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172-PT

PROFICIENCY TESTING 2013

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: 29 JULY 2013

DATE REPORT: 27 JANUARY 2014

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 6 reference serum samples of porcine origin, either free from detectable SAL-specific antibodies (n=2; coded 'PT2013SALSERNS1' and 'PT2013SALSERNS2') or containing detectable SAL-specific antibodies (n=4; coded 'PT2013SALSERPS1', 'PT2013SALSERPS2', 'PT2013SALSERPS3' and 'PT2013SALSERPS4'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants were given 20 aliquots: 3 aliquots of the reference serum samples PT2013SALSERNS1, PT2013SALSERNS2, PT2013SALSERPS1 and PT2013SALSERPS2, and 4 aliquots of the reference serum samples PT2013SALSERPS3 and PT2013SALSERPS4. 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 during pre-verification using the Swine Salmonella Antibody ELISA Test Kit from IDEXX. All reference serum samples were derived from pigs included in an *in vivo* infection experiment with *Salmonella* Typhimurium. PT2013SALSERNS1 and PT2013SALSERNS2 were obtained from 2 uninfected animals (negative control group), whereas the reference serum samples PT2013SALSERPS1, PT2013SALSERPS2, PT2013SALSERPS3 and PT2013SALSERPS4 were obtained from either directly (seeders) or indirectly (contact animals) 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 PT2013SALSERNS1 and PT2013SALSERNS2 were considered as negative sera, and the reference serum samples PT2013SALSERPS1, PT2013SALSERPS2, PT2013SALSERPS3 and PT2013SALSERPS4 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, 3 aliquots of each reference serum sample were tested 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* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference 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 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 29th of July 2013 (80 aliquots in total). All participants acknowledged receipt of the samples on the same day. Analyses were performed between 30th of July and 5th of August 2013 (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 2nd and 8th of August 2013 (Table 1). All participants hereby respected the deadline of 9th of August 2013 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	29/07/2013	05/08/2013	07/08/2013
LAB2	29/07/2013	01/08/2013	08/08/2013
LAB3	29/07/2013	30/07/2013	02/08/2013
LAB4	29/07/2013	01/08/2013	02/08/2013

IV.3. Compliance with the procedure

Except LAB3, 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 3 out of 4 participating laboratories (LAB1, LAB2 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence achieved 100% of

agreement. In contrast, LAB3 misclassified 1 aliquot of reference serum samples and obtained 95% 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 the results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by 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)	1 (5.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	19 (95.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1, LAB2 and LAB4 since these participants correctly identified all reference serum samples. In contrast, LAB3 misclassified 1 out of 4 aliquots of the positive reference serum sample PT2013SALSERPS3 (NEG instead of POS).

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 CODA-CERVA (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013SALSERPS1	POS	POS	1
2	1	2	PT2013SALSERPS2	POS	POS	1
3	1	3	PT2013SALSERNS2	NEG	NEG	1
4	1	4	PT2013SALSERPS1	POS	POS	1
5	1	5	PT2013SALSERPS3	POS	POS	1
6	1	6	PT2013SALSERNS1	NEG	NEG	1
7	1	7	PT2013SALSERPS3	POS	POS	1
8	1	8	PT2013SALSERPS4	POS	POS	1
9	1	9	PT2013SALSERNS2	NEG	NEG	1
10	1	10	PT2013SALSERPS2	POS	POS	1
11	1	11	PT2013SALSERPS4	POS	POS	1
12	1	12	PT2013SALSERNS1	NEG	NEG	1
13	1	13	PT2013SALSERNS2	NEG	NEG	1
14	1	14	PT2013SALSERPS3	POS	POS	1
15	1	15	PT2013SALSERPS4	POS	POS	1
16	1	16	PT2013SALSERPS2	POS	POS	1
17	1	17	PT2013SALSERPS3	POS	POS	1
18	1	18	PT2013SALSERPS1	POS	POS	1
19	1	19	PT2013SALSERPS4	POS	POS	1
20	1	20	PT2013SALSERNS1	NEG	NEG	1
21	2	1	PT2013SALSERNS1	NEG	NEG	1
22	2	2	PT2013SALSERPS3	POS	POS	1
23	2	3	PT2013SALSERPS4	POS	POS	1
24	2	4	PT2013SALSERNS2	NEG	NEG	1
25	2	5	PT2013SALSERPS2	POS	POS	1
26	2	6	PT2013SALSERPS4	POS	POS	1
27	2	7	PT2013SALSERNS1	NEG	NEG	1
28	2	8	PT2013SALSERNS2	NEG	NEG	1
29	2	9	PT2013SALSERPS3	POS	POS	1
30	2	10	PT2013SALSERPS4	POS	POS	1
31	2	11	PT2013SALSERPS2	POS	POS	1
32	2	12	PT2013SALSERPS3	POS	POS	1
33	2	13	PT2013SALSERPS1	POS	POS	1
34	2	14	PT2013SALSERPS4	POS	POS	1
35	2	15	PT2013SALSERNS1	NEG	NEG	1
36	2	16	PT2013SALSERPS1	POS	POS	1
37	2	17	PT2013SALSERPS2	POS	POS	1
38	2	18	PT2013SALSERNS2	NEG	NEG	1
39	2	19	PT2013SALSERPS1	POS	POS	1
40	2	20	PT2013SALSERPS3	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013SALSERPS4	POS	POS	1
42	3	2	PT2013SALSERNS1	NEG	NEG	1
43	3	3	PT2013SALSERNS2	NEG	NEG	1
44	3	4	PT2013SALSERPS3	**POS**	**NEG**	**0**
45	3	5	PT2013SALSERPS4	POS	POS	1
46	3	6	PT2013SALSERPS2	POS	POS	1
47	3	7	PT2013SALSERPS3	POS	POS	1
48	3	8	PT2013SALSERPS1	POS	POS	1
49	3	9	PT2013SALSERPS4	POS	POS	1
50	3	10	PT2013SALSERNS1	NEG	NEG	1
51	3	11	PT2013SALSERPS1	POS	POS	1
52	3	12	PT2013SALSERPS2	POS	POS	1
53	3	13	PT2013SALSERNS2	NEG	NEG	1
54	3	14	PT2013SALSERPS1	POS	POS	1
55	3	15	PT2013SALSERPS3	POS	POS	1
56	3	16	PT2013SALSERNS1	NEG	NEG	1
57	3	17	PT2013SALSERPS3	POS	POS	1
58	3	18	PT2013SALSERPS4	POS	POS	1
59	3	19	PT2013SALSERNS2	NEG	NEG	1
60	3	20	PT2013SALSERPS2	POS	POS	1
61	4	1	PT2013SALSERPS2	POS	POS	1
62	4	2	PT2013SALSERPS3	POS	POS	1
63	4	3	PT2013SALSERPS1	POS	POS	1
64	4	4	PT2013SALSERPS4	POS	POS	1
65	4	5	PT2013SALSERNS1	NEG	NEG	1
66	4	6	PT2013SALSERPS1	POS	POS	1
67	4	7	PT2013SALSERPS2	POS	POS	1
68	4	8	PT2013SALSERNS2	NEG	NEG	1
69	4	9	PT2013SALSERPS1	POS	POS	1
70	4	10	PT2013SALSERPS3	POS	POS	1
71	4	11	PT2013SALSERNS1	NEG	NEG	1
72	4	12	PT2013SALSERPS3	POS	POS	1
73	4	13	PT2013SALSERPS4	POS	POS	1
74	4	14	PT2013SALSERNS2	NEG	NEG	1
75	4	15	PT2013SALSERPS2	POS	POS	1
76	4	16	PT2013SALSERPS4	POS	POS	1
77	4	17	PT2013SALSERNS1	NEG	NEG	1
78	4	18	PT2013SALSERNS2	NEG	NEG	1
79	4	19	PT2013SALSERPS3	POS	POS	1
80	4	20	PT2013SALSERPS4	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, 3 out of 4 participating laboratories (LAB1, LAB2 and LAB4) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement), whereas LAB3 misclassified 1 aliquot of the positive reference serum sample PT2013SALSERPS3 (95% of agreement) (Table 2 and Table3).

All participating laboratories used the Swine Salmonella Antibody ELISA Test Kit from IDEXX, but 3 different batches were used: A811 (LAB1), A451 (LAB2 and LAB4) and B101 (LAB3). LAB2 and LAB4 performed the short incubation protocol, whereas LAB1 performed the long incubation protocol. LAB3 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 CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of SAL-specific antibodies in porcine serum 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 R (box plots) and SAS 9.2. (summary statistics). 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.

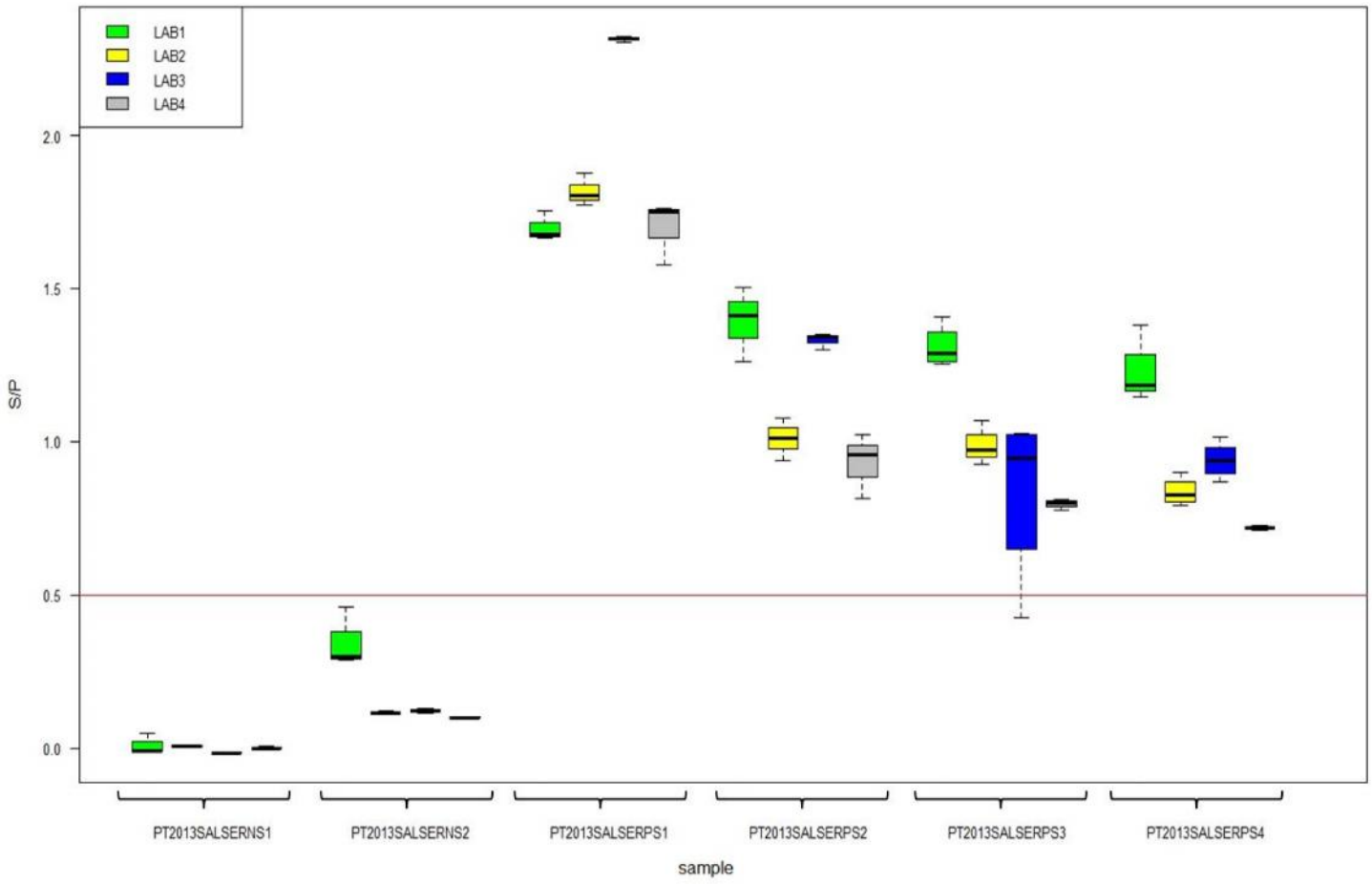

Figure 1. Box plots showing the S/P ratio per reference serum sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. All participating laboratories used the same ELISA kit (3 different batches). Hereby, LAB2 and LAB4 performed the short incubation protocol of the same batch, whereas LAB1 performed the long incubation protocol of another batch (LAB3 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 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 1,59 for the reference samples PT2013SALSERNS1, PT2013SALSERNS2, PT2013SALSERPS1 and PT2013SALSERPS2 (p=4 and n=3) and 1,50 for the reference samples PT2013SALSERPS3 and PT2013SALSERPS4 (p=4 and n=4).

LAB2 and LAB4 obtained a satisfactory between-laboratory consistency for all reference serum samples, whereas LAB1 and LAB3 showed an increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB1 for the negative reference serum sample PT2013SALSERNS2 (h=1,50), and LAB3 for the negative reference serum sample PT2013SALSERNS1 (h=-1,43) and for the positive reference serum sample PT2013SALSERPS1 (h=1,47). All participating laboratories used the same ELISA kit (3 different batches). Hereby, LAB2 and LAB4 performed the short incubation protocol, whereas LAB1 performed the long incubation protocol (LAB3 did not provide information about the used incubation protocol).

Only LAB2 obtained a satisfactory within-laboratory consistency for all reference serum samples. In contrast, LAB1, LAB3 and LAB4 showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB1 for the negative reference serum samples PT2013SALSERNS1 (k=1,97) and PT2013SALSERNS2 (k=1,99) and for the positive reference serum sample PT2013SALSERPS4 (k=1,62), LAB3 for the positive reference serum sample PT2013SALSERPS3 (k=1,90) and LAB4 for the positive reference serum sample PT2013SALSERPS1 (k=1,64).

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 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.

Statistically significant differences between laboratories were only observed at the sample level, but not at the global or status level.

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
PT2013SALSERNS1	**1**	3	0,001136	0,011	0,001	0,049	0,017	0,018	0,004	0,82	**1,97**	303,12
PT2013SALSERNS1	2	3	0,000002	0,007	0,001	0,049	0,017	0,018	0,004	0,52	0,08	17,29
PT2013SALSERNS1	**3**	3	0,000006	-0,016	0,001	0,049	0,017	0,018	0,004	**-1,43**	0,14	-15,61
PT2013SALSERNS1	4	3	0,000024	0,002	0,001	0,049	0,017	0,018	0,004	0,09	0,29	203,77
PT2013SALSERNS2	**1**	3	0,009578	0,349	0,171	0,645	0,049	0,083	0,066	**1,50**	**1,99**	28,08
PT2013SALSERNS2	2	3	0,000045	0,116	0,171	0,645	0,049	0,083	0,066	-0,47	0,14	5,81
PT2013SALSERNS2	3	3	0,000059	0,120	0,171	0,645	0,049	0,083	0,066	-0,43	0,16	6,40
PT2013SALSERNS2	4	3	0,000002	0,101	0,171	0,645	0,049	0,083	0,066	-0,59	0,03	1,46
PT2013SALSERPS1	1	3	0,002314	1,698	1,881	0,877	0,063	0,180	0,169	-0,62	0,76	2,83
PT2013SALSERPS1	2	3	0,002820	1,816	1,881	0,877	0,063	0,180	0,169	-0,22	0,84	2,92
PT2013SALSERPS1	**3**	3	0,000101	2,315	1,881	0,877	0,063	0,180	0,169	**1,47**	0,16	0,43
PT2013SALSERPS1	**4**	3	0,010749	1,697	1,881	0,877	0,063	0,180	0,169	-0,63	**1,64**	6,11
PT2013SALSERPS2	1	3	0,014616	1,392	1,166	0,680	0,089	0,157	0,129	0,98	1,36	8,69
PT2013SALSERPS2	2	3	0,004955	1,009	1,166	0,680	0,089	0,157	0,129	-0,68	0,79	6,98
PT2013SALSERPS2	3	3	0,000721	1,331	1,166	0,680	0,089	0,157	0,129	0,72	0,30	2,02
PT2013SALSERPS2	4	3	0,011108	0,931	1,166	0,680	0,089	0,157	0,129	-1,02	1,19	11,32
PT2013SALSERPS3	1	4	0,004823	1,310	0,982	0,423	0,149	0,196	0,128	1,40	0,47	5,30
PT2013SALSERPS3	2	4	0,003628	0,986	0,982	0,423	0,149	0,196	0,128	0,02	0,40	6,11
PT2013SALSERPS3	**3**	4	0,080000	0,835	0,982	0,423	0,149	0,196	0,128	-0,63	**1,90**	33,86
PT2013SALSERPS3	4	4	0,000194	0,797	0,982	0,423	0,149	0,196	0,128	-0,79	0,09	1,75
PT2013SALSERPS4	**1**	4	0,011081	1,223	0,929	0,782	0,065	0,139	0,123	1,36	**1,62**	8,61
PT2013SALSERPS4	2	4	0,002155	0,834	0,929	0,782	0,065	0,139	0,123	-0,44	0,71	5,56
PT2013SALSERPS4	3	4	0,003622	0,939	0,929	0,782	0,065	0,139	0,123	0,05	0,93	6,41
PT2013SALSERPS4	4	4	0,000047	0,719	0,929	0,782	0,065	0,139	0,123	-0,97	0,11	0,95

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).