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**172-TEST**

**(ISO 43-1)**

## **Inter-laboratory Trials 2006**

***Enzootic Bovine Leucosis***  
***Detection of EBL-specific antibodies in milk***

**COORDINATION CENTRE FOR VETERINARY DIAGNOSTICS**

**DATE ILT: 20-21 NOVEMBER 2006**

**DATE REPORT: 20 DECEMBER 2006**

## I. Introduction

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

## II. Aim

This inter-laboratory trial, which is focusing on the detection of Enzootic Bovine Leucosis-specific antibodies in tank milk aims to assess the analytical accuracy of tests conducted by participants.

## III. Material and methods

### III.1. Conduct of diagnostic tests

In the framework of this inter-laboratory trial, reference milk samples containing or free from specific antibodies against Enzootic Bovine Leucosis must be tested by means of an ELISA test. The procedure for these tests must be fully described in the SOPs of the participating laboratories

### III.2. Reference samples

Reference samples for Enzootic Bovine Leucosis were used in this inter-laboratory trial.

Replicates of nine reference milks either free from Enzootic Bovine Leucosis-specific detectable antibodies ( $n = 4$ ; coded 'ILT06-EBLNM1', 'ILT06-EBLNM2', 'ILT06-EBLNM3', and 'ILT06-EBLNM4') or containing Enzootic Bovine Leucosis-specific detectable antibodies ( $n = 5$ ; coded 'ILT06-EBLPM1', 'ILT06-EBLPM2', 'ILT06-EBLPM3', 'ILT06-EBLPM4', and 'ILT06-EBLPM7') were used and aliquots were sent to the participating laboratories. These nine reference milks were tested 10 times by the reference laboratory of the Veterinary and Agrochemical Research Centre (VAR). Validated results were issued between 18 October 2006 and 7 November 2006, i.e., before the start of the inter-laboratory trial. On all occasions, the reference milks encoded 'ILT06-EBLNM1', 'ILT06-EBLNM2', 'ILT06-EBLNM3', and 'ILT06-EBLNM4' scored 'Negative' (absence of detectable antibodies against Enzootic Bovine Leucosis) and the reference milks encoded 'ILT06-EBLPM1', 'ILT06-EBLPM2', 'ILT06-EBLPM3', 'ILT06-EBLPM4', and 'ILT06-EBLPM7' scored 'Positive' (presence of detectable antibodies against Enzootic Bovine Leucosis). These reference milks were thus considered as reliable samples to evaluate the absence or presence of specific antibodies against Enzootic Bovine Leucosis in tank milk of bovine origin.

### 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, negative result when the reference sample is truly negative, non-interpretable result when the reference sample is truly non-interpretable) or *failure* (positive result when the reference sample is truly negative or non-interpretable, negative result when the reference sample is truly positive or non-interpretable, non-interpretable result when the reference sample is truly negative or positive).

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

The levels of agreement achieved by the participating laboratories are expressed as the percentages of successes among all tests carried out for this trial.

### *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. 36 aliquots of reference milk samples free from Enzootic Bovine Leucosis-specific detectable antibodies: ILT06-EBLNM1 samples (n = 9), ILT06-EBLNM2 samples (n = 9), ILT06-EBLNM3 samples (n = 9), and ILT06-EBLNM4 samples (n = 9)
- ii. 44 aliquots of reference milk samples containing Enzootic Bovine Leucosis-specific detectable antibodies: ILT06-EBLPM1 samples (n = 9), ILT06-EBLPM2 samples (n = 9), ILT06-EBLPM3 samples (n = 9), ILT06-EBLPM4 samples (n = 9), and ILT06-EBLPM7 samples (n = 8)

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

All 80 aliquots of reference samples were sent on 20 November 2006 to each of the four participating laboratories (320 aliquots in total). The four laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 20 (LAB2) and 22 (LAB1, LAB3 and LAB4) November 2006.

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

Results from participating laboratories have been received on 23 (LAB1) and 24 (LAB2, LAB3, and LAB4) November 2006.

### ***IV.3. Compliance with the procedure***

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

Three participating laboratories have reached 100% of agreement for the detection of Enzootic Bovine Leucosis-specific detectable antibodies in reference milks. One participating laboratory reached only 90.0% of agreement for the detection of Enzootic Bovine Leucosis-specific detectable antibodies in reference milks (Table 1).

**Table 1.** Outcome of the assessment of Enzootic Bovine Leucosis reference milk samples: Agreement between results generated by the participating laboratories (LABNR) and the status of reference samples as determined by repeated screening of samples free from Enzootic Bovine Leucosis-specific detectable antibodies ('ILT06-EBLNM1', 'ILT06-EBLNM2', 'ILT06-EBLNM3', and 'ILT06-EBLNM4') or containing Enzootic Bovine Leucosis-specific detectable antibodies ('ILT06-EBLPM1', 'ILT06-EBLPM2', 'ILT06-EBLPM3', 'ILT06-EBLPM4', and 'ILT06-EBLPM7'). The purpose of the inter-laboratory trial is to determine the presence/absence of Enzootic Bovine Leucosis-specific detectable antibodies in reference milks.

Success while screening the samples (0 = Failure, 1 = Success)				
Variable	LABNR			
	1 (N=80)	2 (N=80)	3 (N=80)	4 (N=80)
	N (%)	N (%)	N (%)	N (%)
0	8 (10.0)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)
1	72 (90.0)	80 (100.0)	80 (100.0)	80 (100.0)

#### ***IV.5. Variability among participating laboratories***

The responses of the four participating laboratories that provided their results for Enzootic Bovine Leucosis reference milks are displayed in Table 2.

**Table 2.** The responses (RESULT) of the participating laboratories (LABNR) with the identification (SAMPLE) of the reference milk samples, the position (LABPOSIT) of the reference milk samples in the block, and the results (STATUS) obtained by repeated screening. Reference samples include samples free from Enzootic Bovine Leucosis-specific detectable antibodies ('ILT06-EBLNM1', 'ILT06-EBLNM2', 'ILT06-EBLNM3', and 'ILT06-EBLNM4') or containing Enzootic Bovine Leucosis-specific detectable antibodies ('ILT06-EBLPM1', 'ILT06-EBLPM2', 'ILT06-EBLPM3', 'ILT06-EBLPM4', and 'ILT06-EBLPM7').

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	ILT06-EBLPM2	POS	POS	1
2	1	2	ILT06-EBLNM4	NEG	NEG	1
3	1	3	ILT06-EBLNM2	NEG	NEG	1
4	1	4	ILT06-EBLPM1	POS	POS	1
5	1	5	ILT06-EBLNM1	NEG	NEG	1
6	1	6	ILT06-EBLPM7	<b>POS</b>	<b>NI</b>	<b>0</b>
7	1	7	ILT06-EBLPM3	POS	POS	1
8	1	8	ILT06-EBLNM1	NEG	NEG	1
9	1	9	ILT06-EBLNM4	NEG	NEG	1
10	1	10	ILT06-EBLPM7	<b>POS</b>	<b>NI</b>	<b>0</b>
11	1	11	ILT06-EBLPM4	POS	POS	1
12	1	12	ILT06-EBLNM3	NEG	NEG	1
13	1	13	ILT06-EBLPM1	POS	POS	1
14	1	14	ILT06-EBLPM2	POS	POS	1
15	1	15	ILT06-EBLPM3	POS	POS	1
16	1	16	ILT06-EBLPM2	POS	POS	1
17	1	17	ILT06-EBLNM1	NEG	NEG	1
18	1	18	ILT06-EBLNM1	NEG	NEG	1
19	1	19	ILT06-EBLNM4	NEG	NEG	1
20	1	20	ILT06-EBLPM3	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	1	21	ILT06-EBLPM3	POS	POS	1
22	1	22	ILT06-EBLPM1	POS	POS	1
23	1	23	ILT06-EBLNM3	NEG	NEG	1
24	1	24	ILT06-EBLPM4	POS	POS	1
25	1	25	ILT06-EBLNM2	NEG	NEG	1
26	1	26	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
27	1	27	ILT06-EBLPM2	POS	POS	1
28	1	28	ILT06-EBLNM2	NEG	NEG	1
29	1	29	ILT06-EBLNM3	NEG	NEG	1
30	1	30	ILT06-EBLPM1	POS	POS	1
31	1	31	ILT06-EBLNM1	NEG	NEG	1
32	1	32	ILT06-EBLNM4	NEG	NEG	1
33	1	33	ILT06-EBLPM4	POS	POS	1
34	1	34	ILT06-EBLNM1	NEG	NEG	1
35	1	35	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
36	1	36	ILT06-EBLPM3	POS	POS	1
37	1	37	ILT06-EBLNM1	NEG	NEG	1
38	1	38	ILT06-EBLNM4	NEG	NEG	1
39	1	39	ILT06-EBLNM3	NEG	NEG	1
40	1	40	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
41	1	41	ILT06-EBLPM1	POS	POS	1
42	1	42	ILT06-EBLNM2	NEG	NEG	1
43	1	43	ILT06-EBLPM2	POS	POS	1
44	1	44	ILT06-EBLNM4	NEG	NEG	1
45	1	45	ILT06-EBLPM3	POS	POS	1
46	1	46	ILT06-EBLPM4	POS	POS	1
47	1	47	ILT06-EBLNM1	NEG	NEG	1
48	1	48	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
49	1	49	ILT06-EBLNM3	NEG	NEG	1
50	1	50	ILT06-EBLPM4	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
51	1	51	ILT06-EBLPM1	POS	POS	1
52	1	52	ILT06-EBLPM2	POS	POS	1
53	1	53	ILT06-EBLPM4	POS	POS	1
54	1	54	ILT06-EBLPM3	POS	POS	1
55	1	55	ILT06-EBLNM2	NEG	NEG	1
56	1	56	ILT06-EBLPM1	POS	POS	1
57	1	57	ILT06-EBLNM3	NEG	NEG	1
58	1	58	ILT06-EBLNM2	NEG	NEG	1
59	1	59	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
60	1	60	ILT06-EBLNM4	NEG	NEG	1
61	1	61	ILT06-EBLNM3	NEG	NEG	1
62	1	62	ILT06-EBLPM2	POS	POS	1
63	1	63	ILT06-EBLNM2	NEG	NEG	1
64	1	64	ILT06-EBLPM3	POS	POS	1
65	1	65	ILT06-EBLPM1	POS	POS	1
66	1	66	ILT06-EBLNM4	NEG	NEG	1
67	1	67	ILT06-EBLPM2	POS	POS	1
68	1	68	ILT06-EBLNM2	NEG	NEG	1
69	1	69	ILT06-EBLPM4	POS	POS	1
70	1	70	ILT06-EBLNM3	NEG	NEG	1
71	1	71	ILT06-EBLPM3	POS	POS	1
72	1	72	ILT06-EBLNM4	NEG	NEG	1
73	1	73	ILT06-EBLNM1	NEG	NEG	1
74	1	74	ILT06-EBLPM2	POS	POS	1
75	1	75	ILT06-EBLPM4	POS	POS	1
76	1	76	ILT06-EBLPM1	POS	POS	1
77	1	77	ILT06-EBLPM7	<b>POS</b>	<b>NEG</b>	<b>0</b>
78	1	78	ILT06-EBLNM3	NEG	NEG	1
79	1	79	ILT06-EBLNM2	NEG	NEG	1
80	1	80	ILT06-EBLPM4	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	2	1	ILT06-EBLPM1	POS	POS	1
82	2	2	ILT06-EBLPM7	POS	POS	1
83	2	3	ILT06-EBLNM3	NEG	NEG	1
84	2	4	ILT06-EBLNM2	NEG	NEG	1
85	2	5	ILT06-EBLPM4	POS	POS	1
86	2	6	ILT06-EBLPM2	POS	POS	1
87	2	7	ILT06-EBLNM4	NEG	NEG	1
88	2	8	ILT06-EBLNM2	NEG	NEG	1
89	2	9	ILT06-EBLPM1	POS	POS	1
90	2	10	ILT06-EBLNM1	NEG	NEG	1
91	2	11	ILT06-EBLPM7	POS	POS	1
92	2	12	ILT06-EBLPM3	POS	POS	1
93	2	13	ILT06-EBLNM1	NEG	NEG	1
94	2	14	ILT06-EBLNM4	NEG	NEG	1
95	2	15	ILT06-EBLPM7	POS	POS	1
96	2	16	ILT06-EBLPM4	POS	POS	1
97	2	17	ILT06-EBLNM3	NEG	NEG	1
98	2	18	ILT06-EBLPM1	POS	POS	1
99	2	19	ILT06-EBLPM2	POS	POS	1
100	2	20	ILT06-EBLPM3	POS	POS	1
101	2	21	ILT06-EBLPM2	POS	POS	1
102	2	22	ILT06-EBLNM1	NEG	NEG	1
103	2	23	ILT06-EBLNM1	NEG	NEG	1
104	2	24	ILT06-EBLNM4	NEG	NEG	1
105	2	25	ILT06-EBLPM3	POS	POS	1
106	2	26	ILT06-EBLPM3	POS	POS	1
107	2	27	ILT06-EBLPM1	POS	POS	1
108	2	28	ILT06-EBLNM3	NEG	NEG	1
109	2	29	ILT06-EBLPM4	POS	POS	1
110	2	30	ILT06-EBLNM2	NEG	NEG	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
111	2	31	ILT06-EBLPM7	POS	POS	1
112	2	32	ILT06-EBLPM2	POS	POS	1
113	2	33	ILT06-EBLNM2	NEG	NEG	1
114	2	34	ILT06-EBLNM3	NEG	NEG	1
115	2	35	ILT06-EBLPM1	POS	POS	1
116	2	36	ILT06-EBLNM1	NEG	NEG	1
117	2	37	ILT06-EBLNM4	NEG	NEG	1
118	2	38	ILT06-EBLPM4	POS	POS	1
119	2	39	ILT06-EBLNM1	NEG	NEG	1
120	2	40	ILT06-EBLPM7	POS	POS	1
121	2	41	ILT06-EBLPM3	POS	POS	1
122	2	42	ILT06-EBLNM1	NEG	NEG	1
123	2	43	ILT06-EBLNM4	NEG	NEG	1
124	2	44	ILT06-EBLNM3	NEG	NEG	1
125	2	45	ILT06-EBLPM7	POS	POS	1
126	2	46	ILT06-EBLPM1	POS	POS	1
127	2	47	ILT06-EBLNM2	NEG	NEG	1
128	2	48	ILT06-EBLPM2	POS	POS	1
129	2	49	ILT06-EBLNM4	NEG	NEG	1
130	2	50	ILT06-EBLPM3	POS	POS	1
131	2	51	ILT06-EBLPM4	POS	POS	1
132	2	52	ILT06-EBLNM1	NEG	NEG	1
133	2	53	ILT06-EBLPM7	POS	POS	1
134	2	54	ILT06-EBLNM3	NEG	NEG	1
135	2	55	ILT06-EBLPM4	POS	POS	1
136	2	56	ILT06-EBLPM1	POS	POS	1
137	2	57	ILT06-EBLPM2	POS	POS	1
138	2	58	ILT06-EBLPM4	POS	POS	1
139	2	59	ILT06-EBLPM3	POS	POS	1
140	2	60	ILT06-EBLNM2	NEG	NEG	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
141	2	61	ILT06-EBLPM1	POS	POS	1
142	2	62	ILT06-EBLNM3	NEG	NEG	1
143	2	63	ILT06-EBLNM2	NEG	NEG	1
144	2	64	ILT06-EBLPM7	POS	POS	1
145	2	65	ILT06-EBLNM4	NEG	NEG	1
146	2	66	ILT06-EBLNM3	NEG	NEG	1
147	2	67	ILT06-EBLPM2	POS	POS	1
148	2	68	ILT06-EBLNM2	NEG	NEG	1
149	2	69	ILT06-EBLPM3	POS	POS	1
150	2	70	ILT06-EBLPM1	POS	POS	1
151	2	71	ILT06-EBLNM4	NEG	NEG	1
152	2	72	ILT06-EBLPM2	POS	POS	1
153	2	73	ILT06-EBLNM2	NEG	NEG	1
154	2	74	ILT06-EBLPM4	POS	POS	1
155	2	75	ILT06-EBLNM3	NEG	NEG	1
156	2	76	ILT06-EBLPM3	POS	POS	1
157	2	77	ILT06-EBLNM4	NEG	NEG	1
158	2	78	ILT06-EBLNM1	NEG	NEG	1
159	2	79	ILT06-EBLPM2	POS	POS	1
160	2	80	ILT06-EBLPM4	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	3	1	ILT06-EBLPM3	POS	POS	1
162	3	2	ILT06-EBLNM4	NEG	NEG	1
163	3	3	ILT06-EBLNM1	NEG	NEG	1
164	3	4	ILT06-EBLPM2	POS	POS	1
165	3	5	ILT06-EBLPM4	POS	POS	1
166	3	6	ILT06-EBLPM1	POS	POS	1
167	3	7	ILT06-EBLPM7	POS	POS	1
168	3	8	ILT06-EBLNM3	NEG	NEG	1
169	3	9	ILT06-EBLNM2	NEG	NEG	1
170	3	10	ILT06-EBLPM4	POS	POS	1
171	3	11	ILT06-EBLPM2	POS	POS	1
172	3	12	ILT06-EBLNM4	NEG	NEG	1
173	3	13	ILT06-EBLNM2	NEG	NEG	1
174	3	14	ILT06-EBLPM1	POS	POS	1
175	3	15	ILT06-EBLNM1	NEG	NEG	1
176	3	16	ILT06-EBLPM7	POS	POS	1
177	3	17	ILT06-EBLPM3	POS	POS	1
178	3	18	ILT06-EBLNM1	NEG	NEG	1
179	3	19	ILT06-EBLNM4	NEG	NEG	1
180	3	20	ILT06-EBLPM7	POS	POS	1
181	3	21	ILT06-EBLPM4	POS	POS	1
182	3	22	ILT06-EBLNM3	NEG	NEG	1
183	3	23	ILT06-EBLPM1	POS	POS	1
184	3	24	ILT06-EBLPM2	POS	POS	1
185	3	25	ILT06-EBLPM3	POS	POS	1
186	3	26	ILT06-EBLPM2	POS	POS	1
187	3	27	ILT06-EBLNM1	NEG	NEG	1
188	3	28	ILT06-EBLNM1	NEG	NEG	1
189	3	29	ILT06-EBLNM4	NEG	NEG	1
190	3	30	ILT06-EBLPM3	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
191	3	31	ILT06-EBLPM3	POS	POS	1
192	3	32	ILT06-EBLPM1	POS	POS	1
193	3	33	ILT06-EBLNM3	NEG	NEG	1
194	3	34	ILT06-EBLPM4	POS	POS	1
195	3	35	ILT06-EBLNM2	NEG	NEG	1
196	3	36	ILT06-EBLPM7	POS	POS	1
197	3	37	ILT06-EBLPM2	POS	POS	1
198	3	38	ILT06-EBLNM2	NEG	NEG	1
199	3	39	ILT06-EBLNM3	NEG	NEG	1
200	3	40	ILT06-EBLPM1	POS	POS	1
201	3	41	ILT06-EBLNM1	NEG	NEG	1
202	3	42	ILT06-EBLNM4	NEG	NEG	1
203	3	43	ILT06-EBLPM4	POS	POS	1
204	3	44	ILT06-EBLNM1	NEG	NEG	1
205	3	45	ILT06-EBLPM7	POS	POS	1
206	3	46	ILT06-EBLPM3	POS	POS	1
207	3	47	ILT06-EBLNM1	NEG	NEG	1
208	3	48	ILT06-EBLNM4	NEG	NEG	1
209	3	49	ILT06-EBLNM3	NEG	NEG	1
210	3	50	ILT06-EBLPM7	POS	POS	1
211	3	51	ILT06-EBLPM1	POS	POS	1
212	3	52	ILT06-EBLNM2	NEG	NEG	1
213	3	53	ILT06-EBLPM2	POS	POS	1
214	3	54	ILT06-EBLNM4	NEG	NEG	1
215	3	55	ILT06-EBLPM3	POS	POS	1
216	3	56	ILT06-EBLPM4	POS	POS	1
217	3	57	ILT06-EBLNM1	NEG	NEG	1
218	3	58	ILT06-EBLPM7	POS	POS	1
219	3	59	ILT06-EBLNM3	NEG	NEG	1
220	3	60	ILT06-EBLPM4	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
221	3	61	ILT06-EBLPM1	POS	POS	1
222	3	62	ILT06-EBLPM2	POS	POS	1
223	3	63	ILT06-EBLPM4	POS	POS	1
224	3	64	ILT06-EBLPM3	POS	POS	1
225	3	65	ILT06-EBLNM2	NEG	NEG	1
226	3	66	ILT06-EBLPM1	POS	POS	1
227	3	67	ILT06-EBLNM3	NEG	NEG	1
228	3	68	ILT06-EBLNM2	NEG	NEG	1
229	3	69	ILT06-EBLPM7	POS	POS	1
230	3	70	ILT06-EBLNM4	NEG	NEG	1
231	3	71	ILT06-EBLNM3	NEG	NEG	1
232	3	72	ILT06-EBLPM2	POS	POS	1
233	3	73	ILT06-EBLNM2	NEG	NEG	1
234	3	74	ILT06-EBLPM3	POS	POS	1
235	3	75	ILT06-EBLPM1	POS	POS	1
236	3	76	ILT06-EBLNM4	NEG	NEG	1
237	3	77	ILT06-EBLPM2	POS	POS	1
238	3	78	ILT06-EBLNM2	NEG	NEG	1
239	3	79	ILT06-EBLPM4	POS	POS	1
240	3	80	ILT06-EBLNM3	NEG	NEG	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
241	4	1	ILT06-EBLNM4	NEG	NEG	1
242	4	2	ILT06-EBLPM2	POS	POS	1
243	4	3	ILT06-EBLNM2	NEG	NEG	1
244	4	4	ILT06-EBLPM4	POS	POS	1
245	4	5	ILT06-EBLNM3	NEG	NEG	1
246	4	6	ILT06-EBLPM3	POS	POS	1
247	4	7	ILT06-EBLNM4	NEG	NEG	1
248	4	8	ILT06-EBLNM1	NEG	NEG	1
249	4	9	ILT06-EBLPM2	POS	POS	1
250	4	10	ILT06-EBLPM4	POS	POS	1
251	4	11	ILT06-EBLPM1	POS	POS	1
252	4	12	ILT06-EBLPM7	POS	POS	1
253	4	13	ILT06-EBLNM3	NEG	NEG	1
254	4	14	ILT06-EBLNM2	NEG	NEG	1
255	4	15	ILT06-EBLPM4	POS	POS	1
256	4	16	ILT06-EBLPM2	POS	POS	1
257	4	17	ILT06-EBLNM4	NEG	NEG	1
258	4	18	ILT06-EBLNM2	NEG	NEG	1
259	4	19	ILT06-EBLPM1	POS	POS	1
260	4	20	ILT06-EBLNM1	NEG	NEG	1
261	4	21	ILT06-EBLPM7	POS	POS	1
262	4	22	ILT06-EBLPM3	POS	POS	1
263	4	23	ILT06-EBLNM1	NEG	NEG	1
264	4	24	ILT06-EBLNM4	NEG	NEG	1
265	4	25	ILT06-EBLPM7	POS	POS	1
266	4	26	ILT06-EBLPM4	POS	POS	1
267	4	27	ILT06-EBLNM3	NEG	NEG	1
268	4	28	ILT06-EBLPM1	POS	POS	1
269	4	29	ILT06-EBLPM2	POS	POS	1
270	4	30	ILT06-EBLPM3	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
271	4	31	ILT06-EBLPM2	POS	POS	1
272	4	32	ILT06-EBLNM1	NEG	NEG	1
273	4	33	ILT06-EBLNM1	NEG	NEG	1
274	4	34	ILT06-EBLNM4	NEG	NEG	1
275	4	35	ILT06-EBLPM3	POS	POS	1
276	4	36	ILT06-EBLPM3	POS	POS	1
277	4	37	ILT06-EBLPM1	POS	POS	1
278	4	38	ILT06-EBLNM3	NEG	NEG	1
279	4	39	ILT06-EBLPM4	POS	POS	1
280	4	40	ILT06-EBLNM2	NEG	NEG	1
281	4	41	ILT06-EBLPM7	POS	POS	1
282	4	42	ILT06-EBLPM2	POS	POS	1
283	4	43	ILT06-EBLNM2	NEG	NEG	1
284	4	44	ILT06-EBLNM3	NEG	NEG	1
285	4	45	ILT06-EBLPM1	POS	POS	1
286	4	46	ILT06-EBLNM1	NEG	NEG	1
287	4	47	ILT06-EBLNM4	NEG	NEG	1
288	4	48	ILT06-EBLPM4	POS	POS	1
289	4	49	ILT06-EBLNM1	NEG	NEG	1
290	4	50	ILT06-EBLPM7	POS	POS	1
291	4	51	ILT06-EBLPM3	POS	POS	1
292	4	52	ILT06-EBLNM1	NEG	NEG	1
293	4	53	ILT06-EBLNM4	NEG	NEG	1
294	4	54	ILT06-EBLNM3	NEG	NEG	1
295	4	55	ILT06-EBLPM7	POS	POS	1
296	4	56	ILT06-EBLPM1	POS	POS	1
297	4	57	ILT06-EBLNM2	NEG	NEG	1
298	4	58	ILT06-EBLPM2	POS	POS	1
299	4	59	ILT06-EBLNM4	NEG	NEG	1
300	4	60	ILT06-EBLPM3	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
301	4	61	ILT06-EBLPM4	POS	POS	1
302	4	62	ILT06-EBLNM1	NEG	NEG	1
303	4	63	ILT06-EBLPM7	POS	POS	1
304	4	64	ILT06-EBLNM3	NEG	NEG	1
305	4	65	ILT06-EBLPM4	POS	POS	1
306	4	66	ILT06-EBLPM1	POS	POS	1
307	4	67	ILT06-EBLPM2	POS	POS	1
308	4	68	ILT06-EBLPM4	POS	POS	1
309	4	69	ILT06-EBLPM3	POS	POS	1
310	4	70	ILT06-EBLNM2	NEG	NEG	1
311	4	71	ILT06-EBLPM1	POS	POS	1
312	4	72	ILT06-EBLNM3	NEG	NEG	1
313	4	73	ILT06-EBLNM2	NEG	NEG	1
314	4	74	ILT06-EBLPM7	POS	POS	1
315	4	75	ILT06-EBLNM4	NEG	NEG	1
316	4	76	ILT06-EBLNM3	NEG	NEG	1
317	4	77	ILT06-EBLPM2	POS	POS	1
318	4	78	ILT06-EBLNM2	NEG	NEG	1
319	4	79	ILT06-EBLPM3	POS	POS	1
320	4	80	ILT06-EBLPM1	POS	POS	1

#### IV. 5. Variability among the participating laboratories

The variability among the participating laboratories concerned the S/P ratio% is illustrated in two graphical enclosures. Enclosure 1: "Graph 1 ILT-EBLMILK LABNR BY SAMPLE" = Boxplot of S/P ratio % of all participating laboratories (LABNR) grouped by sample (=X-as). Enclosure 2: "Graph 2 ILT-EBLMILK SAMPLE BY LABNR" = Boxplot of S/P ratio % of all samples grouped by the participating laboratories (LABNR (=X-as)). The box plots shown are a graphical representation of the summary for a variable. It is based on the quartiles of a variable. The rectangular box corresponds to the lower quartile (25% Q1) and the upper quartile (75%Q3). The line in the middle is the median. The plus sign in the middle is the mean. We can visually compare the lengths of the whiskers. If one is clearly longer than the other the distribution may be skewed. The highest and lower level indicated gives respectively the highest and lowest value observed in the dataset.

## V. Discussion

The purpose of this trial is to assess performances of participating laboratories when analyzing:

- (i) reference milks free from Enzootic Bovine Leucosis-specific detectable antibodies,
- (ii) reference milks containing Enzootic Bovine Leucosis-specific detectable antibodies.

Three of the four participating laboratories (LAB2, LAB3 and LAB4) provided responses that were in full agreement with the true status of the reference milks. One participating laboratory (LAB1) misclassified 8 reference milk samples in the ELISA test. This misclassification was due to 1 sample, ILT06-EBLPM7, which was classified 6 times as negative and 2 times as NI while the status of the sample was positive. The mean S/P % of this sample for LAB1 was 48,3 (range 6,2 (min)-66 (max) while this was much higher in LAB 2, 3 and 4 with respectively a mean S/P % of 84,3 (range 77,4 - 95,6), 100,8 (72,9 – 123,4) and 87,2 (76,7-92,6). In addition, verifications results by the VAR confirmed the 'weak positive status' of the sample ILT-06-EBLPM7 (mean S/P ratio % = 92,5, min S/P ratio % = 76 and max S/P ratio % = 122). Therefore, the ILT06-EBLPM7 sample (8 aliquots) was included in the inter-laboratory trial to verify if the participating laboratories could classify these 'cut off'-samples correctly. Therefore it is expected that the result of sample ILT06-EBLPM7 may fluctuate around the cut off value and might result in NI and negative results. Only in LAB1, the positive cut off level of 70% S/P was never reached.

## 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 samples (Section III.3.3. of this Report). Consequently, all four participating laboratories achieved a satisfactory performance.

The Committee

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