Brucellosis milk ILPT 2013

EU Reference Laboratory for Brucellosis
ANSES – Maisons-Alfort, France

EU Brucellosis Ring-Trials organised (past & present)

- 2007/2008: 1st EU Bovine Brucellosis Serum Proficiency Ring-Trial
- 2008/2009: 1st EU Bovine Brucellosis Milk iELISA Proficiency Ring-Trial
- 2009: 2nd EU Bovine Brucellosis Milk iELISA Proficiency Ring-Trial
- 2009/2010: EU Bovine Brucellosis Serum Collaborative Ring-trial for CFT harmonisation (+ proficiency for the RBT)
- 2009/2010: EU Ovine/Caprine Brucellosis serum iELISA Collaborative Ring-trial (EU Sheep & Goat BSS)
- 2010/2011: EU Collaborative Ring-Trial CFT Harmonisation (EUPigBSS, EUSBSS & EUGBSS)
- 2011 : EU Bovine Brucellosis Milk iELISA Proficiency Ring-Trial
- 2012 : EU Bovine Brucellosis Serum Proficiency Ring-Trial
- 2013 : 4th EU Bovine Brucellosis Milk iELISA Proficiency Ring-Trial
  - ☞ *further assessment of milk iELISA consistency performed throughout the EU*
  - ☞ *improvement of the support provided by the EURL to the NRL network*
  - ☞ *improvement of quality control of the reagents*

Participation

- Participants : **22 EU MS NRLs + EURL**

EFTA, EU candidate countries and Balkan countries were asked to participate in, but finally none of them took part in the trial

- 5 kit suppliers
- Method: EU approved milk test in cattle = **I-ELISA**

kits that should have been approved by a European NRL according to the Annex C of the 64/432 EC Directive.

- RT led simultaneously at French level (17 labs)

Panel preparation (August-sept. 2013)

Samples with Ab.

[1] pos. serum

[2] pos. serum

[3] pos. serum

Two-fold dilutions in serum + 1/10 bulk milk

Kit D, batch 2 — Pre-selection

Direct dilution in serum + 1/10 bulk milk

Kit D, batch 2 / 8 repetition — Selection

Max. kits available on the market — Confirmation

Samples without Ab.

neg. sera

1/10 bulk milk

1/10 bulk milk

neg. bulk milks

Serum selection (August-sept. 2013)

Samples with Ab. :

- **2** different bovine **positive (pos.)** sera (from infected animals) diluted in negative (neg.) serum used to spike the neg. milk (**1/10**) to get different levels of Ab.
  - One pos. serum (**batch 49**) used for the preparation of a level with strong pos. result (**1/250**) ;
  - One pos. serum (**104**) used for the preparation of three different levels:
    - a level with pos. result (**1/400**)
    - a level with pos. result close to the cut-off (**1/700**)
    - a level with neg. result (but containing *Brucella* Ab.) (**1/4000**)

Samples without Ab. :

- A neg. serum diluted in bulk milk
- A neg. bulk milk

→ *For the dilution : pooled neg. sera / neg. bulk milk*
→ *For dilution in milk: 9 vol. neg. milk / 1 vol. of pos. serum*

Panel preparation

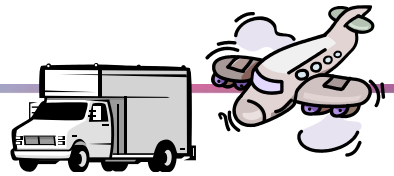
Sample	Internal identification number	Dilution in pooled neg. sera	Dilution in neg. bulk milk	Qualitative results	Level
pos. serum diluted in neg. serum (pooled neg. sera), and in neg. bulk milk (1/10)	batch 49	1/250	1/10	Strong pos.	6
	Batch 104	1/400	1/10	pos./Doubtful/ neg.*	5
		1/700	1/10	pos. ~cut-off /doubtful/neg.*	4
		1/4000	1/10	neg.	3
neg. serum diluted in neg. bulk milk (1/10)	batch 11-01	-	1/10	neg.	2
neg. bulk milk	batch 13-01	-	-	neg.	1

☞ **6 levels of samples**

- Test with all the kits received from the suppliers
  - Consistent results on level 1, 2, 3, 6
  - Some differences on level 4 & 5
- Results of preliminary testing = complement for participants' results evaluation
- Preparation of the bulk; distribution in tubes (storage -20° C); codification
- **Homogeneity challenge**: 10 samples; 2 technicians; 1 Kit → **validated (CV<10%)**

Panel preparation

- Samples were in duplicate or in triplicate → **repeatability**
  - Samples without Ab. / **duplicates** : **2* Level 1 + 2* Level 2**
  - Samples with Ab. / **triplicates** : **3* Level 3 + 3*Level 4 + 3* Level 5 + 3* Level 6**
- Panel of **16 bulk milk samples**
- Instructions:
  - Test the samples in **their usual working conditions**, 8 days after reception
- Laboratories were asked to provide for all samples:
  - OD measurements,
  - Calculated index and,
  - Qualitative interpretation according to the instructions for use for each kit
- Various kits were used by the participants during this ILPT : mostly commercial except one (kit A), that is produced and standardised by a NRL.



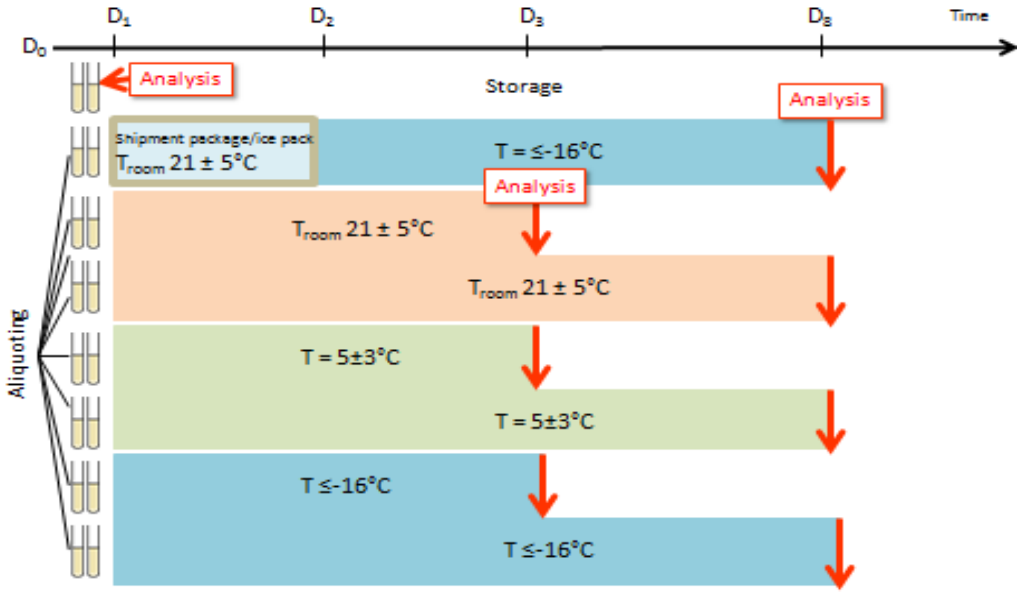
Organization

- Announcement : September 2013
- Shipment : **2013 October, 15**

  room T + ice pack

  → *In parallel : stability challenge*

  3 samples/ level in 8 ≠ conditions



- **No problems** were reported during the shipment except for **one lab.**

  → one broken tube and clotted milk in others tubes (panel received 7 days after the shipment)

  → shipment of a new panel was thus organized for this laboratory, without delay.

- Result Form sent within 2 weeks upon reception of the parcel (DDL 8/11)

→ **instructions followed by all participants ☺**

Analysis

- Expected / accepted results were defined :
  - ☞ according to results of EURL & participants (French, European labs and others);
  - ✋ Expectations especially adjusted for samples close to the threshold (levels 3 & 4)
- Analysis concerned :
  - **qualitative and quantitative** results
    - ☞ Sensitivity, specificity and repeatability, coherence between dilutions of the same serum
    - ☞ Consistency qualitative/quantitative (interpretation)
  - **traceability** (sample codes) → 1 lab (1 sample number)
- Information about reagents? Controls?...
- Assessment **of the proficiency compared to the previous ring trials** (when critical failure has been noticed)

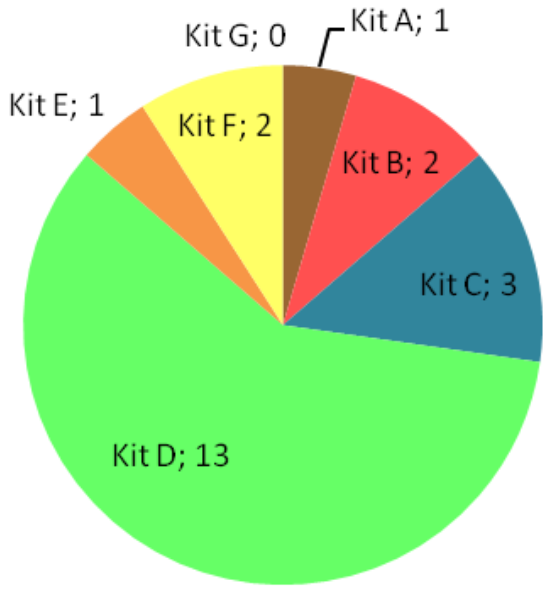
Analysis

- Kits used : 5 commercial + 1 home-made (kit A/1 NRL)
  → French labs used 2 kits (all labs Kit D, but one kit B)
- **4 kits** tested by the EURL (Kits B, D, E and F) during preliminary testing
- Results of participants, EURL and suppliers were taken into account to propose expected results

If few results available...caution!

Kit G was not used by any of the participants.

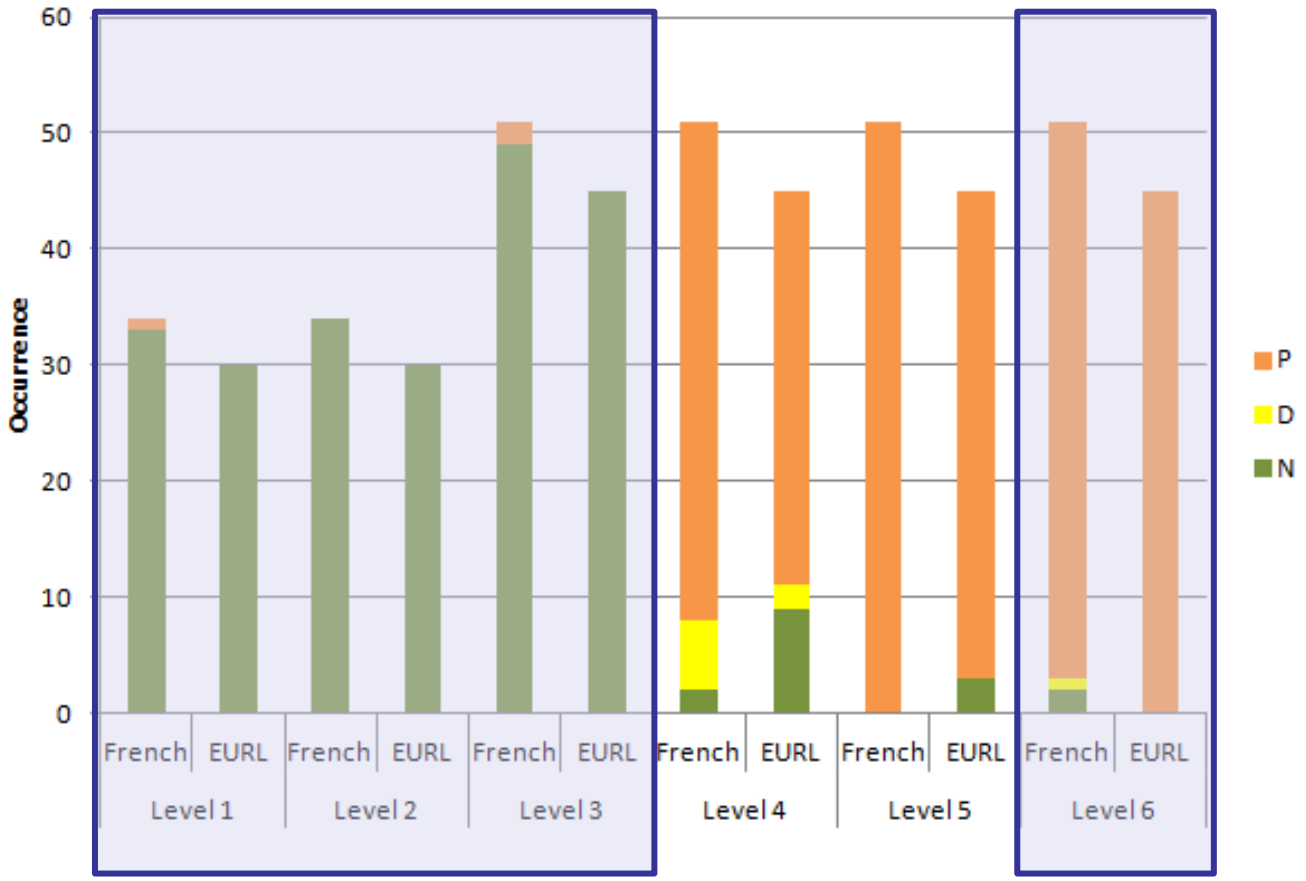
Kit C is another kit supplied by the supplier of kit D.



- Results sorted out by kit

Analysis

- French results (17 labs;kits B/**D**) / EURL (kits B/**D**/E/F/G) results

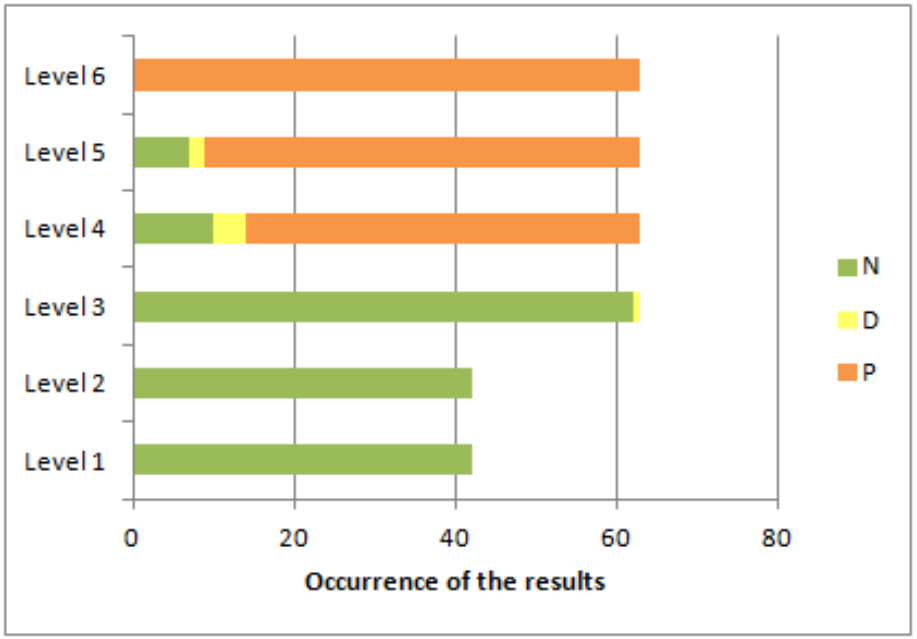


Results (qualitative)

Lab. code	Supplier	Batch	Level 1		Level 2		Level 3			Level 4			Level 5			Level 6		
lab 16	Kit A	Batch 1	N	N	N	N	N	D	N	D	D	P	P	D	D	P	P	P
lab 1	Kit B	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 26	Kit B	Batch 2	N	N	N	N	N	N	N	P	P	D	P	P	P	P	P	P
lab 29	Kit C	Batch 1	N	N	N	N	N	N	N	N	N	N	P	P	N	P	P	P
lab 30	Kit C	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 9	Kit C	Batch 3	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 2	Kit D	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 23	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 27	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 47	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 32	Kit D	Batch 3	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 20	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 24	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 48	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 4	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 19	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 21	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 31	Kit D	Batch 5	N	N	N	N	N	N	N	P	D	N	P	P	P	P	P	P
lab 34	Kit E	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 25	Kit F	Batch 1	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P
lab 37	Kit F	Batch 2	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P

Results

- Consistency qualitative quantitative : all labs were satisfactory except one (interpr. of one result Neg. ↔ Dbt.)
- Qualitative results:



Results for several levels were expected identical **whatever the kit used**:

- On **levels 1 and 2** : **neg.** results expected in samples (without Ab.)
- On **level 3** (very low level of Ab.) : **neg.** results expected
- On **level 6 (**very high level of Ab.) : **pos.** results expected

Results

Lab. code	Supplier	Batch	Level 1		Level 2		Level 3			Level 6		
lab 16	Kit A	Batch 1	N	N	N	N	N	D	N	P	P	P
lab 1	Kit B	Batch 1	N	N	N	N	N	N	N	P	P	P
lab 26	Kit B	Batch 2	N	N	N	N	N	N	N	P	P	P
lab 29	Kit C	Batch 1	N	N	N	N	N	N	N	P	P	P
lab 30	Kit C	Batch 2	N	N	N	N	N	N	N	P	P	P
lab 9	Kit C	Batch 3	N	N	N	N	N	N	N	P	P	P
lab 2	Kit D	Batch 1	N	N	N	N	N	N	N	P	P	P
lab 23	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P
lab 27	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P
lab 47	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P
lab 32	Kit D	Batch 3	N	N	N	N	N	N	N	P	P	P
lab 20	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P
lab 24	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P
lab 48	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P
lab 4	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P
lab 19	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P
lab 21	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P
lab 31	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P
lab 34	Kit E	Batch 1	N	N	N	N	N	N	N	P	P	P
lab 25	Kit F	Batch 1	N	N	N	N	N	N	N	P	P	P
lab 37	Kit F	Batch 2	N	N	N	N	N	N	N	P	P	P
	expected results		N	N	N	N	N	N	N	P	P	P

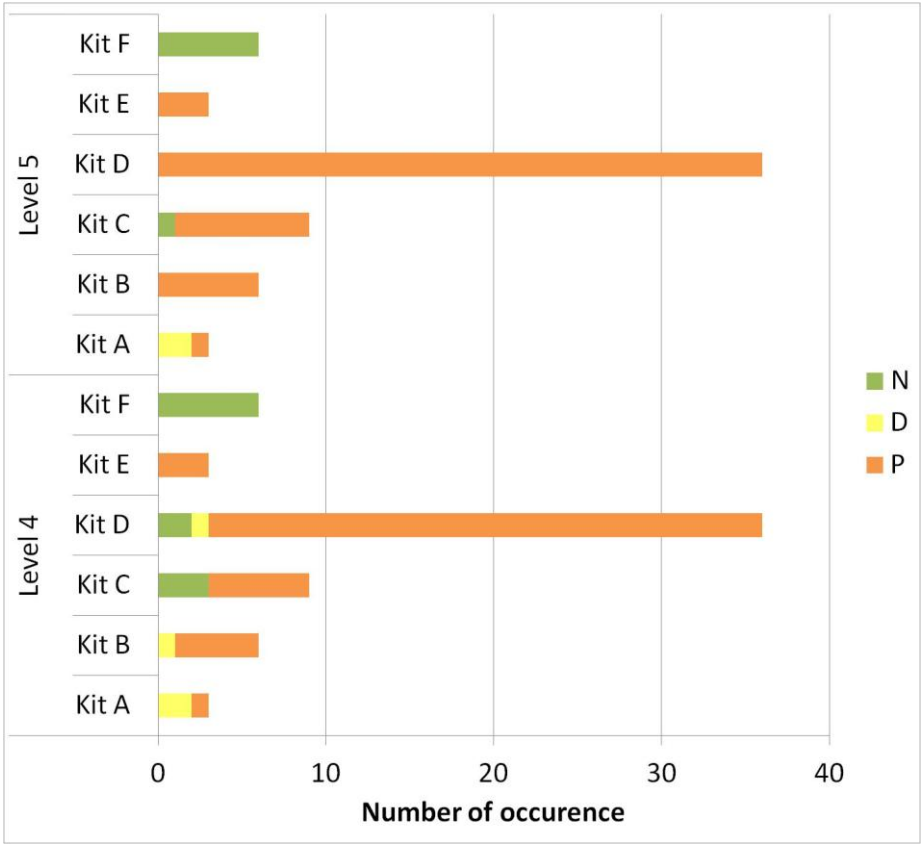
On levels 1, 2, 3 & 6 : consistent results

Slight excess of Se? for 1 lab. on 1 sample of level 3 (lack of consistency between results of 3, 4 & 5)

Results

- On levels 4 & 5 (~cut-off) : expected/accepted results **according to the kit used**
  - considering the results obtained by participants and EURL
  - considering results of French laboratories (that used mostly kit D but also kit B).

*Results obtained for these two levels were mostly **pos.**, as initially expected.*



Results Kit B & D

Lab. code	Supplier	Batch	Level 4			Level 5		
lab 1	Kit B	Batch 1	P	P	P	P	P	P
lab 26	Kit B	Batch 2	P	P	D	P	P	P
lab 2	Kit D	Batch 1	P	P	P	P	P	P
lab 23	Kit D	Batch 2	P	P	P	P	P	P
lab 27	Kit D	Batch 2	P	P	P	P	P	P
lab 47	Kit D	Batch 2	P	P	P	P	P	P
lab 32	Kit D	Batch 3	P	P	P	P	P	P
lab 20	Kit D	Batch 4	P	P	P	P	P	P
lab 24	Kit D	Batch 4	P	P	P	P	P	P
lab 48	Kit D	Batch 4	P	P	P	P	P	P
lab 4	Kit D	Batch 5	P	P	P	P	P	P
lab 19	Kit D	Batch 5	P	P	P	P	P	P
lab 21	Kit D	Batch 5	P	P	P	P	P	P
lab 31	Kit D	Batch 5	P	D	N	P	P	P

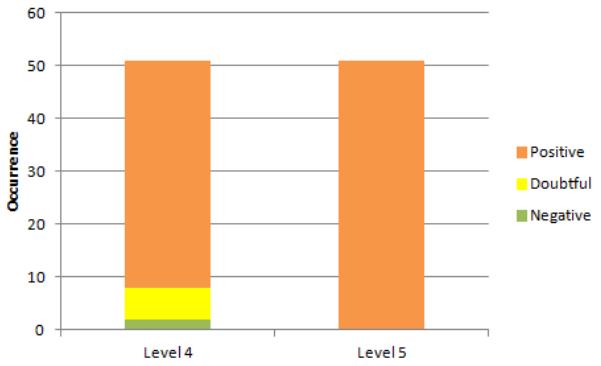
Sample level	Expected results	Accepted results
	Qualitative	Qualitative
4	Positive	Doubtful
5	Positive	Id.

Lack of Se for 1 lab.
(one sample, level 4)
Due to interpretation troubles !
Quantitative result is doubftul

EURL

EURL results		Niveau 4			Niveau 5		
Kit B	Batch 2	D	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 2	P	P	P	P	P	P
Kit D	Batch 4	D	P	P	P	P	P

French



Results Kit E & C

Sample level	Expected results	Accepted results
	Qualitative	Qualitative
4	Positive	Negative (close to the cut-off)
5	Positive	Id.

Lab. code	Supplier	Batch	Level 4			Level 5		
lab 29	Kit C	Batch 1	N	N	N	P	P	N
lab 30	Kit C	Batch 2	P	P	P	P	P	P
lab 9	Kit C	Batch 3	P	P	P	P	P	P
lab 34	Kit E	Batch 1	P	P	P	P	P	P

EURL results		Niveau 4			Niveau 5		
Kit E	Batch 2	N	N	N	P	P	P

Critical lack of Se for 1 lab. / one sample of level 5

Results Kit F

Sample level	Expected results Qualitative	Accepted results Qualitative
4	Negative	Id.
5	Negative	Positive close to the cut-off

Lab. code	Supplier	Batch	Level 4			Level 5		
lab 25	Kit F	Batch 1	N	N	N	N	N	N
lab 37	Kit F	Batch 2	N	N	N	N	N	N

EURL results		Niveau 4			Niveau 5		
Kit F	Batch 3	N	N	N	N	N	N

Lower Se of the kit ?

But compliance with EU requirements

Results

Kit A : 1 lab, expected results ?

Consistency with other results but lack of consistency between levels (4 & 5) prepared with increasing dilutions of the same serum (see quantitative)

Lab. code	Supplier	Batch	Level 1		Level 2		Level 3			Level 4			Level 5			Level 6		
lab 16	Kit A	Batch 1	N	N	N	N	N	D	N	D	D	P	P	D	D	P	P	P
lab 1	Kit B	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 26	Kit B	Batch 2	N	N	N	N	N	N	N	P	P	D	P	P	P	P	P	P
lab 29	Kit C	Batch 1	N	N	N	N	N	N	N	N	N	N	P	P	N	P	P	P
lab 30	Kit C	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 9	Kit C	Batch 3	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 2	Kit D	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 23	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 27	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 47	Kit D	Batch 2	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 32	Kit D	Batch 3	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 20	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 24	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 48	Kit D	Batch 4	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 4	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 19	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 21	Kit D	Batch 5	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 31	Kit D	Batch 5	N	N	N	N	N	N	N	P	D	N	P	P	P	P	P	P
lab 34	Kit E	Batch 1	N	N	N	N	N	N	N	P	P	P	P	P	P	P	P	P
lab 25	Kit F	Batch 1	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P
lab 37	Kit F	Batch 2	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P

Results (quantitative)

Repeatability (levels 4,5 & 6) : 4 NRLs occasionally obtained a CV exceeding 20%.

All the concerned laboratories (16, 24, 25, and 47) obtained a CV exceeding 20% on only one level.

washing or pipetting troubles?

Low cut-off does not favour repeatability

Lab. code	Supplier	Batch	Positive cutoff	Doubtful cutoff	Negative cutoff	Niveau 4			CV %	Niveau 5			CV %	Niveau 6			CV %
lab 16	Kit A	Batch 1	>=10%	8-9.9%	<8%	9,1	8,3	10,3	10,90	12,5	8,2	9,4	22,11	12,7	13,6	12,3	5,17
lab 1	Kit B	Batch 1	>=50%	45-50%	<=45%	58,6	57,5	58,3	0,98	93,8	94	84,6	5,91	217,8	217,8	218,2	0,11
lab 26	Kit B	Batch 2	>=50%	45-50%	<=45%	53,4	51,2	48,3	5,02	77,8	76,6	78,3	1,13	292,7	290,5	303,4	2,34
lab 29	Kit C	Batch 1	>30%	-	<30%	14,47	12,72	12,51	8,13	30,68	30,14	22,3	16,93	161,7	169,42	170,84	2,94
lab 30	Kit C	Batch 2	>=30%	-	<30%	35,679	34,744	33,366	3,36	51,476	50,148	40,502	12,64	142,96	136,61	134,01	3,34
lab 9	Kit C	Batch 3	>=30%	-	<30%	32,7	32,1	30,9	2,87	50,1	51,1	48,6	2,52	153,2	153,2	143,6	3,70
lab 2	Kit D	Batch 1	>=55%	45-55%	<=45%	74,3	71,2	76,4	3,54	116,2	124,2	122	3,42	189,5	185,8	190	1,22
lab 23	Kit D	Batch 2	>=55%	45-55%	<=45%	63,557	55,488	57,958	7,01	87,925	96,432	98,408	5,91	166,905	167,234	156,037	3,90
lab 27	Kit D	Batch 2	>=55%	45-55%	<=45%	75	74	78,7	3,26	131	133	125	3,21	219	212	203	[illegible]
lab 47	Kit D	Batch 2	>=55%	45-55%	<=45%	63,874	64,921	59,511	4,57	116,318	113,874	99,04	8,52	174,433	106,733	162,653	24,45
lab 32	Kit D	Batch 3	>=55%	45-55%	<=45%	71,99	74,76	71,07	2,65	120,21	111,49	118,75	4,00	224,7	228,66	220,47	1,82
lab 20	Kit D	Batch 4	>=55%	45-55%	<=45%	61,33	64,232	60,206	[illegible]	97,472	105,056	96,067	4,86	159,551	145,693	179,869	10,63
lab 24	Kit D	Batch 4	>=55%	45-55%	<=45%	108,9	75,4	75	22,51	124,3	118,7	140,5	8,86	197,2	268,8	209,2	17,04
lab 48	Kit D	Batch 4	>=55%	45-55%	<=45%	72	68	65	5,14	111	109	110	0,91	170	190	165	7,56
lab 4	Kit D	Batch 5	>=55%	45-55%	<=45%	74,4	72,99	68	4,68	119,35	125,95	113,71	5,12	187,27	189,89	193,84	1,74
lab 19	Kit D	Batch 5	>=55%	45-55%	<=45%	63,5	59,2	56,8	5,67	104,6	104,4	101	1,96	153,4	171,6	168,5	5,92
lab 21	Kit D	Batch 5	>=55%	45-55%	<=45%	73,17	64,68	67,8	6,26	112,3	119,3	120,4	3,74	192,4	189,6	181,1	3,14
lab 31	Kit D	Batch 5	>=55%	45-55%	<=45%	59,052	45,901	53,843	12,51	83,305	76,9	86,465	5,93	131,042	141,033	121,563	7,42
lab 34	Kit E	Batch 1	>= 25%	-	<= 25%	53,20	58,80	47,00	11,14	71,61	58,30	54,90	14,34	108,00	95,78	99,90	[illegible]
lab 25	Kit F	Batch 1	>=10%	-	<10%	5,686	5,956	4,778	11,28	6,797	6,023	5,114	14,09	47,222	20,962	15,242	61,33
lab 37	Kit F	Batch 2	>=10%	-	<10%	9,2	8,3	8	7,35	9,7	9,4	9,4	1,82	33	33,7	30	6,10

Results (quantitative)

Consistency between levels 3, 4 & 5 (same serum) : 3 NRLs obtained overlapping results

Lab. code	Supplier	Batch	Positive cutoff	Doubtful cutoff	Negative cutoff	Niveau 3			Niveau 4			CV %	Niveau 5							
lab 16	Kit A	Batch 1	>=10%	8-9.9%	<8%	6.7	8.7	7.5	9.1	8.3	10.3	10.90	12.5	8.2	9.4	22.11	12.7	13.6	12.3	5.17
lab 1	Kit B	Batch 1	>=50%	45-50%	<=45%	23.2	24.2	20	58.6	57.5	58.3	0.98	93.8	94	84.6	5.91	217.8	217.8	218.2	0.11
lab 26	Kit B	Batch 2	>=50%	45-50%	<=45%	25.2	27.2	27.5	53.4	51.2	48.3	5.02	77.8	76.6	78.3	1.13	292.7	290.5	303.4	2.34
lab 29	Kit C	Batch 1	>30%	-	<30%	3.91	4.35	4.78	14.47	12.72	12.51	8.13	30.68	30.14	22.3	16.93	161.7	169.42	170.84	2.94
lab 30	Kit C	Batch 2	>=30%	-	<30%	9.3012	7.5295	6.4469	35.679	34.744	33.366	3.36	51.476	50.148	40.502	12.64	142.96	136.61	134.01	3.34
lab 9	Kit C	Batch 3	>=30%	-	<30%	9.3	9.2	8.4	32.7	32.1	30.9	2.87	50.1	51.1	48.6	2.52	153.2	153.2	143.6	3.70
lab 2	Kit D	Batch 1	>=55%	45-55%	<=45%	13.2	14.2	11.2	74.3	71.2	76.4	3.54	116.2	124.2	122	3.42	189.5	185.8	190	1.22
lab 23	Kit D	Batch 2	>=55%	45-55%	<=45%	13.172	11.032	8.782	63.557	55.488	57.958	7.01	87.925	96.432	98.408	5.91	166.905	167.234	156.037	3.90
lab 27	Kit D	Batch 2	>=55%	45-55%	<=45%	18	16	15.9	75	74	78.7	3.26	131	133	125	3.21	219	212	203	3.80
lab 47	Kit D	Batch 2	>=55%	45-55%	<=45%	13.002	13.351	12.653	63.874	64.921	59.511	4.57	116.318	113.874	99.04	8.52	174.433	106.733	162.653	24.45
lab 32	Kit D	Batch 3	>=55%	45-55%	<=45%	12.28	12.41	10.56	71.99	74.76	71.07	2.65	120.21	111.49	118.75	4.00	224.7	228.66	220.47	1.82
lab 20	Kit D	Batch 4	>=55%	45-55%	<=45%	9.27	6.18	2.715	61.33	64.232	60.206	3.35	97.472	105.056	96.067	4.86	159.551	145.693	179.869	10.63
lab 24	Kit D	Batch 4	>=55%	45-55%	<=45%	11.8	12.6	13	108.9	75.4	75	22.51	124.3	118.7	140.5	8.86	197.2	268.8	209.2	17.04
lab 48	Kit D	Batch 4	>=55%	45-55%	<=45%	15	16	14	72	68	65	5.14	111	109	110	0.91	170	190	165	7.56
lab 4	Kit D	Batch 5	>=55%	45-55%	<=45%	16.95	17.61	14.16	74.4	72.99	68	4.68	119.35	125.95	113.71	5.12	187.27	189.89	193.84	1.74
lab 13	Kit D	Batch 5	>= 55%	45-55%	<= 45%	17	22	23	95	100	88	6.39	183	125	178	19.84	260	267	235	6.62
lab 19	Kit D	Batch 5	>=55%	45-55%	<=45%	13.5	13.6	12.6	63.5	59.2	56.8	5.67	104.6	104.4	101					
lab 21	Kit D	Batch 5	>=55%	45-55%	<=45%	16.99	16.23	16.9	73.17	64.68	67.8	6.26	112.3	119.3	120.4					
lab 31	Kit D	Batch 5	>=55%	45-55%	<=45%	14.475	11.315	9.863	59.052	45.901	53.843	12.51	83.205	76.9	86.465	5.93	131.042	141.033	121.563	7.42
lab 34	Kit E	Batch 1	>= 25%	-	<= 25%	19.40	16.80	18.20	53.20	58.80	47.00	11.14	71.61	58.30	54.90	14.34	108.00	95.78	99.90	6.14
lab 25	Kit F	Batch 1	>=10%	-	<10%	3.937	4.071	3.466	5.686	5.956	4.778	11.28	6.797	6.023	5.114	14.09	47.222	20.962	15.242	61.33
lab 37	Kit F	Batch 2	>=10%	-	<10%	7.6	6.7	6.4	9.2	8.3	8	7.35	9.7	9.4	9.4	1.82	33	33.7	30	6.10

Method? Repeatability? Kit?

Method?Repeatability?

Method? Repeatability? Discrimination of kit F (cut-off)?

Conclusion

- **Very satisfactory,** strong influence of the kit used,
- Minor problems except for 1 lab (lack of Se) → Follow-up (other panel) ☺

- **Traceability problems**, **discrepancies between qualitative and quantitative** results → easily corrected

- **Importance of including an internal pos. control in the analysis**

→ indexes calculated from C+, additional internal control in each plate

→ check the repeatability between plates and Se

- Compared to last ring trial,
  - ↑ participants this time (22 *vs*. 18)
  - same variety of kits used
  - as for the kits tested by the EURL, no kit standardisation problems
  - as regards troubles faced by the laboratories, less sensitivity troubles observed this time

Thanks for your attention!!