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172-TEST

(ISO 43-1)

## **PROFICIENCY-TESTING 2007**

*Salmonella* isolation

**COORDINATION CENTRE FOR VETERINARY DIAGNOSTICS**

**DATE ILT: 26-27 March 2007**

**DATE REPORT: 11 May 2007**

## I. Introduction

Details relevant to the proficiency-test are available in the Procedure PRO/6.5/01 'Beheer van de interlaboratoriumproeven / Gestion des essais interlaboratoires'.

## II. Aim

This proficiency-test, which is focusing on the isolation of *Salmonella*, aims to assess the analytical accuracy of the corresponding tests conducted by the participants.

## III. Material and methods

### III.1. Conduct of diagnostic tests

In the framework of this proficiency-test, reference faeces samples for *Salmonella* must be tested by means of isolation tests. The procedure for these tests must be fully described in the SOPs of the participating laboratories.

### III.2. Reference samples

Reference faeces samples for *Salmonella* originated from 1 bovine animal were used in this proficiency-test. Each Reference faeces samples consisted of 10 gram faeces/aliquot. Before the proficiency-test, forty of these aliquots were tested on 4 days by the reference laboratory, and all were found negative for *Salmonella*.

Seventy aliquots were used as such in the proficiency-test and were considered as negative samples (ILT07-SALBACNF1).

Thirty five aliquots were inoculated with 70 µl of a *Salmonella* Enteritidis (ATCC 13076) culture dilution  $10^{-5}$  (=140 cfu/70µl) and were considered as strong positive samples (ILT07-SALBACPF1). *Salmonella* Enteritidis ATCC 13076 is chosen as reference strain since it is commercially available and is used by the reference laboratory in first line controls to test the fertility of the growth media. However, strain ATCC 13076 have the disadvantage that it is a not mobile *Salmonella* strain.

Thirty five aliquots were inoculated with 70 µl of a *Salmonella* Enteritidis (ATCC 13076) culture dilution  $10^{-6}$  (=14 cfu/70µl) were considered as weak positive samples (ILT07-SALBACPF2).

On the 26/03/2007 the 10x ILT07-SALBACNF1, 5x ILT07-SALBACPF1 and 5x ILT07-SALBACPF2 were sent out to each of the participating laboratories. The same and the following day, the reference laboratory tested similar positive and negative samples 10 times to validate and verify the samples in order to classify the samples to their status. Validated results were issued on 30th March and 2nd April 2007. The reference faeces samples encoded 'ILT07-SALBACNF1' scored 10 times 'Negative'. The reference faeces sample encoded 'ILT07-SALBACPF1' and 'ILT07-SALBACPF2' scored each 10 times 'Positive'.

Consequently, the negative and positive (strong and weak) reference faeces were considered as reliable samples to evaluate the absence (ILT07-SALBACNF1) or presence (ILT07-SALBACPF1 and ILT07-SALBACPF2) of *Salmonella* in faeces.

### **III.3. Classification of results, levels 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 (=presence of *Salmonella*), negative result when the reference sample is truly negative (=absence of *Salmonella*) or *failure* (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive).

#### *III.3.2. Levels of agreement*

The levels of agreement achieved by the participating laboratories are expressed as the percentages of successes for all 20 samples carried out for this proficiency-test.

#### *III.3.3. Threshold for qualification*

Following the procedure, a participating laboratory is only qualified if the level of agreement is at least 90%.

## **IV. Results**

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Coordination Centre for Veterinary Diagnostics (CCVD) of the Veterinary and Agrochemical Research Centre (VAR).

### **IV.1. Reference samples**

#### *IV.1.1. Allocation of samples to participating laboratories*

All participating laboratories were given:

- i. 10 aliquots of reference faeces samples ILT07-SALBACNF1,
- ii. 5 aliquots of reference faeces samples ILT07-SALBACPF1,
- iii. 5 aliquots of reference faeces samples ILT07-SALBACPF2.

#### *IV.1.2. Transfer and start of the analyses*

The 20 aliquots of reference faeces samples were sent on 26 March 2007 to each of the seven participating laboratories (140 aliquots in total). The seven laboratories acknowledged receipt of the samples on the same day. The analyses were started on 26 (LAB1, LAB3, LAB4, and LAB5), and 27 (LAB2, LAB6, and LAB7) March 2007.

### **IV.2. Dates at which results were returned to the CCVD**

Results from participating laboratories have been received on 30 (LAB4) March and on 3 (LAB2), 5 (LAB5, and LAB6), 6 (LAB7), and 10 (LAB1, and LAB3) April 2007.

### IV.3. Compliance with the procedure

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

### IV.4. Levels of agreement

Six participating laboratories have reached 100% of agreement for the isolation of *Salmonella*. One participating laboratory reached only 50% of agreement for the isolation of *Salmonella* (Table 1).

**Table 1.** Agreement between results generated by the participating laboratories based on the status of samples.

Success while screening the samples (0 = Failure, 1 = Success)							
Variable	LABNR						
	1 (N=20)	2 (N=20)	3 (N=20)	4 (N=20)	5 (N=20)	6 (N=20)	7 (N=20)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
<b>0</b>	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)	10 (50.0)	0 ( 0.0)	0 ( 0.0)
<b>1</b>	20 (100.)	20 (100.)	20 (100.)	20 (100.)	10 (50.0)	20 (100.)	20 (100.)

### IV.5. Variability among participating laboratories

The responses of the seven participating laboratories that provided their results for *Salmonella* reference faeces samples are displayed in Table 2.

**Table 2.** The responses (RESULT) of the participating laboratories (LABNR) with the identification (SAMPLE) of the reference faeces samples, the position/identification (LABPOSIT) of the reference faeces samples, and the result (STATUS) obtained by repeated screening.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	ILT07-SALBACNF1	NEG	NEG	1
2	1	2	ILT07-SALBACPF2	POS	POS	1
3	1	3	ILT07-SALBACPF2	POS	POS	1
4	1	4	ILT07-SALBACPF1	POS	POS	1
5	1	5	ILT07-SALBACNF1	NEG	NEG	1
6	1	6	ILT07-SALBACNF1	NEG	NEG	1
7	1	7	ILT07-SALBACNF1	NEG	NEG	1
8	1	8	ILT07-SALBACPF2	POS	POS	1
9	1	9	ILT07-SALBACNF1	NEG	NEG	1
10	1	10	ILT07-SALBACPF2	POS	POS	1
11	1	11	ILT07-SALBACPF1	POS	POS	1
12	1	12	ILT07-SALBACPF2	POS	POS	1
13	1	13	ILT07-SALBACNF1	NEG	NEG	1
14	1	14	ILT07-SALBACNF1	NEG	NEG	1
15	1	15	ILT07-SALBACPF1	POS	POS	1
16	1	16	ILT07-SALBACNF1	NEG	NEG	1
17	1	17	ILT07-SALBACNF1	NEG	NEG	1
18	1	18	ILT07-SALBACNF1	NEG	NEG	1
19	1	19	ILT07-SALBACPF1	POS	POS	1
20	1	20	ILT07-SALBACPF1	POS	POS	1



	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	2	1	ILT07-SALBACNF1	NEG	NEG	1
22	2	2	ILT07-SALBACNF1	NEG	NEG	1
23	2	3	ILT07-SALBACPF1	POS	POS	1
24	2	4	ILT07-SALBACNF1	NEG	NEG	1
25	2	5	ILT07-SALBACPF1	POS	POS	1
26	2	6	ILT07-SALBACNF1	NEG	NEG	1
27	2	7	ILT07-SALBACPF2	POS	POS	1
28	2	8	ILT07-SALBACPF2	POS	POS	1
29	2	9	ILT07-SALBACPF1	POS	POS	1
30	2	10	ILT07-SALBACNF1	NEG	NEG	1
31	2	11	ILT07-SALBACNF1	NEG	NEG	1
32	2	12	ILT07-SALBACNF1	NEG	NEG	1
33	2	13	ILT07-SALBACPF2	POS	POS	1
34	2	14	ILT07-SALBACNF1	NEG	NEG	1
35	2	15	ILT07-SALBACPF2	POS	POS	1
36	2	16	ILT07-SALBACPF1	POS	POS	1
37	2	17	ILT07-SALBACPF2	POS	POS	1
38	2	18	ILT07-SALBACNF1	NEG	NEG	1
39	2	19	ILT07-SALBACNF1	NEG	NEG	1
40	2	20	ILT07-SALBACPF1	POS	POS	1



	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	ILT07-SALBACPF1	POS	POS	1
42	3	2	ILT07-SALBACPF2	POS	POS	1
43	3	3	ILT07-SALBACNF1	NEG	NEG	1
44	3	4	ILT07-SALBACNF1	NEG	NEG	1
45	3	5	ILT07-SALBACPF1	POS	POS	1
46	3	6	ILT07-SALBACNF1	NEG	NEG	1
47	3	7	ILT07-SALBACNF1	NEG	NEG	1
48	3	8	ILT07-SALBACPF1	POS	POS	1
49	3	9	ILT07-SALBACNF1	NEG	NEG	1
50	3	10	ILT07-SALBACPF1	POS	POS	1
51	3	11	ILT07-SALBACNF1	NEG	NEG	1
52	3	12	ILT07-SALBACPF2	POS	POS	1
53	3	13	ILT07-SALBACPF2	POS	POS	1
54	3	14	ILT07-SALBACPF1	POS	POS	1
55	3	15	ILT07-SALBACNF1	NEG	NEG	1
56	3	16	ILT07-SALBACNF1	NEG	NEG	1
57	3	17	ILT07-SALBACNF1	NEG	NEG	1
58	3	18	ILT07-SALBACPF2	POS	POS	1
59	3	19	ILT07-SALBACNF1	NEG	NEG	1
60	3	20	ILT07-SALBACPF2	POS	POS	1



	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	4	1	ILT07-SALBACNF1	NEG	NEG	1
62	4	2	ILT07-SALBACNF1	NEG	NEG	1
63	4	3	ILT07-SALBACPF2	POS	POS	1
64	4	4	ILT07-SALBACNF1	NEG	NEG	1
65	4	5	ILT07-SALBACPF2	POS	POS	1
66	4	6	ILT07-SALBACPF1	POS	POS	1
67	4	7	ILT07-SALBACPF2	POS	POS	1
68	4	8	ILT07-SALBACNF1	NEG	NEG	1
69	4	9	ILT07-SALBACNF1	NEG	NEG	1
70	4	10	ILT07-SALBACPF1	POS	POS	1
71	4	11	ILT07-SALBACNF1	NEG	NEG	1
72	4	12	ILT07-SALBACNF1	NEG	NEG	1
73	4	13	ILT07-SALBACPF1	POS	POS	1
74	4	14	ILT07-SALBACNF1	NEG	NEG	1
75	4	15	ILT07-SALBACPF1	POS	POS	1
76	4	16	ILT07-SALBACNF1	NEG	NEG	1
77	4	17	ILT07-SALBACPF2	POS	POS	1
78	4	18	ILT07-SALBACPF2	POS	POS	1
79	4	19	ILT07-SALBACPF1	POS	POS	1
80	4	20	ILT07-SALBACNF1	NEG	NEG	1



	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	ILT07-SALBACPF1	<u>POS</u>	<u>NEG</u>	<u>0</u>
82	5	2	ILT07-SALBACNF1	NEG	NEG	1
83	5	3	ILT07-SALBACPF1	<u>POS</u>	<u>NEG</u>	<u>0</u>
84	5	4	ILT07-SALBACNF1	NEG	NEG	1
85	5	5	ILT07-SALBACPF1	<u>POS</u>	<u>NEG</u>	<u>0</u>
86	5	6	ILT07-SALBACNF1	NEG	NEG	1
87	5	7	ILT07-SALBACPF1	<u>POS</u>	<u>NEG</u>	<u>0</u>
88	5	8	ILT07-SALBACNF1	NEG	NEG	1
89	5	9	ILT07-SALBACPF1	<u>POS</u>	<u>NEG</u>	<u>0</u>
90	5	10	ILT07-SALBACNF1	NEG	NEG	1
91	5	11	ILT07-SALBACPF2	<u>POS</u>	<u>NEG</u>	<u>0</u>
92	5	12	ILT07-SALBACPF2	<u>POS</u>	<u>NEG</u>	<u>0</u>
93	5	13	ILT07-SALBACPF2	<u>POS</u>	<u>NEG</u>	<u>0</u>
94	5	14	ILT07-SALBACNF1	NEG	NEG	1
95	5	15	ILT07-SALBACNF1	NEG	NEG	1
96	5	16	ILT07-SALBACNF1	NEG	NEG	1
97	5	17	ILT07-SALBACNF1	NEG	NEG	1
98	5	18	ILT07-SALBACPF2	<u>POS</u>	<u>NEG</u>	<u>0</u>
99	5	19	ILT07-SALBACNF1	NEG	NEG	1
100	5	20	ILT07-SALBACPF2	<u>POS</u>	<u>NEG</u>	<u>0</u>



	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
101	6	1	ILT07-SALBACNF1	NEG	NEG	1
102	6	2	ILT07-SALBACNF1	NEG	NEG	1
103	6	3	ILT07-SALBACPF1	POS	POS	1
104	6	4	ILT07-SALBACNF1	NEG	NEG	1
105	6	5	ILT07-SALBACPF1	POS	POS	1
106	6	6	ILT07-SALBACNF1	NEG	NEG	1
107	6	7	ILT07-SALBACPF2	POS	POS	1
108	6	8	ILT07-SALBACPF2	POS	POS	1
109	6	9	ILT07-SALBACPF1	POS	POS	1
110	6	10	ILT07-SALBACNF1	NEG	NEG	1
111	6	11	ILT07-SALBACNF1	NEG	NEG	1
112	6	12	ILT07-SALBACNF1	NEG	NEG	1
113	6	13	ILT07-SALBACPF2	POS	POS	1
114	6	14	ILT07-SALBACNF1	NEG	NEG	1
115	6	15	ILT07-SALBACPF2	POS	POS	1
116	6	16	ILT07-SALBACPF1	POS	POS	1
117	6	17	ILT07-SALBACPF2	POS	POS	1
118	6	18	ILT07-SALBACNF1	NEG	NEG	1
119	6	19	ILT07-SALBACNF1	NEG	NEG	1
120	6	20	ILT07-SALBACPF1	POS	POS	1

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	ILT07-SALBACPF1	POS	POS	1
122	7	2	ILT07-SALBACPF2	POS	POS	1
123	7	3	ILT07-SALBACNF1	NEG	NEG	1
124	7	4	ILT07-SALBACNF1	NEG	NEG	1
125	7	5	ILT07-SALBACPF1	POS	POS	1
126	7	6	ILT07-SALBACNF1	NEG	NEG	1
127	7	7	ILT07-SALBACNF1	NEG	NEG	1
128	7	8	ILT07-SALBACPF1	POS	POS	1
129	7	9	ILT07-SALBACNF1	NEG	NEG	1
130	7	10	ILT07-SALBACPF1	POS	POS	1
131	7	11	ILT07-SALBACNF1	NEG	NEG	1
132	7	12	ILT07-SALBACPF2	POS	POS	1
133	7	13	ILT07-SALBACPF2	POS	POS	1
134	7	14	ILT07-SALBACPF1	POS	POS	1
135	7	15	ILT07-SALBACNF1	NEG	NEG	1
136	7	16	ILT07-SALBACNF1	NEG	NEG	1
137	7	17	ILT07-SALBACNF1	NEG	NEG	1
138	7	18	ILT07-SALBACPF2	POS	POS	1
139	7	19	ILT07-SALBACNF1	NEG	NEG	1
140	7	20	ILT07-SALBACPF2	POS	POS	1

## V. Discussion

The purpose of this proficiency-test was to assess performances of participating laboratories to isolate *Salmonella* from matrices with a high amount of interfering flora, such as faeces samples.

Laboratories participating to this proficiency-test all received 10 samples that were not inoculated with *Salmonella* Enteritidis and 10 samples that were inoculated with either a  $10^{-5}$  or a  $10^{-6}$  culture dilution of *Salmonella* Enteritidis corresponding with 140 and 14 cfu/70  $\mu$ l respectively.

Six participating laboratories provided responses that were in full agreement with the true status of the reference faeces samples. One participating laboratory reached only 50% of agreement. This laboratory described in its procedure for isolation of *Salmonella* spp. the use of only one selective enrichment medium i.e. modified semisolid Rappaport Vassiliadis (MSRV) while all the other laboratories used at least two different enrichment media (the semisolid MSRV and the liquid RVS (Rappaport Vassiliadis Soja) medium) or two liquid enrichment media (RVS and MKTT (Muller-Kauffmann tetrathionate). MSRV only allows the detection of motile *Salmonella* strains whereas RVS and MKTT allow the detection of both motile and non-motile *Salmonella* spp.

The reason for the non-detection of *Salmonella* by one laboratory for the ILT07-SALBACPF1 and ILT07-SALBACPF2 aliquots can be explained by the use of only the MSRV enrichment medium which was unable to detect the non-motile *Salmonella* Enteritidis strain (ATCC 13076) used for spiking the aliquots.

## VI. Conclusions

According to the procedure currently in force, the performances of a participating laboratory are satisfactory if at least 90% of the results provided by this laboratory are in agreement with the status of the reference faeces samples (Section III.3.3. of this Report). Consequently, only six participating laboratories achieved a satisfactory performance.

The Committee

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