CODA-CERVA
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE
GROESELENBERG 99 – B 1180 BRUSSELS (UKKEL)
TEL: +32 (0)2 379 04 11
FAX : + 32 (0)2 379 06 70
HTTP: // WWW.CODA-CERVA.BE

172-PT

PROFICIENCY TESTING 2012

SALMONELLA (SAL)

Detection of SAL-specific antibodies in porcine serum by Enzyme Linked Immunosorbent Assay (ELISA)

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 30 JULY 2012

DATE REPORT: 13 SEPTEMBER 2012

I. Introduction

Details relevant to the proficiency test (PT) are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of SAL-specific antibodies in porcine serum by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be tested by means of a SAL antibody ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 5 reference serum samples of porcine origin, either free from detectable SAL-specific antibodies (n=2; coded 'PT2012SALSERNS1' and 'PT2012SALSERNS2') or containing detectable SAL-specific antibodies (n=3; coded 'PT2012SALSERPS1', 'PT2012SALSERPS2' and 'PT2012SALSERPS3'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants were given 4 aliquots of the 5 reference serum samples, i.e. 20 aliquots. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 3).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained by the Swine Salmonella Antibody ELISA Test Kit from IDEXX (pre-verification). All reference serum samples were derived from pigs included in an *in vivo* infection experiment with *Salmonella* Typhimurium. PT2012SALSERNS1 and PT2012SALSERNS2 were obtained from 2 uninfected animals (negative control group), whereas the reference serum samples PT2012SALSERPS1, PT2012SALSERPS2 and PT2012SALSERPS3 were obtained from either directly or transiently infected pigs. For the qualitative interpretation of the S/P ratios obtained by the Swine Salmonella Antibody ELISA Test Kit from IDEXX, a cut-off of 0,5 has been applied (S/P < 0.5 is NEG, S/P ≥ 0.5 is POS). Taken together, the reference serum samples PT2012SALSERNS1 and PT2012SALSERNS2 were considered as negative sera, and the reference serum samples PT2012SALSERPS1, PT2012SALSERPS2 and PT2012SALSERPS3 as positive sera in SAL antibody ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the Swine Salmonella Antibody ELISA Test Kit from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of SAL-specific antibodies in porcine serum. In addition, all reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) using the Swine Salmonella Antibody ELISA Test Kit from IDEXX.

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* (i.e., the reported result matches with the assigned status) for the 20 aliquots of reference serum samples used for this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference serum samples is at least 90%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the operational unit CVD-ERA of CODA-CERVA.

IV.1. Transfer and start of the analyses of the reference samples

The 20 aliquots of reference serum samples were sent frozen (dry ice) to each of the 4 participating laboratories by national courier on 30th of July 2012 (80 aliquots in total). All participants acknowledged receipt of the samples on the same day. Analyses were performed between 31st of July and 6th of August 2012 (Table 1).

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results were submitted to the operational unit CVD-ERA between 1st and 10th of August 2012 (Table 1). All participants hereby respected the deadline of 17th of August 2012 for submission of the results.

Table 1. Overview of the dates on which (i) the reference serum samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	30/07/2012	31/07/2012	01/08/2012
LAB2	30/07/2012	01/08/2012	03/08/2012
LAB3	30/07/2012	06/08/2012	10/08/2012
LAB4	30/07/2012	01/08/2012	09/08/2012

IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that all participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples and hence achieved 100% of agreement (Table 2).

A quantitative data analysis (including box plots) is shown for educational purposes in Annex 1 and Annex 2.

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the SAL reference laboratory of CODA-CERVA. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	**2**	**3**	**4**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement for the detection of SAL-specific antibodies in reference serum samples, no variability between qualitative laboratory results could be observed.

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum samples (SAMPLE), the positions of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by the SAL reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012SALSERNS2	NEG	NEG	1
2	1	2	PT2012SALSERPS2	POS	POS	1
3	1	3	PT2012SALSERNS1	NEG	NEG	1
4	1	4	PT2012SALSERPS1	POS	POS	1
5	1	5	PT2012SALSERPS3	POS	POS	1
6	1	6	PT2012SALSERPS3	POS	POS	1
7	1	7	PT2012SALSERNS2	NEG	NEG	1
8	1	8	PT2012SALSERPS3	POS	POS	1
9	1	9	PT2012SALSERPS1	POS	POS	1
10	1	10	PT2012SALSERNS1	NEG	NEG	1
11	1	11	PT2012SALSERPS3	POS	POS	1
12	1	12	PT2012SALSERPS2	POS	POS	1
13	1	13	PT2012SALSERNS2	NEG	NEG	1
14	1	14	PT2012SALSERNS2	NEG	NEG	1
15	1	15	PT2012SALSERPS1	POS	POS	1
16	1	16	PT2012SALSERPS2	POS	POS	1
17	1	17	PT2012SALSERNS1	NEG	NEG	1
18	1	18	PT2012SALSERPS2	POS	POS	1
19	1	19	PT2012SALSERPS1	POS	POS	1
20	1	20	PT2012SALSERNS1	NEG	NEG	1
21	2	1	PT2012SALSERPS3	POS	POS	1
22	2	2	PT2012SALSERNS2	NEG	NEG	1
23	2	3	PT2012SALSERPS3	POS	POS	1
24	2	4	PT2012SALSERPS1	POS	POS	1
25	2	5	PT2012SALSERNS1	NEG	NEG	1
26	2	6	PT2012SALSERPS3	POS	POS	1
27	2	7	PT2012SALSERPS2	POS	POS	1
28	2	8	PT2012SALSERNS2	NEG	NEG	1
29	2	9	PT2012SALSERNS2	NEG	NEG	1
30	2	10	PT2012SALSERPS1	POS	POS	1
31	2	11	PT2012SALSERPS2	POS	POS	1
32	2	12	PT2012SALSERNS1	NEG	NEG	1
33	2	13	PT2012SALSERPS2	POS	POS	1
34	2	14	PT2012SALSERPS1	POS	POS	1
35	2	15	PT2012SALSERNS1	NEG	NEG	1
36	2	16	PT2012SALSERNS2	NEG	NEG	1
37	2	17	PT2012SALSERPS2	POS	POS	1
38	2	18	PT2012SALSERNS1	NEG	NEG	1
39	2	19	PT2012SALSERPS1	POS	POS	1
40	2	20	PT2012SALSERPS3	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012SALSERPS3	POS	POS	1
42	3	2	PT2012SALSERPS2	POS	POS	1
43	3	3	PT2012SALSERNS2	NEG	NEG	1
44	3	4	PT2012SALSERNS2	NEG	NEG	1
45	3	5	PT2012SALSERPS1	POS	POS	1
46	3	6	PT2012SALSERPS2	POS	POS	1
47	3	7	PT2012SALSERNS1	NEG	NEG	1
48	3	8	PT2012SALSERPS2	POS	POS	1
49	3	9	PT2012SALSERPS1	POS	POS	1
50	3	10	PT2012SALSERNS1	NEG	NEG	1
51	3	11	PT2012SALSERNS2	NEG	NEG	1
52	3	12	PT2012SALSERPS2	POS	POS	1
53	3	13	PT2012SALSERNS1	NEG	NEG	1
54	3	14	PT2012SALSERPS1	POS	POS	1
55	3	15	PT2012SALSERPS3	POS	POS	1
56	3	16	PT2012SALSERPS3	POS	POS	1
57	3	17	PT2012SALSERNS2	NEG	NEG	1
58	3	18	PT2012SALSERPS3	POS	POS	1
59	3	19	PT2012SALSERPS1	POS	POS	1
60	3	20	PT2012SALSERNS1	NEG	NEG	1
61	4	1	PT2012SALSERPS2	POS	POS	1
62	4	2	PT2012SALSERNS1	NEG	NEG	1
63	4	3	PT2012SALSERPS2	POS	POS	1
64	4	4	PT2012SALSERPS1	POS	POS	1
65	4	5	PT2012SALSERNS1	NEG	NEG	1
66	4	6	PT2012SALSERNS2	NEG	NEG	1
67	4	7	PT2012SALSERPS2	POS	POS	1
68	4	8	PT2012SALSERNS1	NEG	NEG	1
69	4	9	PT2012SALSERPS1	POS	POS	1
70	4	10	PT2012SALSERPS3	POS	POS	1
71	4	11	PT2012SALSERPS3	POS	POS	1
72	4	12	PT2012SALSERNS2	NEG	NEG	1
73	4	13	PT2012SALSERPS3	POS	POS	1
74	4	14	PT2012SALSERPS1	POS	POS	1
75	4	15	PT2012SALSERNS1	NEG	NEG	1
76	4	16	PT2012SALSERPS3	POS	POS	1
77	4	17	PT2012SALSERPS2	POS	POS	1
78	4	18	PT2012SALSERNS2	NEG	NEG	1
79	4	19	PT2012SALSERNS2	NEG	NEG	1
80	4	20	PT2012SALSERPS1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of porcine origin for the detection of SAL-specific antibodies by ELISA.

For the detection of SAL-specific antibodies in the porcine reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement).

All participating laboratories used the Swine Salmonella Antibody ELISA Test Kit from IDEXX, but 4 different batches were used: Y351 (LAB1), X401 (LAB2), Z291 (LAB3) and Z111 (LAB4). LAB1 and LAB3 performed the short incubation protocol, whereas LAB2 performed the long incubation protocol. LAB4 did not provide information about the used incubation protocol.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by the SAL reference laboratory of CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of SAL-specific antibodies in porcine reference serum samples by ELISA.

Head CVD-ERA
Yves Van der Stede

Appendix

Name of the participating laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Loncin, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs SAS 9.2. (summary statistics) and R (box plots). All quantitative data analyses were performed on the normalized data, namely the S/P ratio calculated according to the instructions for this PT: (OD_{Sample} - mean $OD_{Negative\ Kit\ Controls}$) / (mean $OD_{Positive\ Kit\ Controls}$ - mean $OD_{Negative\ Kit\ Controls}$).

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

I. Box plots

Box plots of the S/P ratio per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

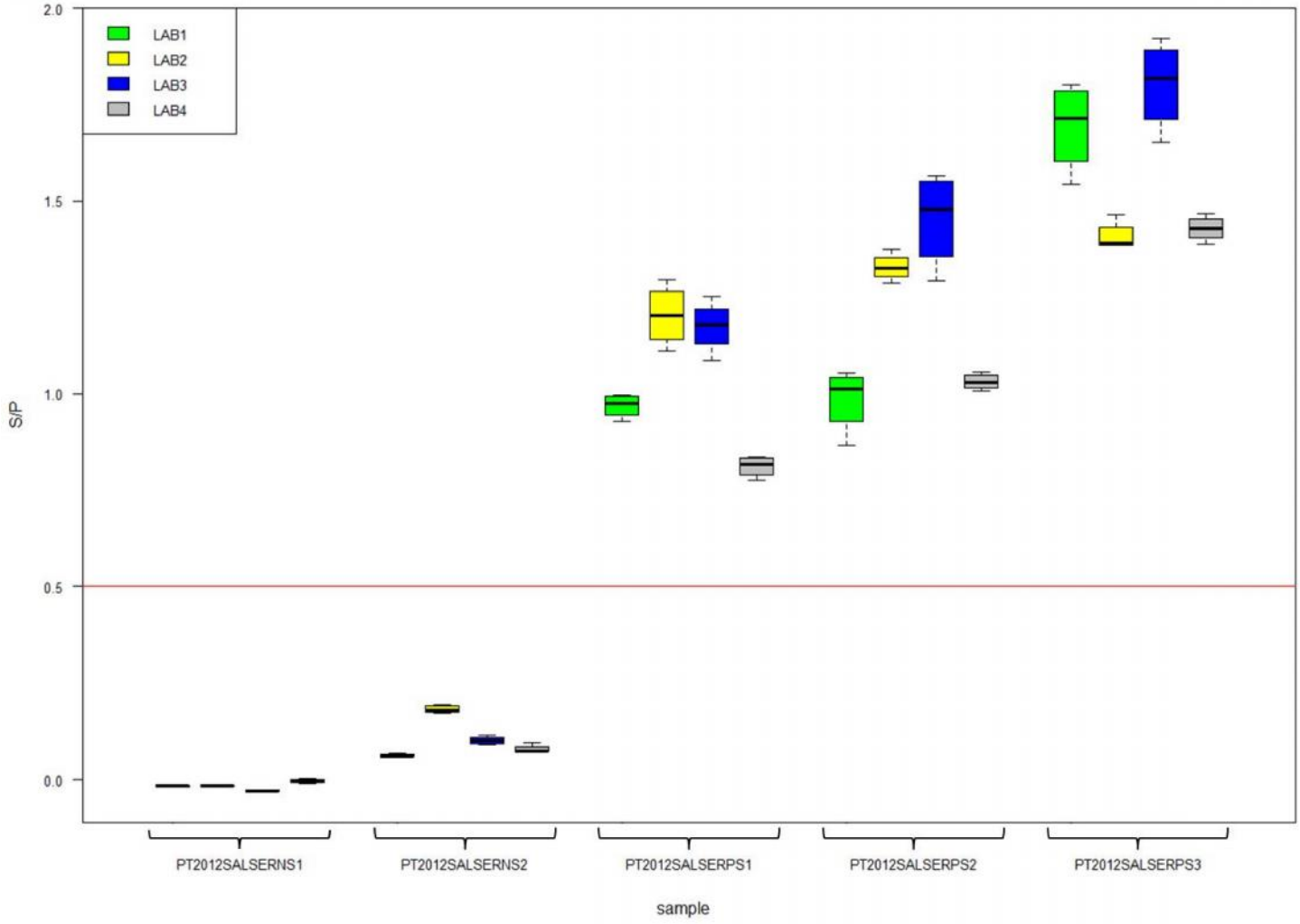

Figure 1. Box plots showing the S/P ratio per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. All participating laboratories used the Swine Salmonella Antibody ELISA Test Kit from IDEXX (4 different batches). LAB1 and LAB3 performed the short incubation protocol, whereas LAB2 performed the long incubation protocol. LAB4 did not provide information about the used incubation protocol. The used cut-off value of 0,5 is shown in red (S/P < 0.5 is NEG, S/P ≥ 0.5 is POS).

II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-lab variability (reproducibility) and within-lab variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated based on the S/P ratio per reference serum sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,40	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic for this PT is 1,42 (p=4), whereas the maximum value for Mandel's k-statistic is 1,50 (p=4 and n=4).

LAB1, LAB3 and LAB4 obtained a satisfactory between-laboratory consistency for all reference serum samples, whereas LAB2 showed a slightly increased value for Mandel's h-statistic for the negative reference serum sample PT2012SALSERNS2 (h=1,43). Each participating laboratory used a different batch of the Swine Salmonella Antibody ELISA Test Kit from IDEXX. LAB1 and LAB3 performed the short incubation protocol, whereas LAB2 performed the long incubation protocol. LAB4 did not provide information about the used incubation protocol.

LAB1 and LAB2 obtained a satisfactory within-laboratory consistency for all reference serum samples. In contrast, LAB3 showed an increased value for Mandel's k-statistic for the positive reference serum sample PT2012SALSERPS2 (k=1,60), whereas LAB4 showed an increased value for Mandel's k-statistic for the negative reference serum sample PT2012SALSERNS1 (k=1,70).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values (in this case the S/P ratio) as a dependent variable, it was investigated whether statistically significant differences exist (α=0,05) between participating laboratories. Comparisons were made at the global level (all reference serum samples were analysed together), status level (all reference serum samples with the same status were analysed together) and sample level (all reference serum samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

No statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level. At the status level, significant differences were observed between laboratories for the positive reference serum samples but not for the negative reference serum samples. For the positive reference serum samples, LAB3 reported S/P ratios that were significantly higher than those reported by LAB4.

Annex 2: Calculations of Mandel's h- and k-statistics (based on S/P ratio)

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2012SALSERNS1	1	4	0,000004	-0,017	-0,016	0,777	0,003	0,006	0,006	-0,02	0,62	-11,61
PT2012SALSERNS1	2	4	0,000000	-0,015	-0,016	0,777	0,003	0,006	0,006	0,11	0,16	-3,28
PT2012SALSERNS1	3	4	0,000007	-0,029	-0,016	0,777	0,003	0,006	0,006	-1,27	0,84	-8,90
PT2012SALSERNS1	**4**	4	0,000027	-0,005	-0,016	0,777	0,003	0,006	0,006	1,18	**1,70**	-115,47
PT2012SALSERNS2	1	4	0,000016	0,062	0,106	0,915	0,009	0,032	0,030	-0,83	0,43	6,34
PT2012SALSERNS2	**2**	4	0,000092	0,182	0,106	0,915	0,009	0,032	0,030	**1,43**	1,04	5,27
PT2012SALSERNS2	3	4	0,000110	0,102	0,106	0,915	0,009	0,032	0,030	-0,08	1,13	10,27
PT2012SALSERNS2	4	4	0,000126	0,079	0,106	0,915	0,009	0,032	0,030	-0,51	1,21	14,14
PT2012SALSERPS1	1	4	0,001025	0,969	1,039	0,776	0,057	0,119	0,105	-0,38	0,57	3,30
PT2012SALSERPS1	2	4	0,006493	1,203	1,039	0,776	0,057	0,119	0,105	0,89	1,42	6,70
PT2012SALSERPS1	3	4	0,004570	1,174	1,039	0,776	0,057	0,119	0,105	0,73	1,19	5,76
PT2012SALSERPS1	4	4	0,000730	0,811	1,039	0,776	0,057	0,119	0,105	-1,24	0,48	3,33
PT2012SALSERPS2	1	4	0,007003	0,986	1,200	0,733	0,078	0,151	0,129	-0,94	1,07	8,49
PT2012SALSERPS2	2	4	0,001320	1,329	1,200	0,733	0,078	0,151	0,129	0,57	0,46	2,73
PT2012SALSERPS2	**3**	4	0,015670	1,454	1,200	0,733	0,078	0,151	0,129	1,12	**1,60**	8,61
PT2012SALSERPS2	4	4	0,000456	1,031	1,200	0,733	0,078	0,151	0,129	-0,74	0,27	2,07
PT2012SALSERPS3	1	4	0,013589	1,694	1,583	0,620	0,086	0,140	0,110	0,57	1,35	6,88
PT2012SALSERPS3	2	4	0,001431	1,408	1,583	0,620	0,086	0,140	0,110	-0,90	0,44	2,69
PT2012SALSERPS3	3	4	0,013703	1,803	1,583	0,620	0,086	0,140	0,110	1,12	1,35	6,49
PT2012SALSERPS3	4	4	0,001134	1,429	1,583	0,620	0,086	0,140	0,110	-0,79	0,39	2,36

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in the normalized data (S/P ratio); **x_i_m** = mean of normalized data (S/P ratio); **x_g_m** = mean of normalized data (S/P ratio) obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).