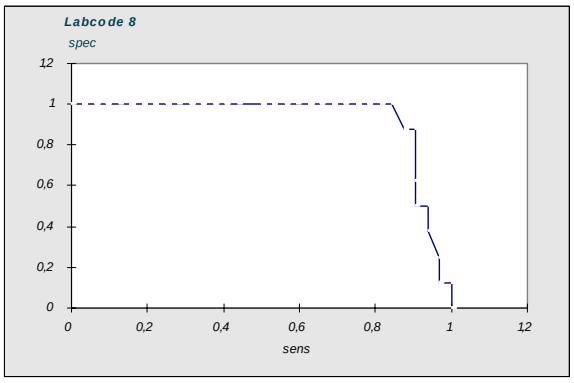

Figure 18 ROC curve Labcode 8

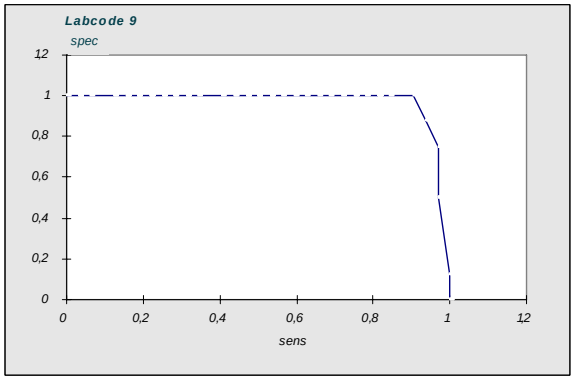

Figure 19 ROC curve Labcode 9

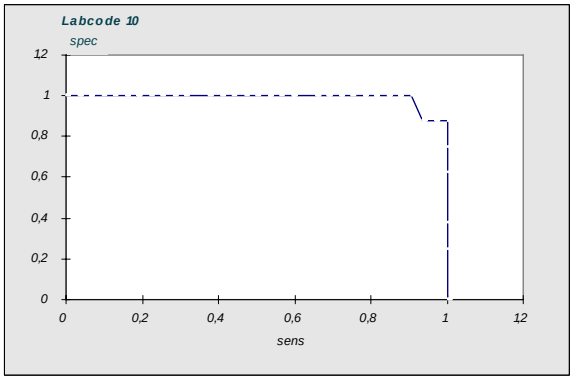

Figure 20 ROC curve Labcode 10

Table 42 ROC-analysis of Salmonella sera per NRL

participant	ROC-area	S.E.M.
1	0.96	0.028
2	0.96	0.030
3	0.93	0.044
4	0.95	0.034
5	0.98	0.020
6	0.95	0.035
7	0.93	0.042
8	0.93	0.042
9	0.97	0.025
10	0.99	0.014

ROC: receiver operating curve
SEM: standard error of the mean

5.2.2 Results per ELISA method

The results per ELISA method are shown in Figure 21-24. Figure 21 shows the results of the NRLs using the HerdCheck from IDEXX. Figure 22 shows the results of the NRLs using the Salmotype PigScreen from Labor Diagnostik Leipzig. Figure 23 shows the results of the NRL using the VetSign ELISA from Guildhay and Figure 24 shows the results from the two NRLs using an in-house ELISA.

The results from the NRLs using the HerdCheck from IDEXX are comparable between the laboratories. On average labcode 8 shows the lowest OD% values and labcode 4 the highest. However, this difference is mainly present in the higher OD% values (>40%, Figure 21).

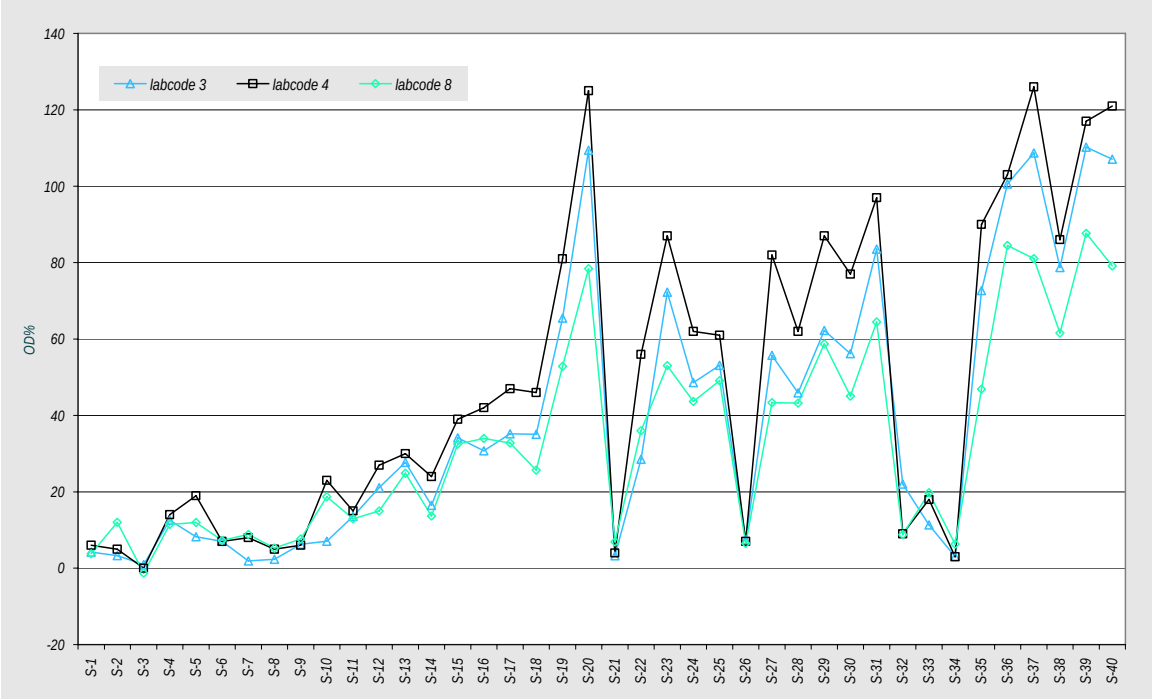

Figure 21 HerdCheck Swine Salmonella results

Of three of the 4 NRLs using the Salmotype ELISA the results for the different sera were also comparable. The OD% values of labcode 10 however, are for almost all sera higher than for the other 3 NRLs (labcode 1, 2 and 6). The results for the other 3 NRLs are almost identical, indicating a high interlaboratory reproducibility, especially in the low OD% ranges (<40%, Figure 22).

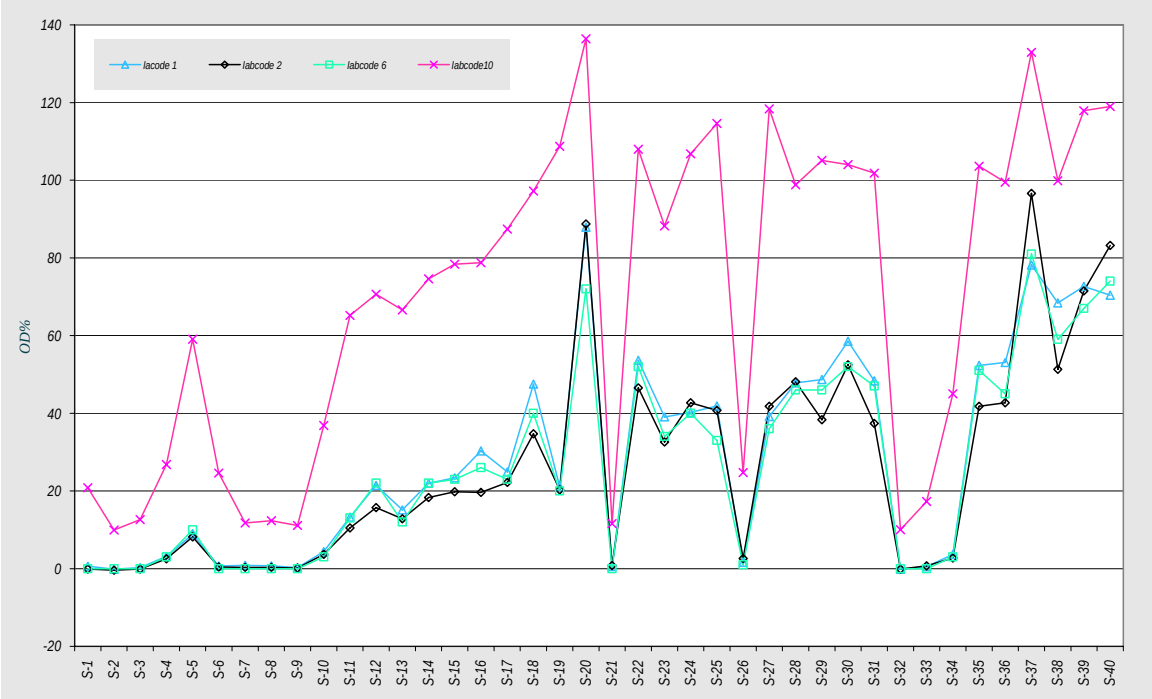

Figure 22 Salmotype PigScreen results

Only one NRL used the VetSign ELISA from Guildhay, so that their results are difficult to compare to the results of the other NRLs. Additional because the results were expressed in S/P ratio and not in OD%, as the results of the other ELISAs. However the shape of the figure is comparable to that of the other NRLs (Figure 23).

Two NRLs (labcode 7 and 9) used their own in-house ELISA method. Also these results are difficult to compare to the results of other NRLs, since another method is used. The calculation of OD% in these two ELISAs also differs from the calculation of the commercially available kits. A regression model is used of a set of reference sera, and the OD% values are calculated from this regression model. The results of both in-house ELISA methods are presented in Figure 24 and show a similar shape as the other ELISA methods.

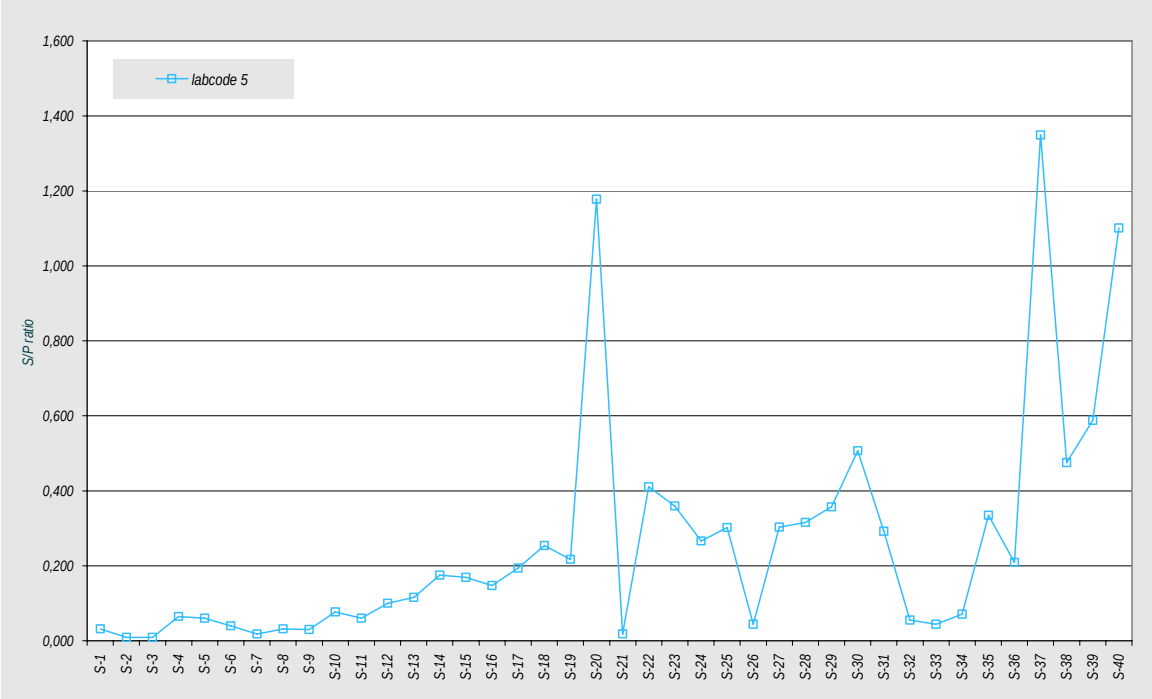

Figure 23 Vetsign Porcine Salmonella results

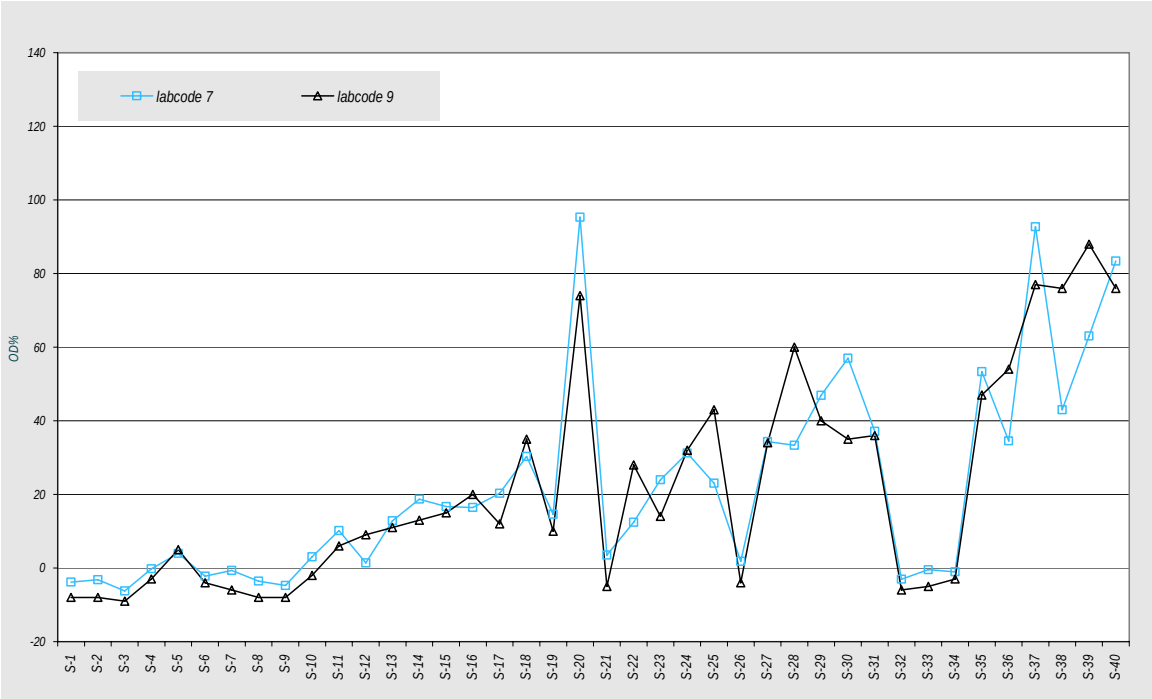

Figure 24 In-House ELISA results

6 Conclusions

Ten National Reference Laboratories for *Salmonella* participated in both the duplicate analysis study and the interlaboratory comparison study. In both studies a variety of ELISAs were used. Although almost all ELISAs are based on the Danish mix-ELISA, there are some differences, what makes it difficult to compare them with each other. One NRL reported their results in S/P ratio; all other NRLs reported their results in OD%. Different cut-off OD% values were used by the different NRLs. Most NRLs used cut-off values of 10 and 20, one NRL used an unusual cut-off value of 15 and one used a cut-off value of 40. Due to the difference is cut-off values used the qualitative results were difficult to compare.

In the duplicate analysis study each laboratory had to send a selection of 60 meat juice samples to the CRL-*Salmonella*, who collected all meat juice samples and sent them to the Animal Health Service (GD Deventer, the Netherlands) where they were tested with the HerdCheck Swine *Salmonella* ELISA from IDEXX. From nine out of the ten participating NRLs the results were statistical significantly different from the results of the CRL. There could be several explanation for the differences found. The samples were sent twice and were also thawed and frozen again at least two times. These thaw-freeze steps could have some effect on the meat juice samples. Before the start of the study the possible effect of repeated thawing and freezing of the meat juice samples was discussed with experts of the Animal Health Service (GD Deventer, the Netherlands), but no substantial influence was expected. If the volume of each sample would have been large enough, the thaw-freeze effect could have been tested by splitting each sample at the CRL and sent one half to the GD and the other half back to the NRL for testing to exclude this thaw-freeze effect. However, the volume of the majority of the samples was not that large to be able to perform such a study. Another explanation for the differences could be that at least half of the NRLs had no longstanding experience with serological detection of *Salmonella* in meat juice. Meat juice is an inhomogeneous material and differences in mixing and place of pipetting (top or bottom of vial) could result in variations in concentration of antibodies detected.

No correlation was found between the serological analysis of the meat juice samples and the bacteriological analysis of the lymph nodes or carcass swabs of the same pigs. This is not very surprising since both analysis represent different stages of infection. Using serological analysis, antibodies are detected which are formed in response on an infection. However, the pig can already be cleared of *Salmonella* and still has an antibody response. It is thought therefore that with the use of serological detection of antibodies against *Salmonella* you can determine the status of a farm, while with bacteriological detection you can determine the status of an individual animal at the slaughterhouse. Interesting are the pigs that are tested negative for antibodies against *Salmonella* using an ELISA, but positive for *Salmonella* in the lymph nodes using bacteriological methods. These infections are either very recent or these pigs are infected with a type of *Salmonella* that is not detected by the ELISAs used. Five of the 10 NRLs have found pigs positive for *Salmonella* in the lymph nodes but negative for antibodies against *Salmonella*. In total 27 pigs were found positive with bacteriological detection, but no antibodies against *Salmonella* could be detected using serological detection. In three of these cases the isolates were not serotyped, one isolate was typed *S.* Londen (group E) and one isolate was typed *S.* Manhatten (group C_2). Both strains from group C_2 and E can not be detected by the ELISAs used. However, all other isolates (22) belonged to group B or C_1, which can be detected with the ELISAs used. These infections can be infections which are acquired recently or it is possible that there are pigs that do not have a measurable antibody response against these *Salmonella* infections.

In the interlaboratory comparison study, ROC analysis indicates that all NRLs performed well, although at different cut-off values for all NRLs. Labcode 10, for example, found on average higher OD% than all other NRLs, therefore the perfect cut-off value for this NRL is higher than that of all other NRLs. Since labcode 10 found higher OD% in both the interlaboratory comparison study and in the duplicate analysis study, this could have been caused by a batch effect of the ELISA test or by problems with the procedure by the NRL. This NRL is advised to check the quality of the test and to further check their procedures. Most NRLs used a cut-off OD% of 10 to 20. However the NRL with labcode 7 used a cut-off value of 40. In the interlaboratory comparison study this laboratory therefore found the least number of positive samples. If this NRL had used a cut-off value of 10-20 the results would have been comparable to all other NRLs.
Two ELISA methods were used by more than one NRL; the HerdCheck Swine Salmonella ELISA and the Salmotype PigScreen. The Salmotype PigScreen ELISA appeared to be the best reproducible between different NRLs (except for labcode 10). Also the HerdCheck Swine ELISA was reproducible, only small differences were found, mainly in the high OD% ranges (>40%).

In conclusion, the majority of the NRLs had no problems with detecting *Salmonella* antibodies in serum using an ELISA. Detection of antibodies in meat juice appeared, however, to be more difficult. The results from the baseline study indicate that the detection of antibodies in meat juice samples is not comparable between different NRLs. This could be due to the design of the study (especially the possible freeze-thaw effect), the inexperience of some laboratories or the inhomogeneity of meat juice or a combination of factors. Therefore, at this point it is not possible to use serological detection of *Salmonella* in meat juice for target setting.

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Annex 1: Protocol duplicate analysis study

Protocol
'Baseline survey on the prevalence of *Salmonella* in slaughter pigs'(2006 - 2007)

SEROLOGY

Introduction

In September 2006 the Commission Decision 'concerning a financial contribution from the Community towards a baseline survey on the prevalence of *Salmonella* in slaughter pigs to be carried out in the Member States' (2006/668/EC) was published. In the technical specification related to this Decision (Annex 1 of the Decision), the more practical aspects of this study are worked out.

New in this study, when compared to former baseline studies, is the possibility to use a serological method in addition to the bacteriological method for the detection of *Salmonella* spp. in pigs. Ten NRLs-*Salmonella* have agreed to perform serology. As no standard method exists for serology, the NRLs are allowed to use their own methods. In an attempt to compare the different methods, the following activities will be performed:

1. The NRLs-*Salmonella* will send during the 1 year baseline study a selection of in total 60 meat juice samples[1] to the CRL-*Salmonella*, where the samples will be analysed with a single serological method;
2. An interlaboratory comparison study will be organised by the CRL-*Salmonella* at the end of the baseline study. In this study the same NRLs-*Salmonella* will participate as the ones participated in the baseline study, using the same method as used during the baseline study. The NRLs will receive a set of 'standard' sera to test with their own method. The selection of sera should be based on the *Salmonella* serotypes most frequently found in pigs, especially during the baseline study, but may be dependent on what is available.

This document concerns item 1. Below more details are given on the selection and mailing of the 60 meat juice samples to the CRL-*Salmonella*.

The serological analyses at the CRL-*Salmonella* will be performed in close cooperation with the Animal Health Service (GD, Deventer, the Netherlands).

[1] According to Commission Decision 2006/668 it is also allowed to analyse blood samples instead of meat juice for serology. As the majority of the NRLs-*Salmonella* have indicated to analyse meat juice samples, this document indicates only meat juice samples. However, if a laboratory analyses blood samples only, please contact the CRL-*Salmonella* to discuss details relevant to this type of samples.

Meat juice samples

- The NRLs-*Salmonella* collect muscle samples for serology on meat juice of the same selected pigs of which bacteriological analyses is performed. These meat juice samples shall be stored frozen (-20 °C) for two years.
- During the 1 year baseline study (1 October 2006 – 1 October 2007) the NRLs select a total of 60 meat juice samples to be sent to the CRL-*Salmonella*.
- Every 3 months a selection of the meat juice samples shall be sent to the CRL-*Salmonella* (see address below).
- The meat juice samples shall be selected (as much as possible) following the criteria as indicated in the table below. In this table the sample results are indicated in OD%, which refers to a set of standard sera, defined according to the Danish Mix ELISA system (Mousing et al., 1997; Nielsen et al., 1995; Nielsen et al., 2001). If a laboratory is not able to calculate their results to OD%, please contact the CRL-*Salmonella*. For the selection of the samples also the fact that the samples are taken in a wide time frame shall be taken into account. Furthermore, it is requested to send in samples from as many of the indicated groups as possible (preferably from all groups) every 3 months.

Number of samples	OD%
10	0-10
10	10-20
10	20-30
10	30-40
10	40-50
10	>50

- The NRLs-*Salmonella* shall send at least 120 µl per meat juice sample to the CRL-*Salmonella.* The samples shall be sent in leak proof screw cap tubes and cooled during transport (e.g. with cooling elements). The transport time shall be kept as short as possible. Therefore a courier service should be used to transport the samples as non-dangerous goods.
- Each NRL will give each meat juice sample a unique code, preferably as follows: country abbreviation followed by a number (1-60). For example for the Netherlands: NL-1, NL-2, …., NL-60.
- Each NRL will inform the CRL-*Salmonella* about the following:
  In general:
    - Serological method (e.g. which ELISA-kit) used at the NRL, including some details on the antigens, etc.;
    - Cut-off value normally used by the NRL.

  Per meat juice sample:
    - Actual OD, S/P ratio and OD% as found by the NRL;
    - Whether the sample was considered positive (+), doubtful (+/-) or negative (-) for *Salmonella* by the NRL, when applying the usual cut-off value;

- The bacteriological results from the same animals (lymph nodes and, if available, carcass swabs).

The NRLs will receive an Excel file in which they can summarise the relevant information. This file contains 3 sheets. On the first sheet some general information is requested, on the second sheet the results per sample shall be given and on the third sheet country abbreviations are indicated.

Reporting

The CRL-*Salmonella* will make an overview on the results of the samples found by the NRL and found by the CRL (actual OD, S/P ratio, OD% and positive or negative for *Salmonella*). In case of large differences between the results found by the NRL and by the CRL, this will be discussed with the NRL.

References

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Nielsen B, Baggesen D, Bager F, Haugegaard J, Lind P (1995). The serological response to Salmonella serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. Vet. Microbiol. 1995 Dec;47(3-4):205-18.

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Contact

For any questions, please contact the CRL-*Salmonella* (see below)

Address information for sending the samples and for questions/information:

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Annex 2: Protocol interlaboratory comparison study

PROTOCOL OF THE INTERLABORATORY COMPARISON STUDY ON SEROLOGICAL METHODS FOR SERA FROM PIGS ORGANISED BY CRL-*SALMONELLA*

Introduction

A baseline study to determine the prevalence of *Salmonella* in slaughter pigs is running from 1 October 2006 to 1 October 2007. In this study serological methods can be used additional to the bacteriological detection of *Salmonella* in lymph nodes. This interlaboratory comparison study is organized by the Community Reference Laboratory (CRL) - *Salmonella* since there is no standard serological method. The aim of this study will therefore be a comparison of different serological methods, by analysing "standard" sera. Ten EU National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*) will participate in this study, each using their own serological method. The performance of the study will take place in week 39 (starting on 24th September 2007) or one week earlier or later. All data will be reported in the test report, send to the CRL-*Salmonella* and will be used for analysis.

Transportation and storage of the *Salmonella* sera to the NRLs.

CRL-*Salmonella* will send the parcels as diagnostic specimens with a door-to-door courier to the NRLs. After arrival the samples have to be stored at 4 ˚C until use.

Serological methods

A total number of 40 sera (numbered S-1 till S-40), supplied by the CRL-*Salmonella*, have to be tested. The serological method routinely performed in your laboratory can be used in this study. The results will be evaluated by the CRL-*Salmonella*.

Instructions for dissolving the sera.

- Add 500 µl of sterile demineralised water to each of the vials.
- Allow the lyophilisate to dissolve during one hour at ambient temperature (do **not** shake, vortex, etc).
- After one hour gently mix the contents of the vial by low speed vortexing.
- Use the samples **immediately** in the ELISA (do **not** freeze-thaw before the test for this study).
- The remaining serum can be stored at -20 ˚C (or lower).

If you have questions or remarks about the interlaboratory comparison study, please contact:

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e-mail: petra.berk@rivm

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Timetable of the interlaboratory comparison study (2007) on serological methods.

Week	Date	Topic
35	27 – 31 August 2007	Mailing of the protocol and test report 2007
37	10 – 14 September 2007	Mailing of the parcels to the participants (NRLs) as diagnostic specimens by door-to-door courier service. After arrival the sera have to be stored at 4˚ C. **If you did not receive the parcel at 14 September 2007, do contact the CRL immediately.**.
39	24 – 28 September 2007	Starting with the serological detection.
41	8 – 12 October 2007	Send the completed test report by email to CRL-*Salmonella*.
42	15 – 19 October 2007	Data input at CRL-*Salmonella* and sending these data by CRL to NRLs by email for checking. Checking the results by the participants and they will inform CRL whether their results are correct. If CRL does not receive a reaction within one week after receipt of this email the CRL will consider the results as correct.

Annex 3: Test report interlaboratory comparison study

TEST REPORT

INTERLABORATORY COMPARISON STUDY ON SEROLOGICAL METHODS 2007

Laboratory code	
Name contact person	
E-mail address contact person	
Name of laboratory	
Name department and/or institute	
Address	
Country	

Please write your remarks and comments on page 6 of the test report!!

rivm

GENERAL QUESTIONS

Shipment of sera	
Date of receipt at your laboratory	
Was your parcel damaged at arrival?	❑ NO ❑ YES

Serological kit	
Name	
Manufacturer	
Batch number	

Explanation of routine results	
Which data are used routinely	❑ Actual OD ❑ OD% ❑ S/P ratio ❑ Other: …….
What is the calculation procedure	
What is the cut-off normally used	

Specific information on the method	
What type of ELISA is used?	❑ Blocking ❑ Indirect ❑ Other: ……..
What types of antigens are used?	❑ LPS ❑ Flagellar proteins ❑ Other: ……..
Which combinations of antigens are used? (e.g. LPS B, LPS C1, LPS-D, etc)	
Please write down a short description of the test (including sample dilution).	

rivm

TEST RESULTS

Labcode	
Date of testing	

Sample no.	Actual OD	OD%	S/P ratio	Serology result* for *Salmonella* (+, -, ±)
S-1				
S-2				
S-3				
S-4				
S-5				
S-6				
S-7				
S-8				
S-9				
S-10				
S-11				
S-12				
S-13				
S-14				
S-15				
S-16				
S-17				
S-18				
S-19				
S-20				

** Result with the cut-off normally used in the laboratory*

TEST RESULTS

Labcode	
Date of testing	

Sample no.	Actual OD	OD%	S/P ratio	Serology result for *Salmonella* (+, -, ±)
S-21				
S-22				
S-23				
S-24				
S-25				
S-26				
S-27				
S-28				
S-29				
S-30				
S-31				
S-32				
S-33				
S-34				
S-35				
S-36				
S-37				
S-38				
S-39				
S-40				

** Result with the cut-off normally used in the laboratory*

REMARKS AND COMMENTS

Name of person(s) carrying out the study	
Date and signature	

Name of person in charge	
Date and signature	

Annex 4: Test report duplicate analysis study

Baseline study on the prevalence of Salmonella in slaughter pigs
Serology
Information on samples for 'duplicate' analyses at CRL-Salmonella

General information

Contact person:	
e-mail address:	
Institute:	
Country:	

Method information

Serological method: (e.g. which ELISA kit)	
Antigens used for serology:	
Cut-off value serology: (normally used)	
Samples serology: (meat juice or blood serum)	
Bacteriological method: (e.g. reference to a standard)	

rivm

Results selected samples baseline study on the prevalence of Salmonella in slaughter pigs

Country:	

Sample number		Serology 'raw' data			Serology result for Salmonella	Bacteriology result for Salmonella			
						Lymph nodes		Carcass swabs	
Country	No.	Actual OD	S/P ratio	OD%	(+, -, +/-)	(+, -, +/-)	Serotyping	(+, -, +/-)	Serotyping
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								
	9								
	10								
	…								
	…								
	…								
	…								
	…								
	…								
	…								
	…								
	…								
	60								

Annex 5: Raw data duplicate analysis study

Results Labcode 1						Results CRL-*Salmonella*	
		Serology results		Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1		2.506	-	-	-	-1	-
2		5.847	-	-	-	6	-
3		102.497	+	-	-	46	+
4		93.309	+	-	-	102	+
5		96.829	+	-	-	49	+
6		40.808	+	-	-	62	+
7		4.296	-	-	-	-3	-
8		10.500	±	-	-	-2	-
9		28.279	+	-	-	12	+
10		146.169	+	-	-	102	+
11		102.974	+	-	-	65	+
12		69.599	+	-	-	73	+
13		58.269	+	-	-	19	+
14		18.957	±	-	-	55	+
15		55.307	+	-	-	54	+
16		104.926	+	-	-	109	+
17		28.974	+	-	-	0	-
18		68.741	+	-	-	47	+
19		25.884	+	-	-	34	+
20		44.962	+	-	-	44	+
21		25.565	+	-	-	21	+
22		32.446	+	-	-	39	+
23		100.202	+	-	+	98	+
24		27.775	+	-	+	37	+
25		81.668	+	-	-	82	+
26		73.682	+	-	-	90	+
27		6.360	-	-	-	-3	-
28		26.868	+	-	-	1	-
29		13.336	±	+	-	-2	-
30		57.94	+	-	-	78	+

Results Labcode 1 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31		8.596	-	-	-	-1	-
32		62.002	+	-	-	2	-
33		8.608	-	-	-	-3	-
34		6.020	-	-	-	-1	-
35		103.949	+	-	-	98	+
36		13.830	±	-	-	1	-
37		95.078	+	-	-	77	+
38		6.395	-	-	-	16	+
39		25.677	+	-	-	49	+
40		103.057	+	-	-	98	+
41		16.948	±	+	+	-2	-
42		7.387	-	-	-	6	-
43		33.171	+	-	-	50	+
44		42.369	+	-	-	66	+
45		11.588	±	-	-	1	-
46		35.344	+	-	-	34	+
47		4.73	-	-	-	20	+
48		24.0	+	-	-	36	+
49		21.4	+	-	+	9	-
50		6.8	-	-	-	9	-
51		40.1	+	+	-	20	+
52		19.1	±	+	-	32	+
53		87.4	+	-	-	104	+
54		18.1	±	-	-	53	+
55		26.96	+	-	-	-3	-
56		42.2	+	-	-	57	+
57		12.66	±	-	-	11	+
58		53.993	+	-	-	29	+
59		21.453	+	-	-	8	-
60		56.224	+	-	-	56	+

Results Labcode 2						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	0.149	9.7	-	-	nd	2	-
2	0.245	16.6	±	+	nd	12	+
3	0.447	31.4	+	-	nd	7	-
4	0.018	-0.2	-	-	nd	0	-
5	0.324	22.3	+	-	nd	15	+
6	0.678	48.4	+	-	nd	41	+
7	0.706	50.5	+	-	nd	14	+
8	1.825	132.9	+	+	nd	105	+
9	0.348	24.5	+	-	nd	6	-
10	0.424	30.0	+	-	nd	0	-
11	0.585	41.7	+	-	nd	7	-
12	0.201	13.3	±	-	nd	3	-
13	0.803	57.6	+	+	nd	40	+
14	0.448	31.5	+	-	nd	25	+
15	0.218	14.8	±	-	nd	7	-
16	0.100	5.7	-	-	nd	12	+
17	0.548	38.8	+	-	nd	-1	-
18	0.691	49.3	+	-	nd	9	-
19	1.117	80.7	+	-	nd	95	+
20	0.488	34.6	+	-	nd	22	+
21	0.667	47.6	+	-	nd	3	-
22	0.164	10.4	±	-	nd	43	+
23	0.029	0.6	-	-	nd	0	-
24	0.348	23.9	+	-	nd	5	-
25	0.018	-0.3	-	-	nd	-2	-
26	0.626	44.5	+	-	nd	43	+
27	0.763	54.6	+	-	nd	34	+
28	0.334	22.9	+	-	nd	1	-
29	0.340	23.3	+	-	nd	0	-
30	0.231	15.2	±	-	nd	6	-

nd = not determined

Results Labcode 2 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	Lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.492	34.6	+	+	nd	4	-
32	0.548	38.7	+	-	nd	7	-
33	0.320	21.9	+	-	nd	5	-
34	0.127	7.7	-	-	nd	-1	-
35	0.955	68.8	+	+	nd	92	+
36	0.471	33.1	+	-	nd	30	+
37	0.299	20.4	+	-	nd	14	+
38	0.788	56.4	+	+	nd	72	+
39	0.604	42.9	+	+	nd	33	+
40	0.720	51.5	+	-	nd	0	-
41	0.256	17.2	±	-	nd	-2	-
42	0.286	19.5	±	-	nd	11	+
43	0.092	4.9	-	-	nd	5	-
44	0.259	17.3	±	-	nd	15	+
45	0.573	40.8	+	-	nd	20	+
46	0.176	11.3	±	+	nd	24	+
47	0.086	5.1	-	-	nd	2	-
48	0.594	42.3	+	-	nd	5	-
49	0.833	59.7	+	-	nd	53	+
50	0.381	26.3	+	-	nd	-1	-
51	0.071	4.5	-	-	nd	21	+
52	0.435	30.5	+	-	nd	1	-
53	0.267	17.9	±	-	nd	22	+
54	0.116	6.7	-	-	nd	2	-
55	0.658	46.9	+	-	nd	21	+
56	0.338	22.3	+	+	nd	7	-
57	0.488	34.8	+	-	nd	6	-
58	0.625	44.6	+	-	nd	11	+
59	0.328	22.7	+	-	nd	6	-
60	0.785	56.3	+	+	nd	49	+

nd = not determined

Results Labcode 3						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	1.40	56.00	+	-	-	62	+
2	0.34	13.70	+	-	-	22	+
3	0.35	14.00	+	-	-	15	+
4	0.52	20.80	+	-	-	47	+
5	1.53	61.30	+	-	-	40	+
6	1.01	40.50	+	-	-	41	+
7	0.28	11.30	+	-	-	18	+
8	1.26	50.40	+	-	-	72	+
9	0.28	11.10	+	-	-	5	-
10	0.28	11.30	+	+	-	7	-
11	1.16	17.70	+	-	-	18	+
12	0.77	14.40	+	-	-	15	+
13	0.46	21.00	+	-	-	12	+
14	0.65	29.60	+	+	-	22	+
15	0.65	29.70	+	-	-	38	+
16	0.83	37.20	+	-	-	58	+
17	0.57	25.00	+	-	-	24	+
18	1.09	48.20	+	-	-	23	+
19	0.86	37.90	+	-	-	18	+
20	0.25	10.80	+	-	-	5	-
21	1.21	53.30	+	-	-	48	+
22	0.35	14.30	+	+	-	16	+
23	2.13	85.40	+	-	-	95	+
24	1.14	45.60	+	-	-	69	+
25	0.29	11.40	+	-	-	6	-
26	0.54	21.80	+	-	-	9	-
27	1.53	61.30	+	-	-	82	+
28	0.58	23.30	+	-	-	16	+
29	0.30	12.00	+	-	-	10	+
30	0.72	28.60	+	-	-	33	+

Results Labcode 3 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.35	14.20	+	+	-	12	+
32	2.0	81.60	+	+	-	98	+
33	1.10	43.50	+	-	-	41	+
34	3.60	122.60	+	-	-	22	+
35	1.15	28.60	+	-	-	12	+
36	3.38	69.20	+	-	-	11	+
37	0.47	11.40	+	-	-	0	-
38	0.38	9.60	-	-	-	7	-
39	0.85	33.80	+	-	-	36	+
40	0.89	36.90	+	-	-	45	+
41	3.00	121.60	+	-	-	113	+
42	0.47	18.90	+	-	-	26	+
43	0.36	14.40	+	-	-	15	+
44	0.11	4.40	-	-	-	12	+
45	0.47	15.20	+	-	-	13	+
46	0.92	20.40	+	-	-	22	+
47	0.38	9.60	+	-	-	9	-
48	0.53	21.20	+	-	-	22	+
49	0.07	2.80	-	-	-	3	-
50	1,07	43.00	+	-	-	55	+
51	0.28	11.20	+	-	-	12	+
52	0.52	20.80	+	-	-	8	-
53	0.11	4.30	-	-	-	-1	-
54	0.20	8.00	-	-	-	3	-
55	0.24	9.60	-	-	-	107	+
56	0.23	9.10	-	-	-	7	-
57	0.02	0.80	-	-	-	-2	-
58	1.54	61.30	+	-	-	55	+
59	0.55	21.90	+	-	-	26	+
60	0.41	18.40	+	-	-	14	+

Results Labcode 4						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	2.619	104	+	-	nd	-2	-
2	0.230	9	-	-	nd	10	+
3	0.059	2	-	-	nd	13	+
4	0.010	0	-	-	nd	3	-
5	0.005	0	-	-	nd	0	-
6	0.130	5	-	-	nd	4	-
7	1.933	77	+	+	nd	106	+
8	0.251	10	+	-	nd	-1	-
9	1.650	66	+	+	nd	85	+
10	0.725	28	+	+	nd	74	+
11	0.330	13	+	+	nd	28	+
12	1.828	73	+	-	nd	90	+
13	0.104	4	-	+	nd	20	+
14	1.204	48	+	+	nd	68	+
15	0.299	11	+	-	nd	2	-
16	1.773	70	+	+	nd	70	+
17	0.540	21	+	-	nd	21	+
18	0.540	21	+	-	nd	64	+
19	0.533	21	+	+	nd	38	+
20	1.144	45	+	-	nd	83	+
21	0.288	11	+	-	nd	28	+
22	0.950	37	+	+	nd	65	+
23	-0.072	-3	-	+	nd	-1	-
24	1.436	57	+	-	nd	79	+
25	1.896	75	+	-	nd	78	+
26	0.986	39	+	-	nd	-2	-
27	0.043	1	-	-	nd	-2	-
28	0.295	11	+	-	nd	6	-
29	-0.045	-2	-	-	nd	-3	-
30	1.119	44	+	-	nd	91	+

nd = not determined

Results Labcode 4 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	Lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.042	1	-	-	nd	2	-
32	0.365	14	+	-	nd	12	+
33	0.748	29	+	-	nd	31	+
34	0.674	26	+	+	nd	30	+
35	0.561	22	+	-	nd	19	+
36	0.548	21	+	-	nd	28	+
37	1.136	45	+	-	nd	46	+
38	0.395	15	+	+	nd	16	+
39	2.662	106	+	-	nd	109	+
40	0.774	30	+	-	nd	74	+
41	0.387	15	+	-	nd	23	+
42	0.490	19	+	-	nd	19	+
43	0.022	0	-	-	nd	-2	-
44	0.972	38	+	+	nd	36	+
45	0.355	14	+	+	nd	42	+
46	1.745	69	+	+	nd	80	+
47	1.122	44	+	-	nd	38	+
48	0.633	25	+	-	nd	24	+
49	0.644	26	+	+	nd	38	+
50	0.781	31	+	+	nd	22	+
51	0.967	38	+	-	nd	42	+
52	1.797	71	+	-	nd	70	+
53	1.409	56	+	-	nd	79	+
54	1.807	72	+	-	nd	85	+
55	0.415	16	+	-	nd	15	+
56	1.804	72	+	+	nd	71	+
57	0.474	18	+	-	nd	-2	-
58	0.499	19	+	-	nd	23	+
59	0.618	24	+	+	nd	35	+
60	0.714	28	+	-	nd	36	+

nd = not determined

Results Labcode 5						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	-0.02		-	-	-	-5	-
2	0		-	-	-	-1	-
3	0.01		-	-	-	1	-
4	0.02		-	-	-	0	-
5	0.03		-	-	-	-3	-
6	0.03		-	-	-	-3	-
7	0.02		-	-	-	-2	-
8	0.01		-	-	-	-2	-
9	0.17		±	-	-	nt	
10	0.11		±	-	-	11	+
11	0.13		±	-	+	33	+
12	0.11		±	-	-	26	+
13	0.22		±	-	-	20	+
14	0.19		±	+	-	37	+
15	0.13		±	-	-	5	-
16	0.20		±	-	+	16	+
17	0.27		+	+	-	70	+
18	0.34		+	-	-	66	+
19	0.32		+	-	+	49	+
20	0.32		+	-	-	30	+
21	0.83		+	+	-	87	+
22	0.49		+	+	-	93	+
23	0.91		+	-	-	95	+
24	0.76		+	-	-	89	+
25	1.03		+	-	-	106	+
26	1.13		+	+	+	113	+
27	1.08		+	+	-	104	+
28	1.16		+	-	-	104	+
29	1.59		+	-	-	112	+
30	2.06		+	+	-	103	+

nt = not tested by the CRL because of a too small amount of meat juice sample

Results Labcode 5 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	1.91		+	-	+	125	+
32	1.47		+	+	-	124	+
33	2.61		+	-	+	162	+
34	1.26		+	-	-	133	+
35	7.9		+	+	-	157	+
36	1.81		+	+	-	143	+
37	1.72		+	+	+	134	+
38	1.31		+	-	-	123	+
39	2.06		+	+	-	145	+
40	0.53		+	+	-	97	+
41	0.26		+	-	-	11	+
42	0.38		+	-	-	59	+
43	0.64		+	+	-	91	+
44	0.71		+	-	-	109	+
45	0.56		+	+	-	97	+
46	0.6		+	-	-	110	+
47	0.21		±	-	+	1	-
48	0.16		±	+	-	27	+
49	0.1		±	+	+	2	-
50	0.21		±	-	-	29	+
51	0.15		±	-	-	78	+
52	0.17		±	-	-	28	+
53	0.18		±	-	-	17	+
54	0.1		-	-	+	6	-
55	0.09		-	-	-	14	+
56	0.01		-	+	-	0	-
57	0.08		-	-	-	2	-
58	0.05		-	+	+	-2	-
59	0.05		-	-	-	-2	-
60	0.04		-	-	-	7	-

Results Labcode 6						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	0.0816	6	-	-	-	2	-
2	0.3061	22	+	-	-	0	-
3	0.0731	5	-	-	-	2	-
4	0.016	1	-	-	-	8	-
5	0.1456	10	±	-	-	1	-
6	0.7077	51	+	+	-	94	+
7	0.2139	15	±	-	-	6	-
8	0.1984	14	±	-	-	4	-
9	0.1401	10	±	-	-	5	-
10	0.2549	18	±	-	-	14	+
11	0.4944	36	+	-	-	7	-
12	0.0357	3	-	-	-	6	-
13	0.2324	17	±	-	-	0	-
14	0.1823	13	±	-	-	3	-
15	0.0035	0.25	-	-	-	-2	-
16	0.3323	24	+	-	-	1	-
17	0.2303	17	±	-	-	8	-
18	0.279	18	±	-	-	-1	-
19	0.0359	3	-	-	-	-2	-
20	0.0556	4	-	-	-	-4	-
21	0.3368	21	+	-	-	7	-
22	0.8499	61	+	-	-	95	+
23	0.051	0	-	-	-	-4	-
24	0.4069	29	+	-	-	34	+
25	0.3687	26	+	-	-	42	+
26	0.1938	14	±	-	-	0	-
27	0.1062	8	-	-	-	0	-
28	0.2102	15	±	-	-	21	+
29	0.0803	6	-	+	-	-1	-
30	0.0084	1	-	+	-	-3	-

Results Labcode 6 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	Lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.344	25	+	-	-	0	-
32	0.165	12	±	-	-	2	-
33	0.304	29	+	-	-	6	-
34	0.008	1	-	-	-	4	-
35	0.124	9	-	-	-	2	-
36	0.098	7	-	-	-	9	-
37	0.112	8	-	-	-	4	-
38	0.133	10	±	-	-	-2	-
39	0.179	13	±	-	-	12	+
40	0.155	11	±	-	-	7	-
41	0.186	13	±	-	-	-1	-
42	0.079	6	-	-	-	4	-
43	0.987	71	+	-	-	8	-
44	0.66	48	+	-	-	-3	-
45	0.05	4	-	-	-	0	-
46	0.195	14	±	+	-	1	-
47	0.222	16	±	-	-	0	-
48	0	0	-	-	-	-3	-
49	0.357	26	+	-	-	1	-
50	0.11	8	-	-	-	3	-
51	0.184	13	±	-	-	0	-
52	0.02	1	-	+	-	-3	-
53	0.098	7	-	-	-	0	-
54	0.212	15	±	-	-	1	-
55	0.061	4	-	-	-	-2	-
56	0.344	25	+	-	-	12	+
57	0.216	16	±	-	-	1	-
58	0.169	12	±	-	-	4	-
59	0	0	-	-	-	4	-
60	0.003	0	-	+	-	-2	-

Results Labcode 7						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	0.041	4.06	-	-	-	-1	-
2	0.096	9.64	-	-	-	5	-
3	0	0	-	+	-	-4	-
4	0.057	5.67	-	-	+	-4	-
5	0.076	7.56	-	+	-	15	+
6	0.151	15.1	-	+	-	24	+
7	0.105	10.53	-	-	-	5	-
8	0.109	10.87	-	-	-	-2	-
9	0.12	11.97	-	-	+	0	-
10	0.195	19.55	-	-	-	4	-
11	0.261	26.09	-	-	-	23	+
12	0.222	22.21	-	-	-	5	-
13	0.226	22.56	-	-	+	44	+
14	0.282	28.19	-	-	-	60	+
15	0.259	25.88	-	-	-	51	+
16	0.327	32.7	-	+	-	11	+
17	0.325	32.467	-	-	-	28	+
18	0.397	39.69	-	-	-	53	+
19	0.349	34.87	-	+	+	-3	-
20	0.337	33.73	-	-	-	-1	-
21	0.451	45.08	+	+	-	66	+
22	0.42359	42.359	+	-	-	28	+
23	0.482	48.19	+	+	-	53	+
24	0.411	41.07	+	+	-	2	-
25	0.417	41.66	+	-	-	60	+
26	0.609	60.9	+	-	-	56	+
27	1.139	113.92	+	+	-	112	+
28	0.728	72.8	+	+	+	108	+
29	0.901	90.11	+	-	-	119	+
30	0.74	73.96	+	+	-	109	+

Results Labcode 7 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.001882	0.1882	-	-	-	-1	-
32	0.0274	2.742	-	-	-	2	-
33	0.0308	3.0783	-	+	+	20	+
34	0.0323	3.2331	-	-	-	-2	-
35	0.0326	3.2567	-	-	-	2	-
36	0.114	11.386	-	-	-	-1	-
37	0.126	12.564	-	+	+	21	+
38	0.159	15.898	-	-	-	39	+
39	0.166	16.638	-	-	-	0	-
40	0.181	18.094	-	-	-	22	+
41	0.207	20.702	-	-	+	69	+
42	0.239	23.938	-	-	-	35	+
43	0.214	21.424	-	-	-	25	+
44	0.282	28.181	-	-	-	62	+
45	0.293	29.273	-	-	-	16	+
46	0.3869	38.69	-	-	-	33	+
47	0.338	33.785	-	-	-	20	+
48	-0.07428	-7.4284	-	-	-	0	-
49	0.343	34.261	-	-	-	39	+
50	0.358	35.771	-	-	+	77	+
51	0.439	43.885	+	-	-	66	+
52	0.46332	46.332	+	-	-	74	+
53	0.492	49.19	+	-	-	79	+
54	0.551	55.057	+	-	-	110	+
55	0.595	59.486	+	+	-	54	+
56	0.668	66.79	+	+	-	24	+
57	0.716	71.61	+	-	-	101	+
58	1.157	115.68	+	-	-	115	+
59	-0.0719	-7.1905	-	+	-	1	-
60	-0.0644	-6.4372	-	-	-	0	-

Results Labcode 8						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	0.108	4.32	-	+	-	2	-
2	0.122	4.88	-	+	nd	3	-
3	0.115	4.6	-	+	-	4	-
4	0.136	5.44	-	+	-	-1	-
5	0.168	6.73	-	+	-	8	-
6	0.399	15.95	+	+	+	29	+
7	0.315	12.61	-	+	-	12	+
8	0.32	12.8	-	+	nd	11	+
9	0.414	16.57	+	+	nd	8	-
10	0.43	17.21	+	+	nd	7	-
11	0.718	28.72	+	-	nd	7	-
12	0.645	25.81	+	-	nd	8	-
13	0.698	27.94	+	+	nd	7	-
14	0.557	22.29	+	+	nd	20	+
15	0.739	29.57	+	-	nd	42	+
16	0.917	36.68	+	+	+	33	+
17	0.965	38.59	+	+	nd	52	+
18	0.933	37.3	+	-	nd	27	+
19	0.931	37.26	+	+	nd	23	+
20	0.857	34.27	+	+	nd	21	+
21	1.065	42.62	+	-	-	87	+
22	1.064	42.56	+	+	-	17	+
23	1.133	45.32	+	+	nd	62	+
24	1.173	46.91	+	+	nd	124	+
25	1.097	43.89	+	-	nd	28	+
26	1.448	57.92	+	+	nd	131	+
27	1.483	59.3	+	+	-	109	+
28	1.375	55	+	+	nd	103	+
29	1.468	58.71	+	+	nd	128	+
30	2.097	83.87	+	+	nd	112	+

nd = not determined

Results Labcode 8 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	Lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.07	2.98	-	-	nd	-1	-
32	0.19	7.41	-	-	nd	0	-
33	0.17	6.71	-	-	-	-1	-
34	0.14	5.8	-	-	nd	1	-
35	0.08	3.25	-	+	nd	-2	-
36	0.34	13.72	-	-	-	2	-
37	0.46	18.34	+	-	nd	14	+
38	0.28	11.34	-	-	nd	2	-
39	0.4	15.81	+	-	nd	27	+
40	0.3	11.82	-	-	nd	-1	-
41	0.56	22.25	+	-	nd	48	+
42	0.52	20.62	+	-	+	14	+
43	0.58	23.02	+	+	nd	42	+
44	0.65	26.1	+	-	nd	10	+
45	0.62	14.95	+	-	nd	-1	-
46	0.82	32.79	+	+	+	12	+
47	0.75	30.01	+	+	nd	56	+
48	0.77	30.77	+	+	nd	24	+
49	0.8	31.98	+	-	-	9	-
50	0.77	30.75	+	-	nd	49	+
51	1.14	45.47	+	-	nd	53	+
52	1.07	42.6	+	-	nd	35	+
53	1.18	27.2	+	+	nd	43	+
54	1.09	43.55	+	-	-	32	+
55	1.09	43.62	+	+	nd	138	+
56	2.19	87.48	+	-	nd	105	+
57	2.11	84.27	+	-	nd	137	+
58	1.38	55.32	+	+	nd	114	+
59	2.03	81.19	+	-	nd	98	+
60	1.5	59.82	+	+	nd	79	+

nd = not determined

Results Labcode 9						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1		0	-	-	-	-1	-
2		0	-	-	-	1	-
3		0	-	-	-	2	-
4		3	-	-	-	12	+
5		4	-	-	-	-2	-
6		11	-	-	-	2	-
7		15	-	-	-	28	+
8		18	-	-	-	1	-
9		19	-	-	-	2	-
10		20	-	-	-	3	-
11		32	+	-	-	47	+
12		37	+	+	-	32	+
13		39	+	-	-	11	+
14		47	+	-	-	26	+
15		51	+	+	-	41	+
16		58	+	-	-	71	+
17		61	+	+	-	35	+
18		62	+	-	-	78	+
19		98	+	-	-	93	+
20		107	+	+	-	90	+
21		112	+	+	-	100	+
22		0	-	-	-	0	-
23		0	-	-	-	4	-
24		0	-	nd	nd	-3	-
25		1	-	nd	nd	2	-
26		0	-	-	-	-1	-
27		20	-	-	-	53	+
28		16	-	-	-	16	+
29		17	-	-	-	26	+
30		29	+	-	-	16	+

nd = not determined

Results Labcode 9 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31		24	+	-	-	33	+
32		21	+	-	-	1	-
33		13	-	nd	nd	18	+
34		13	-	nd	nd	5	-
35		26	+	-	-	20	+
36		33	+	-	-	47	+
37		25	+	nd	nd	65	+
38		31	+	-	-	12	+
39		21	+	nd	nd	25	+
40		26	+	-	-	10	+
41		27	+	-	-	12	+
42		29	+	-	-	23	+
43		32	+	nd	nd	27	+
44		45	+	-	-	33	+
45		51	+	-	-	16	+
46		40	+	nd	nd	33	+
47		45	+	+	-	11	+
48		62	+	+	nd	37	+
49		71	+	-	-	43	+
50		75	+	+	-	130	+
51		65	+	+	-	37	+
52		5	-	-	-	16	+
53		89	+	-	-	nt	
54		72	+	+	-	23	+
55		89	+	nd	nd	19	+
56		106	+	nd	nd	100	+
57		98	+	nd	nd	99	+
58		108	+	+	-	102	+
59		93	+	+	-	nt	
60		79	+	-	-	57	+

nd = not determined

nt = not tested by the CRL because of a too small amount of meat juice sample

Results Labcode 10						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	lymph nodes	Carcass swabs	OD%	result (+, ±, -)
1	0.10	0.30	-	+	-	-1	-
2	0.07	0.01	-	-	-	0	-
3	0.10	0.28	-	+	-	1	-
4	0.40	15.36	±	-	-	0	-
5	0.36	12.58	±	-	-	1	-
6	0.36	12.22	±	-	-	0	-
7	0.51	26.84	+	+	-	17	+
8	0.48	23.39	+	-	-	7	-
9	0.55	30.99	+	-	-	1	-
10	0.55	30.83	+	-	-	2	-
11	1.43	229.02	+	-	-	109	+
12	0.47	25.06	+	-	-	32	+
13	1.19	174.74	+	+	-	87	+
14	0.93	101.75	+	-	-	13	+
15	0.05	0.11	-	-	-	-3	-
16	0.06	0.2	-	-	-	1	-
17	0.10	0.92	-	-	-	1	-
18	0.15	2.92	-	-	-	0	-
19	0.27	10.48	±	-	-	-2	-
20	0.30	13.75	±	-	-	-2	-
21	0.34	17.69	±	-	-	6	-
22	0.29	12.22	±	-	-	22	+
23	0.39	24.21	+	-	-	-2	-
24	0.40	24.66	+	-	-	9	-
25	0.42	27.18	+	nd	nd	2	-
26	0.42	26.41	+	-	-	0	-
27	0.49	38.88	+	-	-	-2	-
28	0.51	41.05	+	+	-	5	-
29	0.55	48.62	+	-	-	nt	
30	0.57	52.52	+	-	-	27	+

nd = not determined

nt = not tested by the CRL because of a too small amount of meat juice sample

Results Labcode 10 (continued)						Results CRL-*Salmonella*	
	Serology results			Bacteriology results			
sample	SP ratio	OD%	result (+, ±, -)	Lymph nodes	Carcass swabs	OD%	result (+, ±, -)
31	0.57	51.37	+	nd	nd	nt	
32	0.57	52.6	+	-	-	5	-
33	0.61	61.24	+	-	-	1	-
34	0.12	6.22	-	-	-	-1	-
35	0.09	3.84	-	+	-	nt	
36	0.11	5.42	-	-	-	-1	-
37	0.22	13.29	±	-	-	0	-
38	0.23	14.53	±	-	-	6	-
39	0.23	14.09	±	-	-	12	+
40	0.35	22.98	+	-	-	21	+
41	0.37	25.03	+	-	-	7	-
42	0.39	26.10	+	-	-	71	+
43	0.56	38.94	+	-	-	8	-
44	0.46	32.02	+	-	-	33	+
45	0.52	36.39	+	-	-	48	+
46	0.48	32.94	+	-	-	4	-
47	0.61	43.15	+	-	-	12	+
48	0.67	47.21	+	-	-	0	-
49	0.66	46.58	+	-	-	75	+
50	0.63	44.22	+	-	-	-1	-
51	0.68	47.98	+	+	-	110	+
52	0.62	43.62	+	-	-	35	+
53	0.58	40.70	+	+	-	nt	
54	0.70	49.90	+	-	-	nt	
55	1.07	77.40	+	nd	nd	18	+
56	1.20	87.32	+	-	-	107	+
57	1.11	80.37	+	nd	nd	111	+
58	---	---	---	---	---	---	---
59	---	---	---	---	---	---	---
60	---	---	---	---	---	---	---

nd = not determined
--- not send in
nt = not tested by the CRL because of a too small amount of meat juice sample

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