Petra Berk | 26 February 2009

Comparability of different ELISA's on the detection of *Salmonella* spp. antibodies in meat juice and serum of pigs

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CRL-*Salmonella*

Background

- Baseline survey on the prevalence of *Salmonella* spp. in slaughter pigs (2006/668/EC)
- New – the possibility to use serological methods on meat juice
- 10 NRLs-*Salmonella* using their own method

Two studies organised by the CRL-*Salmonella*

1. Duplicate analysis study
2. Interlaboratory comparison study at the end of the baselinestudy

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Information on ELISAs used

Labcode	ELISA	Data	Cut-off values used		
			-	±	+
1	Salmotype PigScreen (LDL)	OD %	<10	>10 and <20	>20
2	Salmotype PigScreen (LDL)	OD %	<10	>10 and <20	>20
3	HerdCheck Swine Salmonella (IDEXX)	OD %	<10	>10 and <20	>20
4	HerdCheck Swine Salmonella (IDEXX)	OD %	<10		>10
5	VetSign Porcine Salmonella (Guildhay)	S/P ratio	<0.10	>0.10 and <0.25	>0.25
6	Salmotype PigScreen (LDL)	OD %	<10	>10 and <20	>20
7	In-house	OD %	<40		>40
8	HerdCheck Swine Salmonella (IDEXX)	OD %	<15		>15
9	In-house	OD %	<20		>20

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Calculation of OD % per ELISA

- S/P ratio

$$S/P = \frac{OD_{Sample} - OD_{NC}}{OD_{PC} - OD_{NC}}$$

- HerdCheck Swine Salmonella

$$OD\% = \frac{S/P}{2.5} * 100\%$$

- Salmotype PigScreen

$$OD\% = S/P * 72.1$$

- In-house ELISA's
  - Reference sera, regression model

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Duplicate analyses of meat juice samples collected during the baseline study on fattening pigs

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Materials and Methods

- Selection of 60 meat juice samples with different OD%
- 120 µl meat juice sample send to the CRL-*Salmonella*
- Samples were collected by the CRL and stored at -20°C
- Samples were send to the Animal Health Service (GD) in the Netherlands
- GD analysed all samples with HerdCheck Swine *Salmonella*
- Comparison results GD with NRLs

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

S/P ratio	5
<0.1	14
0.1-0.2	12
0.2-0.3	6
0.3-0.4	4
0.4-0.5	1
>0.5	23
total	60

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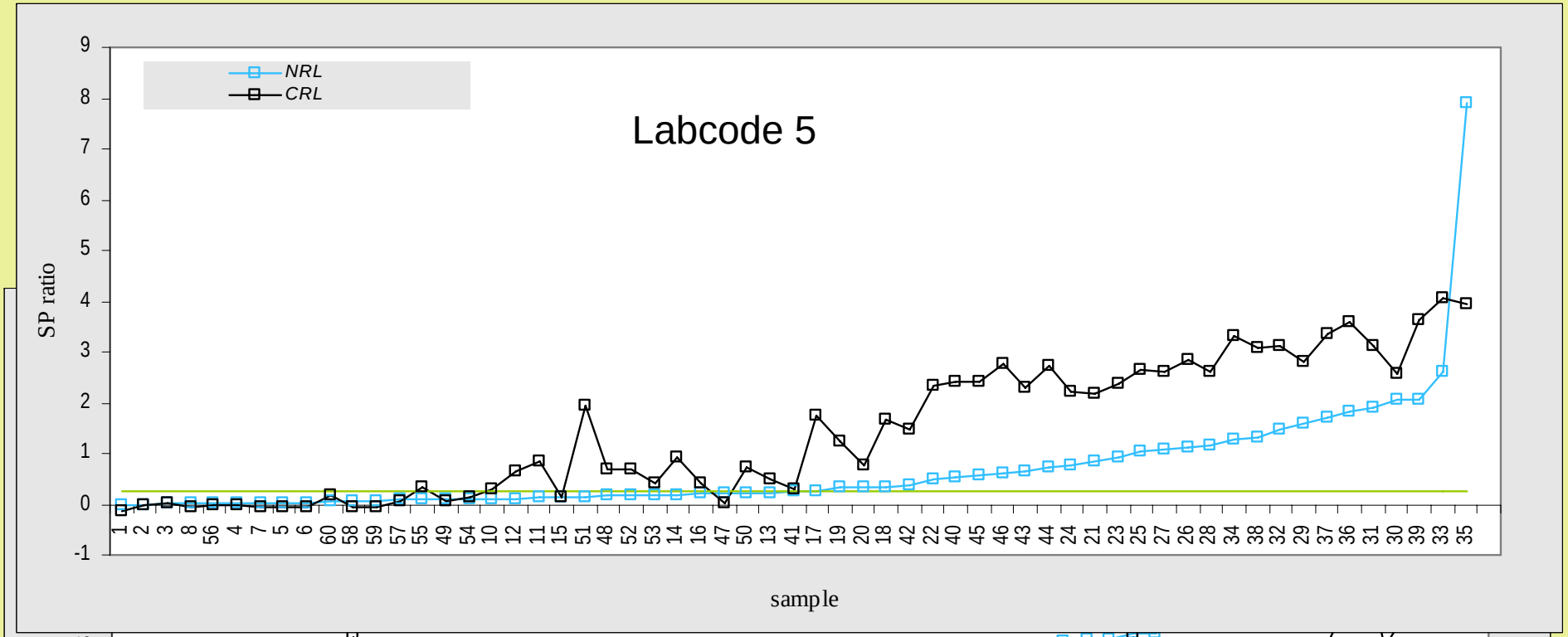
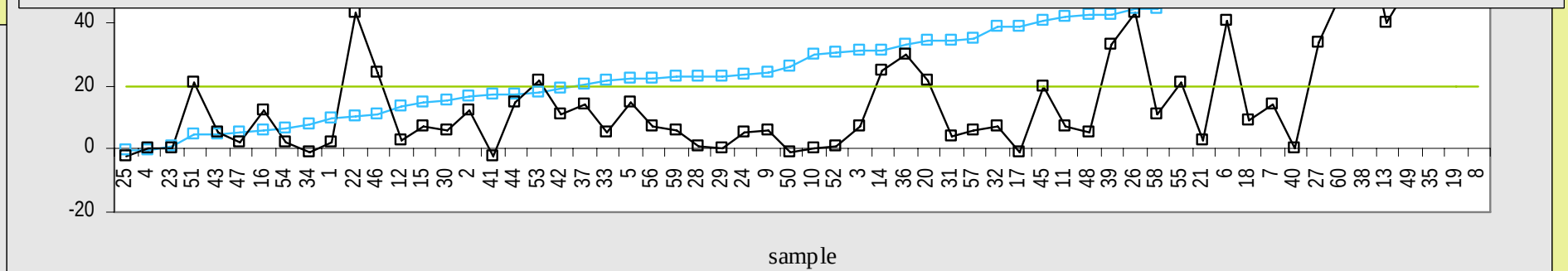

NRL
CRL
Labcode 5
SP ratio
sample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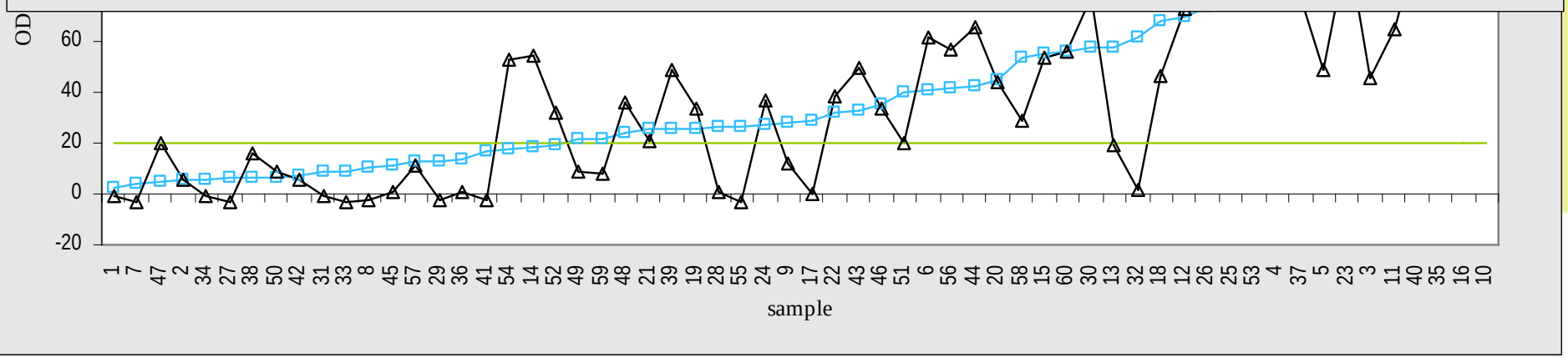
sample

Dependent t-test

Labcode	ELISA	Average OD%		P value
		NRL	CRL*	
1	Salmotype PigScreen	42.84	37.43	0.047
2	Salmotype PigScreen	31.41	18.13	$1.31*10^{-7}$
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	$1.4*10^{-7}$
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

* CRL used HerdCheck Swine ELISA

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Interlaboratory comparison study on serological methods

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CRL-*Salmonella*

Materials and Methods

- 40 sera were sent to the NRLs for serological analysis
  - 2x *Salmonella* negative pigs
  - 2x pigs inoculated with *Yersinia enterocolitica* O3-/O9-
  - 36 pigs inoculated with *Salmonella* spp.

- 4x *S.* Brandenburg (group B)
- 24x *S.* Typhimurium (group B)
- 2x *S.* Livingstone (group C1)
- 4x *S.* Goldcoast (group C2)
- 2x *S.* Panama (group D)

- All NRLs interpreted their results using a cut-off value used routinely

Results specificity panel

No.	Description	exp	participant 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	-	-	-	-	-	-	-	-	-	-	±

No.	Description	participant 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

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Results serum panel (1)

No.	Description	exp	participant 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-4	*S*. Livingstone	+	-	-	-	+	-	-	-	±	-	+
S-10	*S*. Livingstone	+	+	+	+	+	+	+	-	±	+	+
S-20	*S*. Panama	+	-	-	-	-	-	-	-	-	-	+
S-37	*S*. Panama	+	+	+	+	+	±	+	-	+	+	+

No.	Description	participant 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-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

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Results serum panel (2)

			participant									
No.	**Description**	**exp**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
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	+	+	±	+	+	±	+	-	±	+	+

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Results serum panel (3)

No.	Description	participant 1	2	3	4	5*	6	7	8	9	10
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

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Analysis qualitative results

	Labcode									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
number negatives	8	8	7	8	8	8	8	8	8	1
total negatives	8	8	8	8	8	8	8	8	8	8
Specificity	**100%**	**100%**	**88%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**13%**
number positives	23	19	23	29	16	22	8	17	17	31
total positives	32	32	32	32	32	32	32	32	32	32
Sensitivity	**72%**	**59%**	**72%**	**91%**	**50%**	**69%**	**25%**	**53%**	**53%**	**97%**
number correct	31	27	30	37	24	30	16	25	25	32
total	40	40	40	40	40	40	40	40	40	40
accuracy	**78%**	**68%**	**75%**	**93%**	**60%**	**75%**	**40%**	**63%**	**63%**	**80%**

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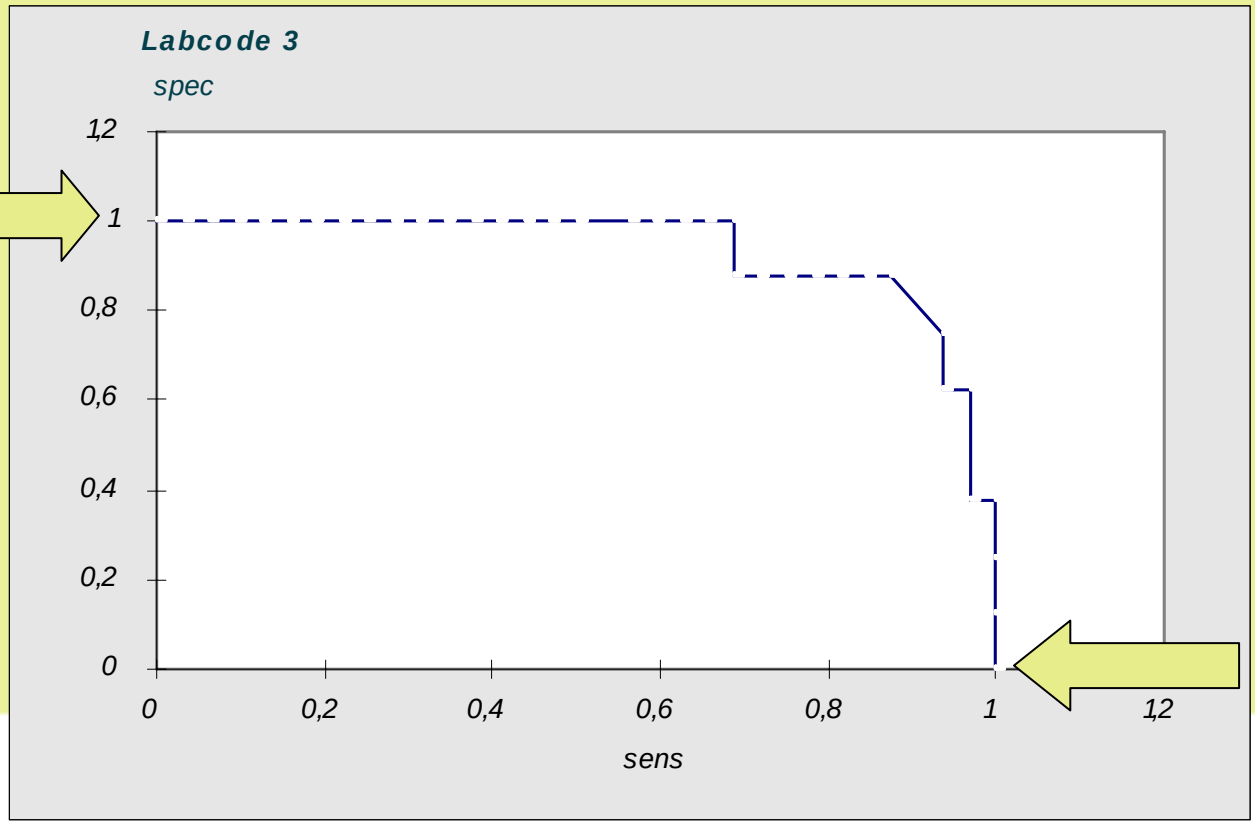
Analysis quantitative Results

- Receiver Operating Characteristics (ROC) plots
  - Sensitivity plotted against the specificity at cut-off's for the whole range of the test
  - Area below the curve is proportional to the accuracy of the test
    - 0.5 = random
    - 1 = perfect

High cut-off



Low cut-off

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labcode	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

All NRLs performed very good and all tests are able to detect the true status of the sample, however at different cut-off values

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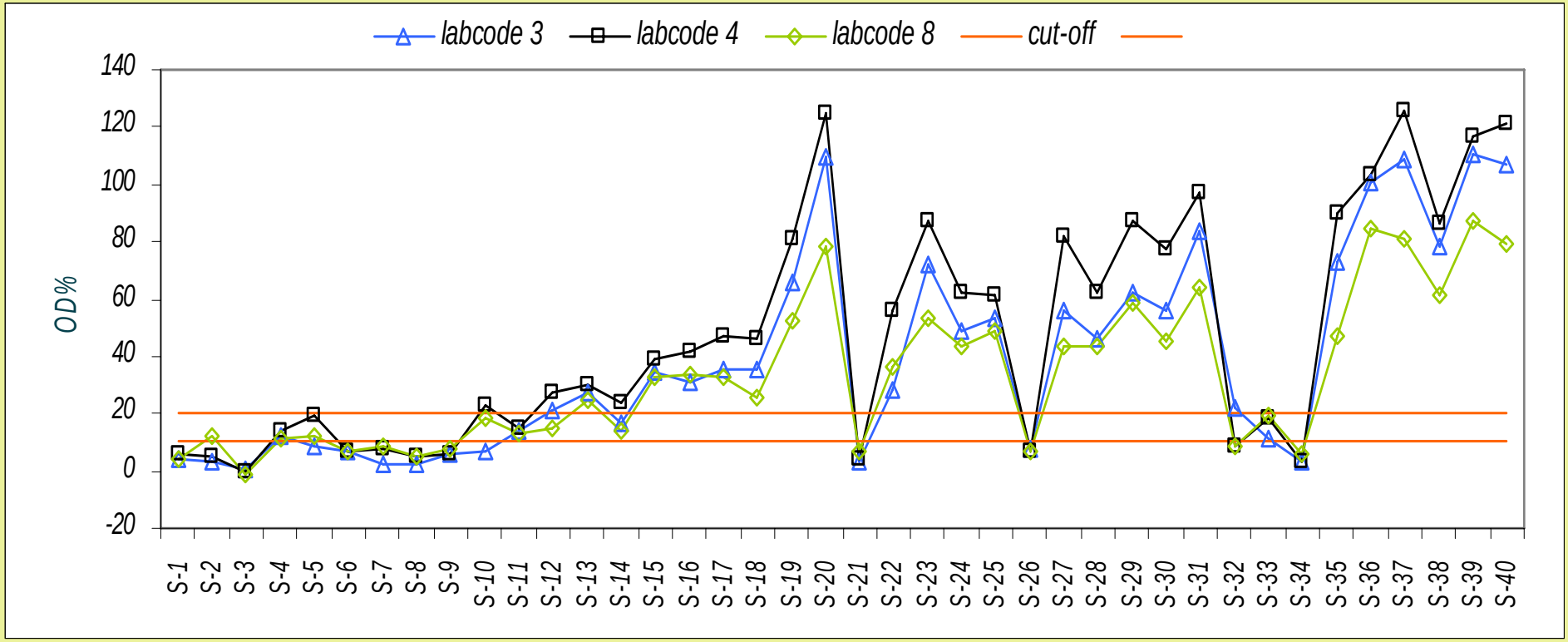
Results per ELISA method (1)

- HerdCheck Swine Salmonella ELISA from IDEXX



Cut-off = 10

Sensitivity: 89%

Specificity: 92%

Accuracy: 89%

Cut-off = 20

Sensitivity: 74%

Specificity: 96%

Accuracy: 78%

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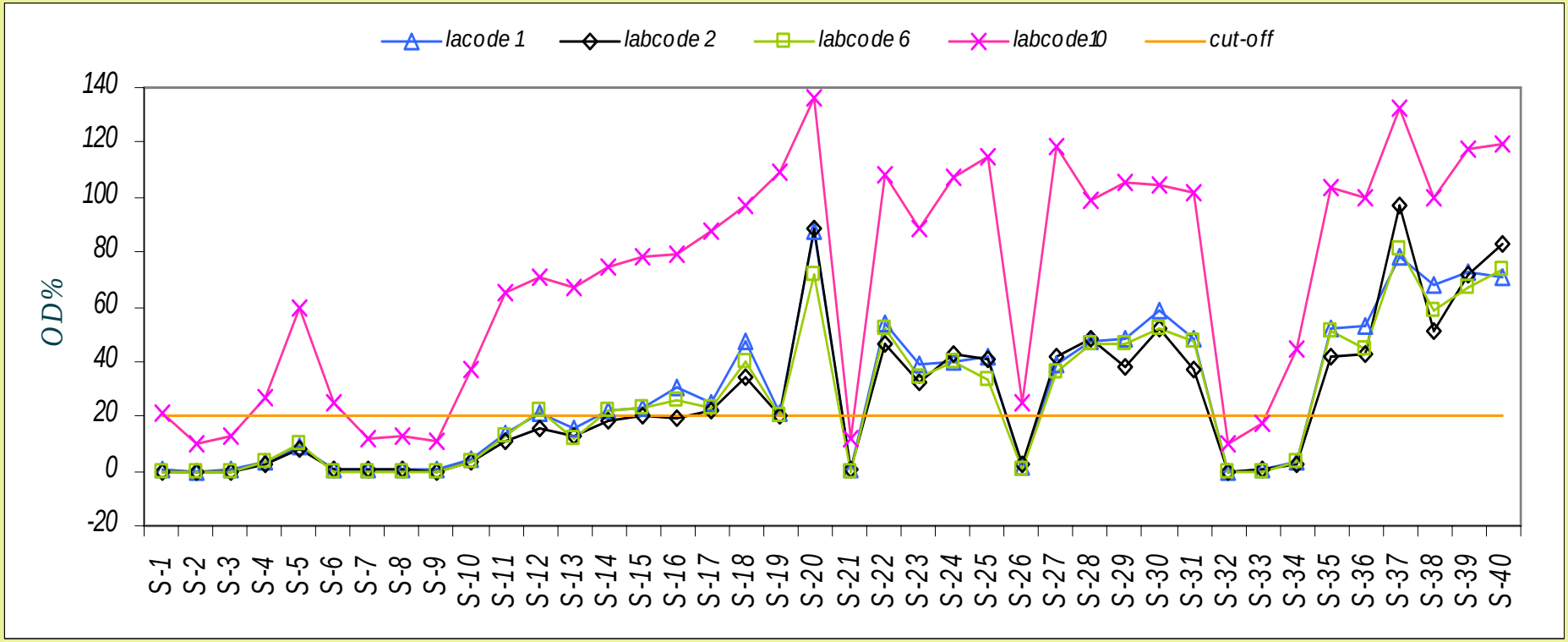
Results per ELISA method (2)

- Salmotype PigScreen from Labor Diagnostik Leipzig



Cut-off = 10

Sensitivity: 84%

Specificity: 75%

Accuracy: 83%

Cut-off = 20

Sensitivity: 77%

Specificity: 97%

Accuracy: 81%

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Conclusions

- General
  - Different ELISA's were used by different NRLs
  - Different cut-off values are used

- Duplicate analysis study
  - In the duplicate analysis study the serological results of the selected meat juice samples of 9 out of 10 NRLs is different from that of the CRL

  - Meatjuice is not homogeneous material
  - Differences in matrix because of extra thaw-freeze step
  - Inexperience of some laboratories with meatjuice

Conclusions

- Interlaboratory comparison study
  - Quantitative ROC analysis indicated that all NRLs performed very good and all tests are able to detect the true status of the sample, however at different cut-off values
  - The NRL with labcode 7 had found the most negative samples, this is due to the high cut-off value used by this NRL (OD % > 40)
  - The OD% of the NRL with labcode 10 is in general higher than that of other NRLs in both studies
  - ELISA methods were comparable between different NRLs using serum samples