

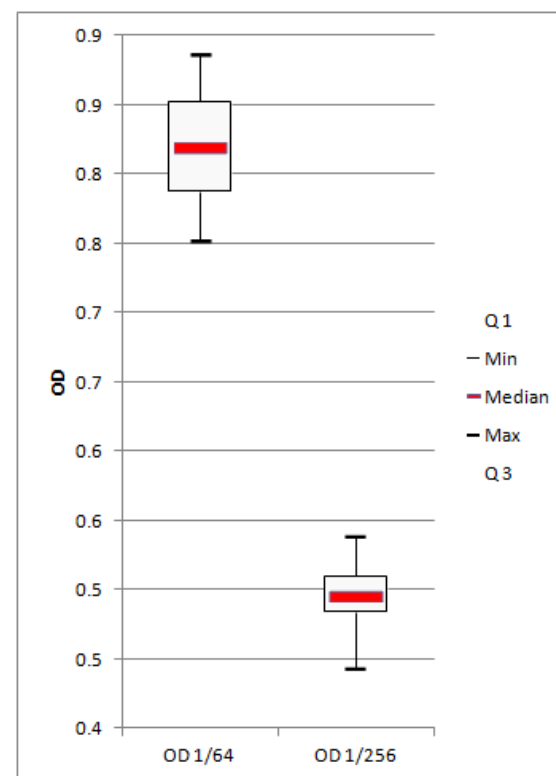
# European Porcine Brucellosis Standard Serum (EUPBSS) iELISA (pig iELISA) validation Ring-Trial



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



- Its origins...
  - Infected herd with *Brucella suis* biovar 2 isolation
  - 200 mL from 5 infected pigs (individually tested) pooled
  - Aliquoted; Freeze-dried (2012)
  - Homogeneity checked (2013)
    - 20 ≠ vials tested at two dilutions, in duplicates around the estimated cut-off (1/64 – 1/256)
    - Comparison of OD mean of each vial
    - CV between the vials **4.85%** - **5.37%** < 10%





## Preliminary testing

Sample	Day 1 (08/04/0213)					Day 2 (09/04/0213)					Day 3 (10/04/0213)				
	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%
EUPigBSS_1/2	1.58	1.63	1.04	P	2.37	Not tested									
EUPigBSS_1/4	1.46	1.52	0.97	P	2.71										
EUPigBSS_1/8	1.34	1.33	0.87	P	0.45										
EUPigBSS_1/16	1.12	1.21	0.76	P	5.19										
EUPigBSS_1/32	0.94	0.97	0.62	P	2.33	1.105	1.171	0.72	P	4.09	1.125	1.100	0.72	P	1.58
EUPigBSS_1/64	0.59	0.63	0.40	P	n/a	0.573	0.561	0.36	P	n/a	0.573	0.573	0.37	P	n/a
EUPigBSS_1/128	0.53	0.41	0.30	P	n/a	0.314	0.351	0.21	N	n/a	0.350	0.381	0.24	N	n/a
EUPigBSS_1/256	0.28	0.25	0.17	N	n/a	0.194	0.202	0.13	N	n/a	0.194	0.190	0.12	N	n/a
EUPigBSS_1/512	0.17	0.14	0.10	N	n/a	0.121	0.140	0.08	N	n/a	0.126	0.130	0.08	N	n/a
ISaBsS_1/4	0.67	0.69	0.44	P	n/a	0.668	0.669	0.42	P	n/a	0.677	0.683	0.44	P	n/a
ISaBsS_1/8	0.54	0.52	0.34	P	n/a	0.601	0.626	0.39	P	n/a	0.564	0.581	0.37	P	n/a
ISaBsS_1/16	0.40	0.39	0.25	P	n/a	0.386	0.427	0.26	P	n/a	0.411	0.387	0.26	P	n/a
ISaBsS_1/32	0.27	0.25	0.17	N	n/a	0.241	0.266	0.16	N	n/a	0.266	0.270	0.17	N	n/a
ISaBsS_1/64	0.17	0.17	0.11	N	n/a	0.151	0.156	0.09	N	n/a	0.153	0.145	0.10	N	n/a
Pig_Neg	0.05	0.05	0.03	N	n/a	0.043	0.045	0.03	N	n/a	0.043	0.047	0.03	N	n/a

Pos. : 1/64 - Neg. : 1/256  
 No variation between vials/ stability of 4°C storage

Sample	Day 1 stored 24 h at 5±3°C (09/04/0213)					Day 2 stored 24 h at 5±3°C (10/04/0213)				
	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%
EUPigBSS_1/16	1.239	1.31	0.81	P	4.05	1.229	1.29	0.82	P	3.51
EUPigBSS_1/32	0.799	0.93	0.55	P	10.75	0.904	0.85	0.57	P	4.54
EUPigBSS_1/64	0.662	0.61	0.40	P	n/a	0.646	0.63	0.41	P	n/a
EUPigBSS_1/128	0.347	0.36	0.22	N	n/a	0.327	0.31	0.21	N	n/a
EUPigBSS_1/256	0.224	0.22	0.14	N	n/a	0.226	0.21	0.14	N	n/a
EUPigBSS_1/512	0.120	0.13	0.08	N	n/a	0.132	0.14	0.09	N	n/a

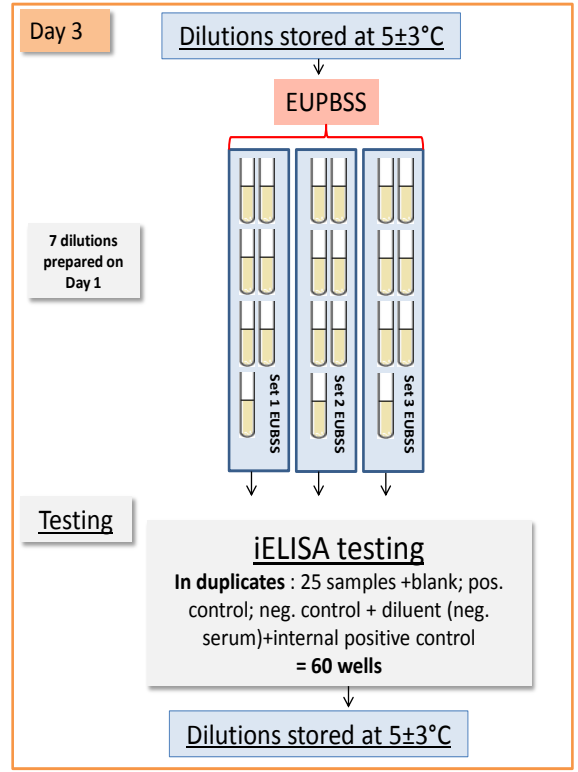
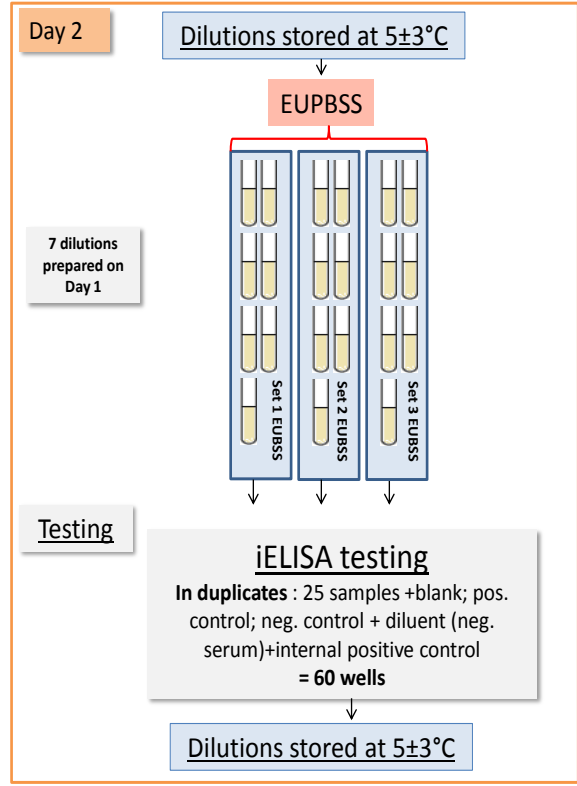
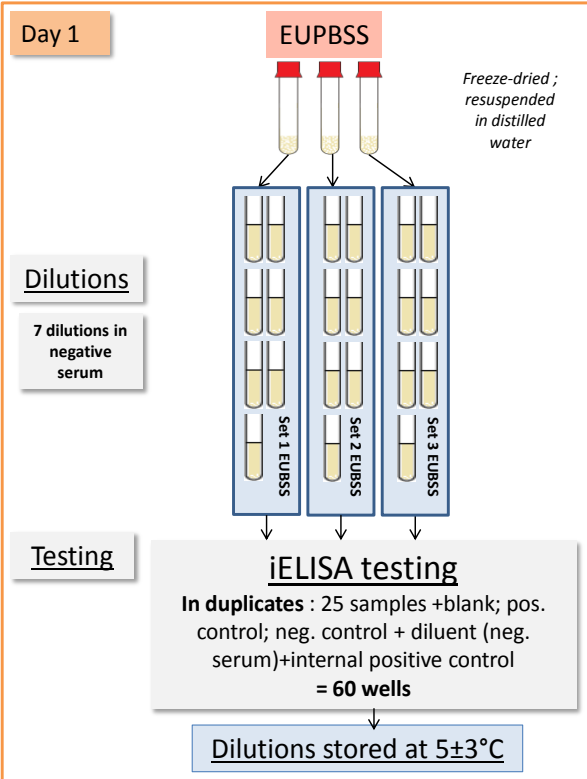
Sample	Day 1 stored 48h at 5±3°C (10/04/0213)				
	DO1	DO2	S/P	Résultat (seuil 0,25)	cv%
EUPigBSS_1/16	1.186	1.15	0.76	P	2.25
EUPigBSS_1/32	0.953	1.00	0.64	P	3.69
EUPigBSS_1/64	0.647	0.68	0.43	P	n/a
EUPigBSS_1/128	0.380	0.35	0.24	N	n/a
EUPigBSS_1/256	0.176	0.18	0.12	N	n/a
EUPigBSS_1/512	0.133	0.14	0.09	N	n/a



- Set the conditions of standardisation **the EUPBSS in iELISA** (pig iELISA)
- Initially comparison with ISaBsS
  - recent changes, ISaBsS = no longer recognized, neither distributed
- participating laboratories → test ≠ dilutions of the EU candidate sera
- laboratories that still have a vial of ISaBsS performed the comparison
- Kits were provided / EURL : ↑ harmonization of test conditions



- The laboratories were provided with:
  - ✓ 4 vials of EUPBSS freeze-dried (1 extra)
  - ✓ 1 iELISA kit Ingezim Brucella Porcino
- In addition :
  - ✓ 10 mL of negative porcine serum (dilution)
  - ✓ If another kit is used routinely to perform iELISA :  
parallel testing
- ☞ 3 consecutive days testing



Intra-vials → homogeneity (3 vials)  
Inter-days → stability (3 days at 4°C)



- Dilutions tested (vial resuspended in 1 mL of distilled water) **in duplicates**

Final dilution	Volume of standard serum		Vol. of neg serum (µl)	Total prepared vol. (µl)	Total required vol. for 3-day testing (µl)	
	Pre-dilution	Volume (µl)				
1/16	no (neat)	10	150	160	30	Direct dilution required (serial dilution not allowed)
1/32	no (neat)	10	310	320	30	
1/64	no (neat)	10	630	640	30	
1/128	1/64*	20	20	40	30	Direct dilution of a pre-dilution allowed
1/256	1/64*	15	45	60	30	
1/512	1/64*	10	70	80	30	
1/1024	1/64*	10	150	160	30	

- Dilution of ISaBsS

Final dilution	Vol. Standard serum (µl)		Vol. of neg serum (µl) <sup>(1)</sup>	Total prepared vol. (µl)	Total required vol. for 3 days-testing (µl) <sup>(2)</sup>	
	Pre-dilution	Volume (µl)				
1/8	no (neat)	10	70	80	30	Direct dilution required (serial dilution not allowed)
1/16	no (neat)	10	150	160	30	
1/304	1/16*	10	180	190	30	
1/400	1/16*	10	240	250	30	

- Following the supplier instructions



- Invitation sent during summer 2013, protocol presented during 2013 WS
  - 23 participating laboratories (20 NRLs + OIE network)
- Package sent early September 2013 → DDL : 31/10/13
  - No trouble during shipment
- Results sent back in due time
- 4 laboratories tested 2 kits in parallel
- 9 labs tested ISaBsS

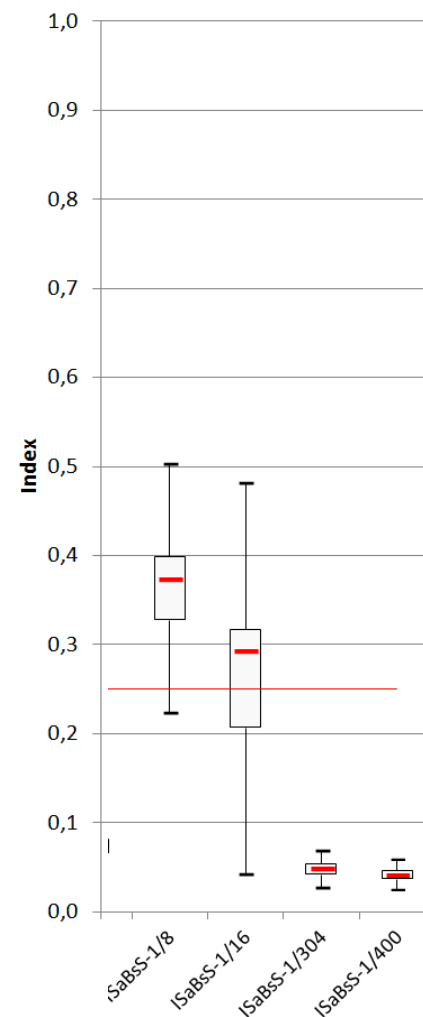




## • Validation criteria

- ISaBsS results... but available for few laboratories, important variability in the results, reliability of the results ?
- OD blank, C+; C-
- Indexes and interpretation checked
- $CV\% (OD_1; OD_2) < 20\%$ 
  - 18 results not included

		ISaBsS			
		1/8	1/16	1/304	1/400
Ingezim	DAY 1	1/0/7	3/0/5	8/0/0	8/0/0
	DAY 2	0/0/8	3/0/5	8/0/0	8/0/0
	DAY 3	1/0/7	4/0/4	8/0/0	8/0/0
Other	DAY 1	0/0/1	0/0/1	1/0/0	1/0/0
	DAY 2	0/0/1	0/0/1	1/0/0	1/0/0
	DAY 3	0/0/1	0/0/1	1/0/0	1/0/0





		EUPBSS Set 1							EUPBSS Set 2							EUPBSS Set 3						
		1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Ingezim	DAY 1	0/0/24	0/0/24	0/0/24	2/0/22	23/0/1	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	3/0/21	23/0/1	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	2/0/22	23/0/1	24/0/0	24/0/0
	DAY 2	0/0/24	0/0/24	1/0/23	1/0/23	23/0/1	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	2/0/22	22/0/2	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	2/0/22	23/0/1	24/0/0	24/0/0
	DAY 3	0/0/24	0/0/24	0/0/24	3/0/21	24/0/0	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	3/0/21	23/0/1	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	5/0/19	23/0/1	24/0/0	24/0/0
Other	DAY 1	0/0/4	0/0/4	1/0/3	1/0/3	2/0/2	4/0/0	4/0/0	0/0/4	0/0/4	1/0/3	1/0/3	3/0/1	4/0/0	4/0/0	0/0/4	0/0/4	1/0/3	2/0/2	3/0/1	4/0/0	4/0/0
	DAY 2	0/0/3	0/0/3	1/0/2	1/0/2	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	1/0/2	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	1/0/2	2/0/1	3/0/0	3/0/0
	DAY 3	0/0/3	0/0/3	0/0/3	2/0/1	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	2/0/1	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	2/0/1	2/0/1	3/0/0	3/0/0

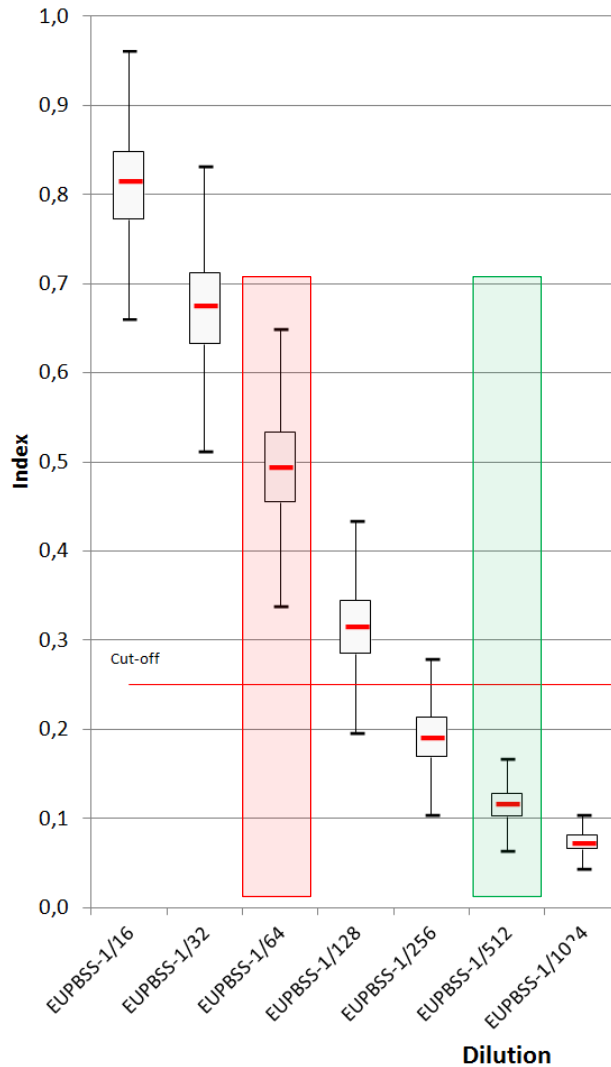
1 home-made kit with lower OD

CV>20% excluded (n =18)

		EUPBSS Set 1							EUPBSS Set 2							EUPBSS Set 3						
		1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Ingezim	DAY 1	0/0/24	0/0/24	0/0/24	2/0/22	22/0/1	24/0/0	23/0/0	0/0/24	0/0/24	0/0/23	3/0/21	23/0/1	23/0/0	24/0/0	0/0/24	0/0/24	0/0/24	2/0/22	23/0/1	24/0/0	22/0/0
	DAY 2	0/0/24	0/0/24	0/0/23	1/0/23	23/0/1	24/0/0	23/0/0	0/0/24	0/0/24	0/0/24	2/0/22	22/0/1	24/0/0	23/0/0	0/0/24	0/0/24	0/0/23	2/0/22	22/0/1	24/0/0	24/0/0
	DAY 3	0/0/24	0/0/23	0/0/23	3/0/21	24/0/0	24/0/0	24/0/0	0/0/24	0/0/24	0/0/23	3/0/21	23/0/1	24/0/0	24/0/0	0/0/24	0/0/24	0/0/24	5/0/18	23/0/1	24/0/0	23/0/0
Other	DAY 1	0/0/4	0/0/4	1/0/3	1/0/3	2/0/2	4/0/0	4/0/0	0/0/4	0/0/4	1/0/2	1/0/3	3/0/1	4/0/0	4/0/0	0/0/4	0/0/4	1/0/3	2/0/2	3/0/1	4/0/0	4/0/0
	DAY 2	0/0/3	0/0/3	1/0/2	1/0/2	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	1/0/2	1/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	1/0/2	1/0/1	2/0/0	3/0/0
	DAY 3	0/0/3	0/0/3	0/0/3	2/0/1	2/0/1	3/0/0	3/0/0	0/0/3	0/0/3	1/0/2	2/0/1	2/0/1	2/0/0	3/0/0	0/0/3	0/0/3	1/0/2	2/0/1	2/0/1	3/0/0	2/0/0



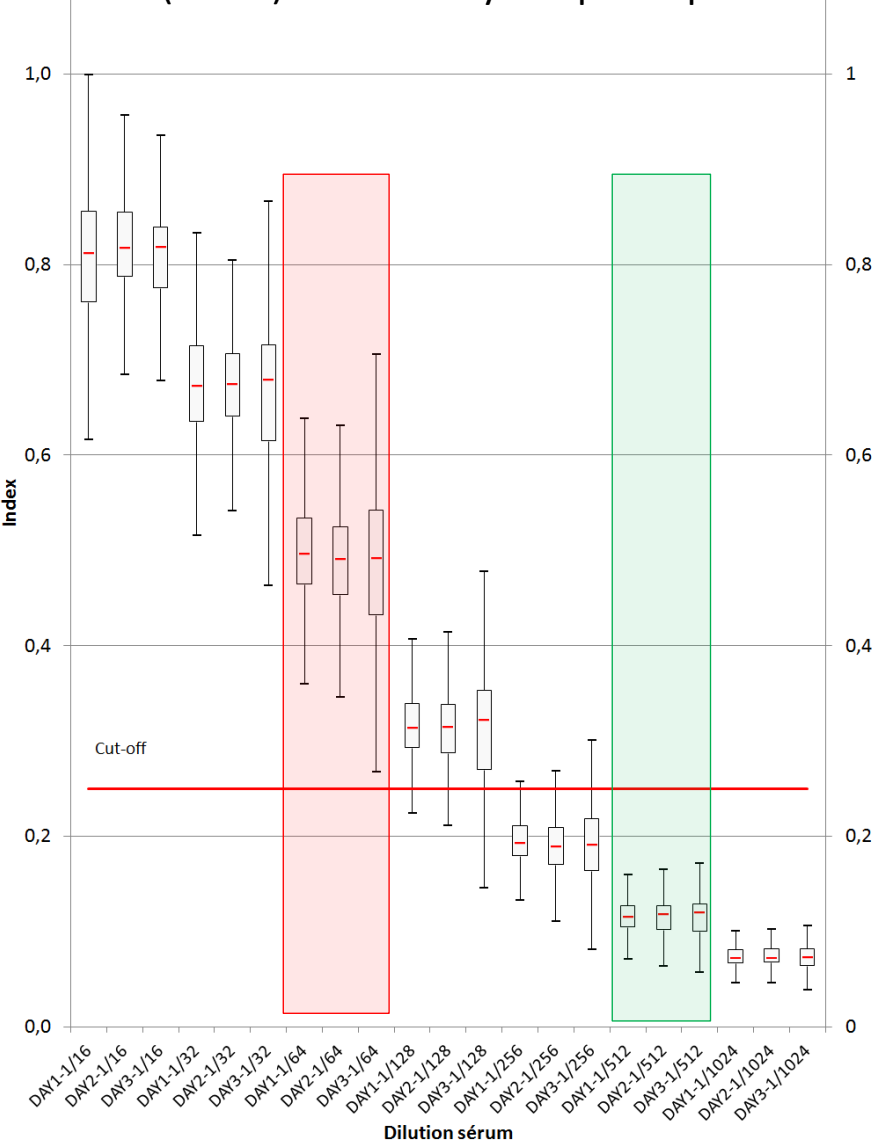
Index (mean) obtained by the participants for each dilution (whatever the testing-day, exc. CV > 20%)



- Positive results at 1/64
- Negative results at 1/512
- Consistent with EURL results



Index (mean) obtained by the participants for each dilution & each testing day (exc. CV > 20%)



- No significant difference **between testing days**
- No significant difference **between the vials**

## Proposed iELISA (pig iELISA) cut-off for EUPBSS

- Pos. At 1/64
- In between : P/N/D
- Neg. at 1/512



# European *Brucella ovis* Standard Serum (EUBoSS)

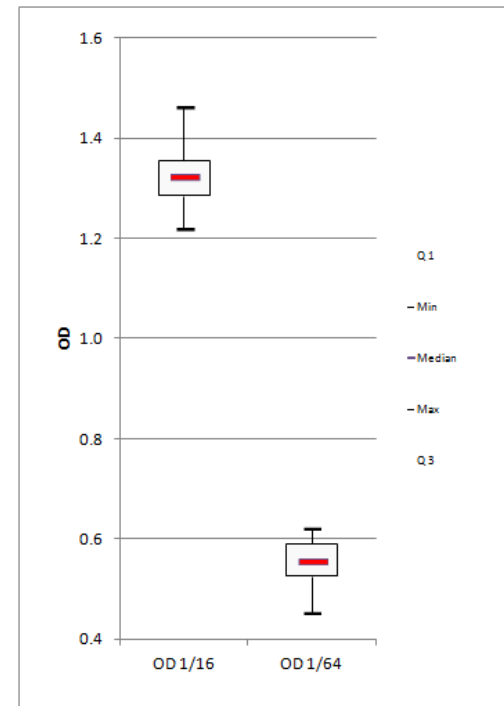
## iELISA & CFT *B. ovis* validation Ring-Trial



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



- Its origins...
  - Infected herd with *Brucella ovis* isolation
  - 1L from 3 infected sheep (individually tested) pooled
  - Aliquoted; freeze-dried (2012);
  - Homogeneity checked (2013)
    - 20 ≠ vials tested at two dilutions, in duplicates around the estimated cut-off (1/16 - 1/64)
    - Comparison of OD mean of each vial
    - CV between the vials **5.30% & 7.93%** < 10%





## Preliminary testing

Sample	Day 1 (08/04/2013)									Day 2 (09/04/2013)						Day 3 (10/04/2013)									
	DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%		DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%		DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%
ISaBoS 1/8	2.203	2.479	213.34	P	P	8.33	0.393	0.712	2.363	2.643	202.16	P	P	7.92	0.449	0.807	2.810	2.283	206.41	P	P	14.64	0.442	0.800	
ISaBoS 1/32	1.069	1.189	99.27	P	P	7.52	0.393	0.712	1.174	1.250	93.97	P	P	4.46	0.449	0.807	1.287	1.101	93.04	P	P	11.03	0.442	0.800	
ISaBoS 1/64	0.533	0.710	51.53	D	P	20.13	0.393	0.712	0.662	0.747	51.44	D	P	8.45	0.449	0.807	0.785	0.744	57.05	D	P	3.76	0.442	0.800	
ISaBoS 1/128	0.350	0.403	28.46	N	D	n/a	0.393	0.712	0.401	0.422	26.87	N	D	n/a	0.449	0.807	0.501	0.472	33.76	D	D	n/a	0.442	0.800	
ISaBoS 1/256	0.215	0.237	14.30	N	D	n/a	0.393	0.712	0.217	0.237	11.42	N	D	n/a	0.449	0.807	0.286	0.264	16.02	N	D	n/a	0.442	0.800	
ISaBoS 1/512	0.143	0.164	7.47	N	N	n/a	0.393	0.712	0.155	0.160	5.62	N	N	n/a	0.449	0.807	0.178	0.190	8.42	N	N	n/a	0.442	0.800	
Ovm Neg	0.078	0.084	0.68	N	N	n/a	0.393	0.712	0.100	0.102	0.85	N	N	n/a	0.449	0.807	0.109	0.325	11.18	N	D	n/a	0.442	0.800	

Sample	Day 3 (10/04/2013)							
	DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%
ISaBoS -20°C 1/8	2.642	2.539	210.10	P	P	2.80	0.442	0.800
ISaBoS -20°C 1/32	1.257	1.317	100.86	P	P	3.29	0.442	0.800
ISaBoS -20°C 1/64	0.765	0.807	58.89	D	P	3.77	0.442	0.800
ISaBoS -20°C 1/128	0.486	0.550	36.40	D	D	n/a	0.442	0.800
ISaBoS -20°C 1/256	0.273	0.295	16.80	N	D	n/a	0.442	0.800
ISaBoS -20°C 1/512	0.181	0.166	7.50	N	N	n/a	0.442	0.800

Sample	Day 1 (08/04/2013)									Day 2 (09/04/2013)						Day 3 (10/04/2013)									
	DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%		DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%		DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%
EUBovisBSS 1/2	2.838	2.980	266.77	P	P	3.45	0.393	0.712	2.643	2.800	220.47	P	P	8.07	0.449	0.807	2.721	2.490	211.36	P	P	6.27	0.442	0.800	
EUBovisBSS 1/4	2.197	2.513	214.65	P	P	9.49	0.393	0.712	1.867	2.041	181.18	P	P	0.807	2.050	1.874	167.42	P	P	6.33	0.442	0.800			
EUBovisBSS 1/8	1.306	1.689	133.92	P	P	18.10	0.393	0.712	1.088	1.216	106.36	P	P	0.449	0.807	1.360	1.416	109.29	P	P	2.85	0.442	0.800		
EUBovisBSS 1/16	0.991	1.162	94.33	P	P	11.20	0.393	0.712	0.806	0.866	76.37	P	P	8.35	0.449	0.807	1.038	0.849	72.07	P	P	14.18	0.442	0.800	
EUBovisBSS 1/32	0.557	0.791	56.42	D	P	24.55	0.393	0.712	0.433	0.433	37.45	D	D	n/a	0.449	0.807	0.530	0.436	33.47	D	D	n/a	0.442	0.800	
EUBovisBSS 1/64	0.322	0.364	25.30	N	D	n/a	0.393	0.712	0.234	0.234	11.37	N	D	n/a	0.449	0.807	0.272	0.245	14.65	N	D	n/a	0.442	0.800	
EUBovisBSS 1/128	0.196	0.226	12.90	N	D	n/a	0.393	0.712	0.157	0.157	7.71	N	D	n/a	0.449	0.807	0.197	0.163	8.04	N	N	n/a	0.442	0.800	
EUBovisBSS 1/256	0.148	0.172	8.07	N	N	n/a	0.393	0.712	0.133	0.133	3.12	N	N	n/a	0.449	0.807	0.134	0.125	3.83	N	N	n/a	0.442	0.800	
EUBovisBSS 1/512	0.097	0.112	2.90	N	N	n/a	0.393	0.712																	

No variation between vials/ stability of 4°C storage  
Pos.: 1/16 - Neg.: 1/512

Sample	Day 1 stored 24 h at 5±3°C (09/04/2013)									Day 2 stored 24 h at 5±3°C (10/04/2013)							
	DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%		DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%
EUBovisBSS 1/4	2.532	2.631	208.74	P	P	2.73	0.449	0.807									
EUBovisBSS 1/8	1.829	1.982	152.08	P	P	5.67	0.449	0.807	1.917	1.800	148.72	P	P	4.45	0.442	0.800	
EUBovisBSS 1/16	1.300	1.392	105.20	P	P	4.85	0.449	0.807	1.278	1.216	97.47	P	P	3.51	0.442	0.800	
EUBovisBSS 1/32	0.798	0.807	59.66	D	P	0.78	0.449	0.807	0.815	0.748	58.47	D	P	6.14	0.442	0.800	
EUBovisBSS 1/64	0.409	0.453	28.51	N	D	n/a	0.449	0.807	0.407	0.469	29.66	N	D	n/a	0.442	0.800	
EUBovisBSS 1/128	0.221	0.270	12.99	N	D	n/a	0.449	0.807	0.248	0.240	13.40	N	D	n/a	0.442	0.800	
EUBovisBSS 1/256	0.160	0.173	6.32	N	N	n/a	0.449	0.807	0.202	0.200	9.80	N	N	n/a	0.442	0.800	
EUBovisBSS 1/512	0.130	0.135	3.48	N	N	n/a	0.449	0.807									

Sample	Day 1 stored 48 h at 5±3°C (10/04/2013)							
	DO1	DO2	DO (%)	Résultat (seuil 30 % - 60 %)	Résultat (seuil 10 % - 50 %)	CV%	DO 30%	DO 60%
EUBovisBSS 1/4								
EUBovisBSS 1/8	1.883	1.793	147.03	P	P	3.46	0.442	0.800
EUBovisBSS 1/16	1.198	1.224	94.50	P	P	1.51	0.442	0.800
EUBovisBSS 1/32	0.826	0.711	57.42	D	P	10.56	0.442	0.800
EUBovisBSS 1/64	0.396	0.452	28.54	N	D	n/a	0.442	0.800
EUBovisBSS 1/128	0.246	0.262	14.27	N	D	n/a	0.442	0.800
EUBovisBSS 1/256	0.165	0.172	7.09	N	N	n/a	0.442	0.800
EUBovisBSS 1/512								



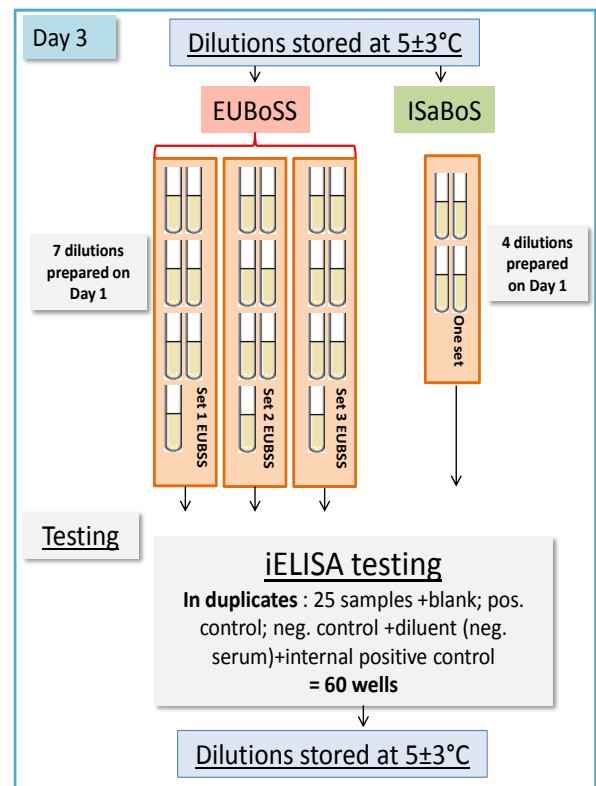
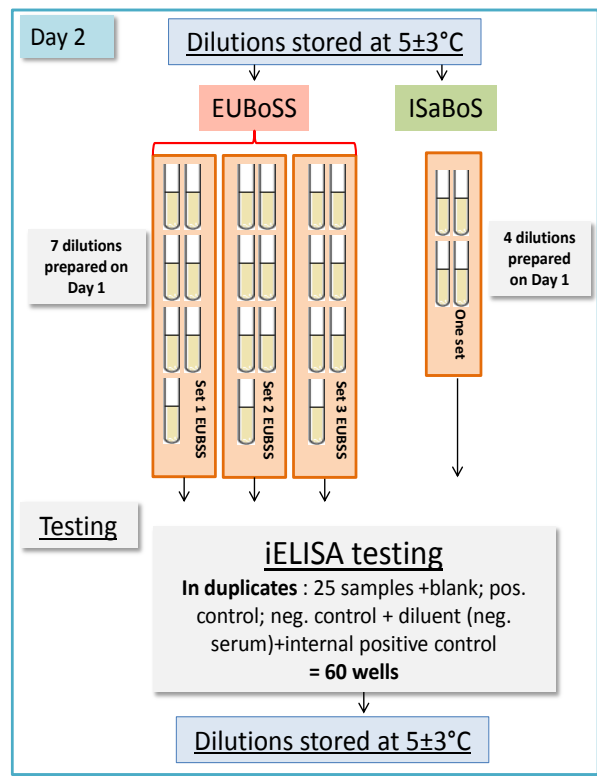
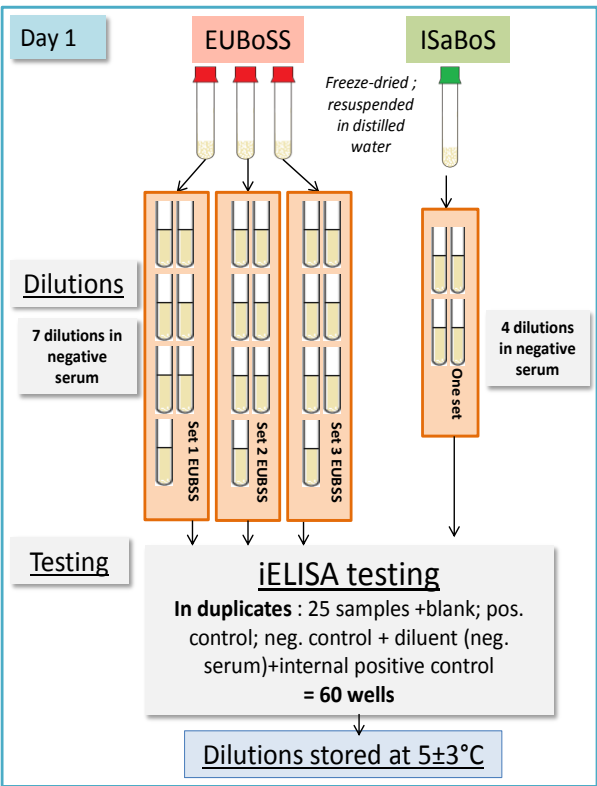




- Set the conditions of standardisation **the EUBoSS in iELISA** (*B. ovis* iELISA)
- In comparison with ISaBoS (limited availability, one vial diluted 1/8)
- participating laboratories → test ≠ dilutions of the EU candidate sera in parallel with ISaBoSS
- Kits were provided / EURL : ↑ harmonization of test conditions



- The laboratories were provided with:
  - ✓ 4 vials of EUBoSS freeze-dried (1 extra)
  - ✓ 1 vial of 500µl diluted ISaBoS (1/8)
  - ✓ An iELISA kit Idexx *Brucella ovis*
- In addition :
  - ✓ 10 mL of negative ovine serum (dilution)
  - ✓ If another kit is used routinely to perform iELISA : parallel testing
  - ✓ If *B. ovis* CFT is performed : parallel testing
- ☞ 3 consecutive days testing



Intra-vials → homogeneity (3 vials)  
Inter-days → stability (3 days at 4°C)





- Dilutions tested (vial resuspended in 1 mL of distilled water) **in duplicates**

Final dilution	Volume of standard serum		Vol. of neg serum (µl)	Total prepared vol. (µl)	Total required vol. for 3-day testing (µl)	
	Pre-dilution	Volume (µl)				
1/8	No (neat)	10	70	80	30	<b>Direct dilution</b> required (serial dilution not allowed)
1/16	No (neat)	10	150	160	30	
1/32	No (neat)	10	310	320	30	
1/64	No (neat)	10	630	640	30	
1/128	1/64*	20	20	40	30	Direct dilution of a pre-dilution allowed
1/256	1/64*	15	45	60	30	
1/512	1/64*	10	70	80	30	

Dilution to perform	Final dilution	Vol. of diluted (1/8) standard serum (µl)	Vol. of neg serum (µl) <sup>(1)</sup>	Total prepared vol. (µl)	Total required vol. for 3 days-testing (µl) <sup>(2)</sup>	
1/2	1/16	25	25	50	30	<b>Direct dilution</b> required (serial dilution not allowed)
1/4	1/32	15	45	60	30	
1/8	1/64	10	70	80	30	
1/64	1/512	10	630	640	30	

- Following the supplier instructions



- Invitation sent during summer 2013, protocol presented during 2013 WS
  - 21 participating laboratories (19 NRLs + OIE network)
- Package sent early September 2013 → DDL : 31/10/13
  - No trouble during shipment
  - 1 lab had dilution troubles → parcel sent again
- Results sent back in due time
- 1 laboratories tested 2 kits in parralel
- 13 labs tested *B. ovis* CFT in parralel



- Validation criteria
  - ISaBoS results (consistence between participants)
    - 3 labs were not included because of important Se discrepancies on ISaBosS & EUBoS
  - OD blank, C+; C-
  - Indexes and interpretation checked
  - CV% ( $OD_1; OD_2$ ) < 20%
    - 22 results not included
- Interpretation was conducted acc. to EURL's recommandations (Praud et al. 2012)

cut off: **30-60%**

Praud et al. *BMC Veterinary Research* 2012, **8**:68  
<http://www.biomedcentral.com/1746-6148/8/68>



(Supplier 10-50%)

RESEARCH ARTICLE

Open Access

Assessment of the diagnostic sensitivity and specificity of an indirect ELISA kit for the diagnosis of *Brucella ovis* infection in rams

Anne Praud<sup>1</sup>, Jean-Luc Champion<sup>2</sup>, Yannick Corde<sup>3</sup>, Antoine Drapeau<sup>3</sup>, Laurence Meyer<sup>4</sup> and Bruno Garin-Bastuji<sup>3\*</sup>





		EUBoSS Set 1							EUBoSS Set 2							EUBoSS Set 3							ISaBOS				
		1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/16	1/32	1/64	1/512	
IDEXX	DAY 1	0/0/21	0/0/21	0/9/12	5/14/2	18/2/1	20/1/0	21/0/0	1/0/20	0/0/21	0/7/14	8/12/1	21/0/0	21/0/0	21/0/0	0/0/21	0/0/21	0/8/13	7/13/1	19/1/1	20/0/1	21/0/0	21/0/0	0/0/21	0/0/21	1/13/7	21/0/0
IDEXX	DAY 2	0/0/21	0/1/20	0/7/14	8/11/2	17/3/1	19/2/0	21/0/0	1/0/20	0/0/21	1/4/16	5/15/1	19/2/0	20/1/0	21/0/0	0/0/21	0/1/20	0/8/13	4/15/2	18/2/1	19/1/1	21/0/0	21/0/0	0/0/21	0/1/20	1/13/7	20/1/0
IDEXX	DAY 3	0/0/21	0/0/21	0/9/12	10/10/1	19/1/1	20/1/0	21/0/0	1/0/20	0/1/20	0/9/12	8/13/0	20/1/0	21/0/0	21/0/0	0/0/21	0/2/19	1/11/9	8/13/0	19/1/1	20/0/1	21/0/0	21/0/0	1/0/20	0/1/20	0/16/5	21/0/0
Other	DAY 1	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 2	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 3	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0

NO improvement of concordance

CV>20% excluded (n =22)

		EUBoSS Set 1							EUBoSS Set 2							EUBoSS Set 3							ISaBOS				
		1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/16	1/32	1/64	1/512	
IDEXX	DAY 1	0/0/21	0/0/21	0/9/12	5/14/2	16/2/1	20/0/0	19/0/0	0/0/20	0/0/19	0/7/14	8/12/1	18/0/0	18/0/0	21/0/0	0/0/21	0/0/21	0/7/12	7/13/1	19/1/1	20/0/1	20/0/0	20/0/0	0/0/21	0/0/21	1/12/7	20/0/0
IDEXX	DAY 2	0/0/21	0/1/20	0/7/14	7/11/2	16/2/1	19/2/0	20/0/0	1/0/20	0/0/20	1/4/16	5/15/1	17/2/0	19/1/0	21/0/0	0/0/20	0/1/20	0/8/13	4/15/2	18/2/1	19/1/1	21/0/0	21/0/0	0/0/21	0/1/20	1/12/7	18/1/0
IDEXX	DAY 3	0/0/21	0/0/21	0/8/12	10/10/1	18/1/1	20/1/0	21/0/0	1/0/20	0/1/20	0/9/12	8/13/0	18/1/0	20/0/0	21/0/0	0/0/21	0/2/19	1/11/9	7/13/0	18/1/1	19/0/1	21/0/0	21/0/0	1/0/20	0/1/20	0/16/5	21/0/0
Other	DAY 1	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 2	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 3	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0

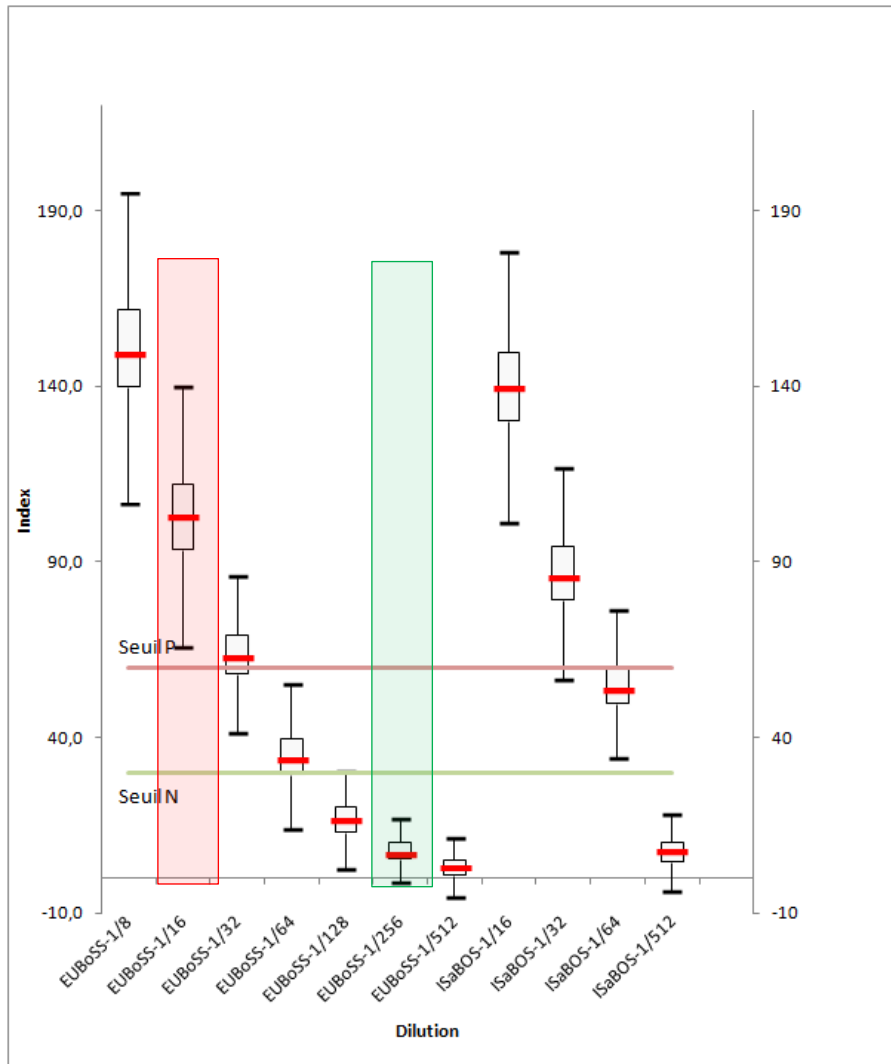
Best concordance

CV>20% excluded (n =22) & 3 labs

		EUBoSS Set 1							EUBoSS Set 2							EUBoSS Set 3							ISaBOS				
		1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/16	1/32	1/64	1/512	
IDEXX	DAY 1	0/0/18	0/0/18	0/6/12	4/12/2	14/2/0	18/0/0	17/0/0	0/0/18	0/0/16	0/5/13	6/11/1	16/0/0	16/0/0	18/0/0	0/0/18	0/0/18	0/5/11	5/12/1	17/1/0	18/0/0	18/0/0	18/0/0	0/0/18	0/0/18	0/11/6	17/0/0
IDEXX	DAY 2	0/0/18	0/0/18	0/6/12	6/11/1	15/1/0	18/0/0	17/0/0	0/0/18	0/0/18	0/4/14	4/14/0	16/1/0	18/0/0	18/0/0	0/0/18	0/0/18	0/7/11	2/15/1	17/1/0	18/0/0	18/0/0	18/0/0	0/0/18	0/0/18	0/12/5	16/0/0
IDEXX	DAY 3	0/0/18	0/0/18	0/7/10	8/10/0	17/1/0	18/0/0	18/0/0	0/0/18	0/0/18	0/8/10	7/11/0	17/1/0	18/0/0	18/0/0	0/0/18	0/1/17	1/9/8	6/12/0	17/1/0	17/0/0	18/0/0	18/0/0	0/0/18	0/1/17	0/14/4	18/0/0
Other	DAY 1	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 2	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0
Other	DAY 3	0/0/1	0/0/1	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	0/0/1	0/0/1	1/0/0	1/0/0



Index (mean) obtained by the participants for each dilution (whatever the testing-day, exc. CV > 20%)

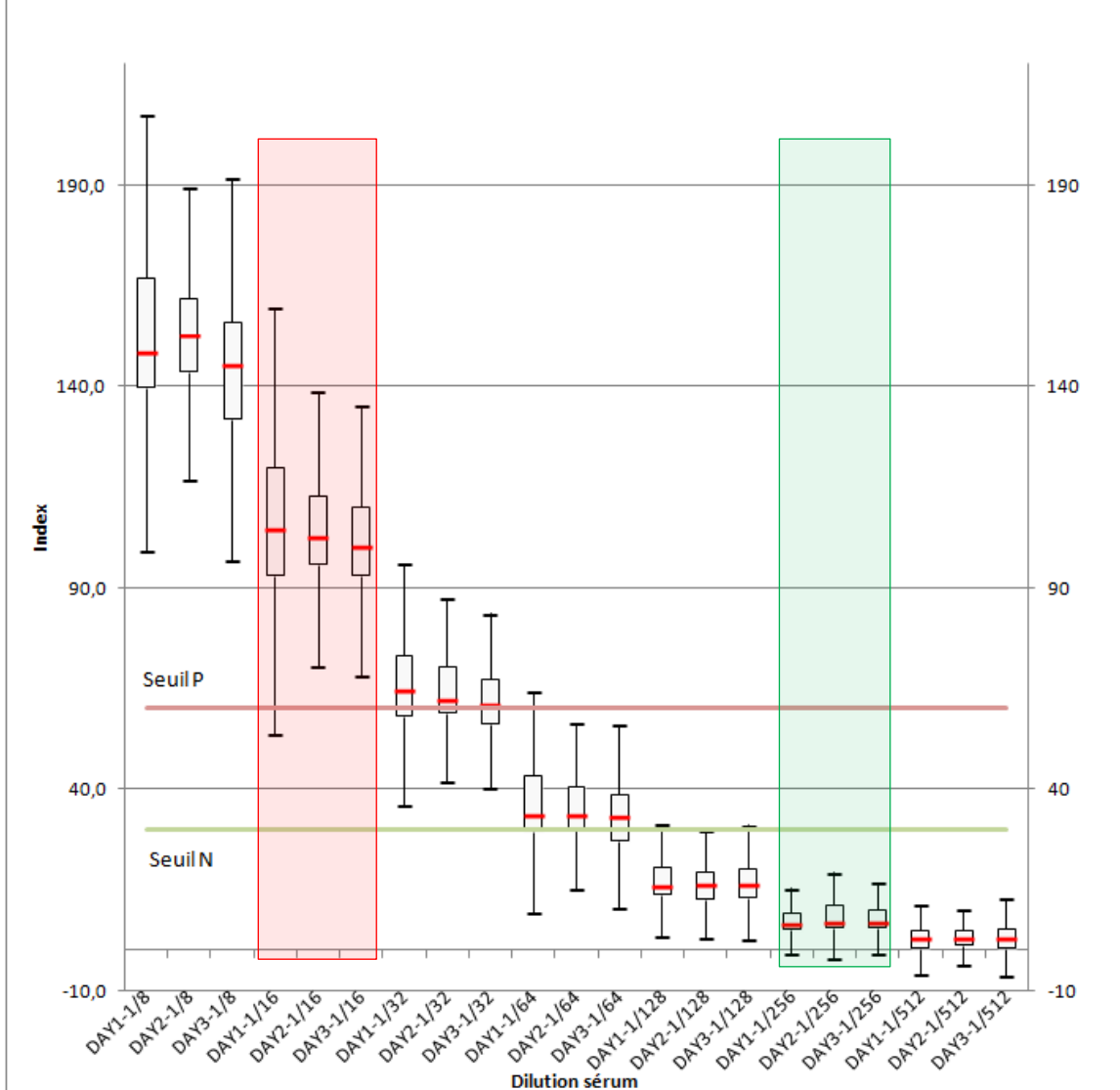


- Positive results at 1/16
- Negative results at 1/256
- Consistent with EURL results





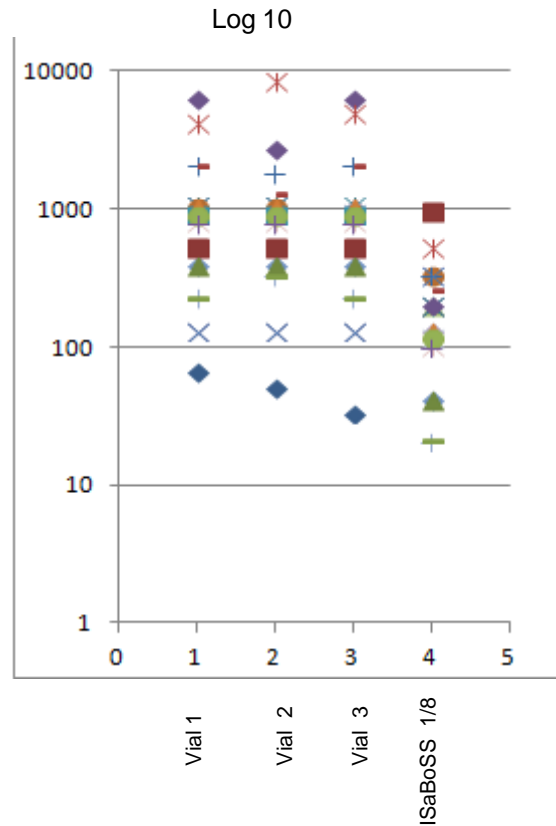
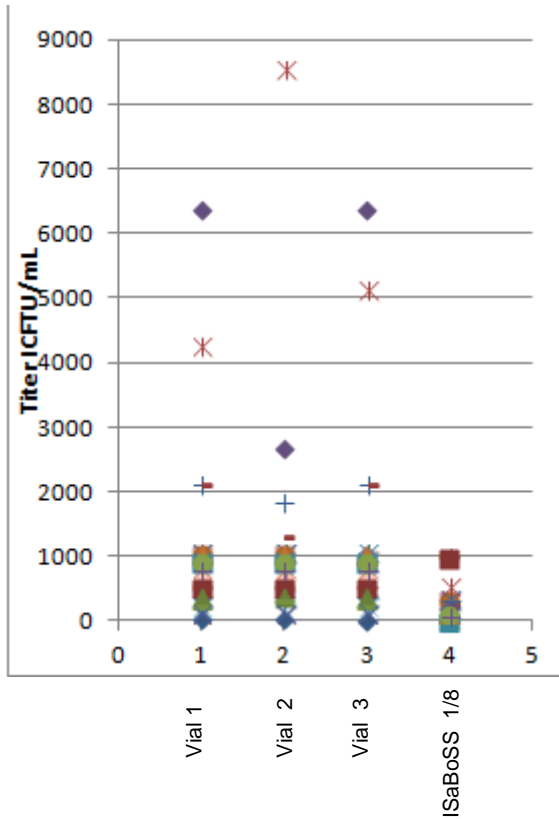
Index (mean) obtained by the participants for each dilution & each testing day (exc. CV > 20%)



- No significant difference between **testing days**
- No significant difference between **vials**



## CFT results (For information)



High titre ranges (>1 dilution) : various protocol, antigens, ... lack of harmonization

👉 Need of a validation trial with **standardized protocol**



## Proposed iELISA cut-off for EUBoSS

- Pos. at 1/32
- In between : P/N/D
- Neg. at 1/256

Proposed CFT validation trial for EUBoSS  
with **standardized protocol**





Thanks for your attention