Analytical calibration of batches of ELISA kits used for the indirect diagnosis of Q fever in ruminants: work in progress

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Background

Three commercially available ELISA kits are widely used serological methods for Q fever in ruminants

✓ Problem: a significant rate of discordant results between the methods

⇒ Major gap for Q fever **epidemiological investigations** [1] and **surveillance** [2]
⇒ Impact on **abortion diagnosis**, as serological analyzes are recommended in addition to qPCR [1, 2]
⇒ Difficulties for diagnostic and reference labs to **ensure reliable and comparable data at network level**

Objectives

A global project was undertaken to **assess and improve** their diagnostic and analytical performances. A previous study, using a Bayesian latent class approach, revealed that **diagnostic performances** are variable among kits: some tests are more sensitive but less specific and vice versa, without pointing a better test for diagnostic applications at herd level, and [3, 4]. This evaluation of the kits encourages to consider the animal species and the epidemiological situation to choose the kit to use.

☛ **Here, the objective** is to define additional decision rules for **validation of kit batches** to better calibrate their **analytical performances** around the **interpretation cut-off (ICO)**, which is the critical area of the method [4, 5, 6].

Explanation of the main concept

Interpretation cut-off (ICO) is the critical area where positive and negative results are segregated

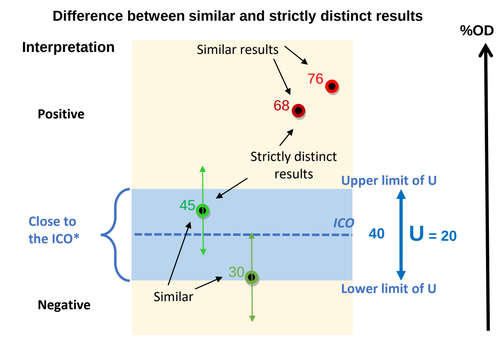
Semi-quantitative or qualitative ELISA results derive from quantitative data

Calibration of the quantitative data is thus crucial

Especially at the ICO → **Trueness of the value**; **Uncertainty of the measure**

U = 2 x Standard Deviation (k=2)

Other options = confidence intervals (CI), ranges (R), coefficient of variation (CV)



*Close to the ICO is an expression used by some diagnostic labs and that should be adopted in the new version of the U47-019 French standard [6]

To obtain reliable diagnostic interpretations relative to the ICO
To allow differentiation between similar and strictly distinct results
→ Statistical studies then become possible

Practical example of the importance of calibration for qualitative methods based on quantitative measures

- **Qualitative interpretation:** A radar used for "speed limit exceeded" alert. This **qualitative information is dependent on the quantitative measure**, especially trueness and precision near the cut-off.
- **Decision at a cut-off :** A car speed control radar to establish fines for overtaking, a ± 5 km/h **tolerance margin is required**. The driver is penalized if the **upper limit** is reached.

Materials and Methods

STEP 1 : to propose an experimental scheme and a reference material (RM) to the three suppliers, in order to assess the analytical performances (trueness, repeatability, reproducibility) in the ICO area

A common procedure was applied by each kit producer

Based on a common RM (same RM distributed to lab users)

Common process thanks to the pivotal role of the Reference laboratory

Two contrasted tracers prepared from the RM (two dilutions selected in the ICO area fixed by each kit producer) and tested according to a common experimental scheme

Each batch of each kit :
- 3 independent analyses of 30 replicates
- each of the 2 contrasted tracers

Estimating statistical parameters (calculations using a common Excel file)

- Mean (trueness)
- Repeatability Standard Deviation (SD_r)
- Inter-series SD (SD_b)
- Reproducibility (SD_R, limits of ± 2xSD_R)
- Coefficients of variation

and recording on the batch certificate (common presentation format)

Batch certificate example (data from the 2 dilutions of the RM tested by a kit producer)

MRE dilution *Niveaux du MRE calibrant FQ*	Titre *Titre*	Low limit of 2 Standard Deviation *Limite basse de 2 écart-types*	High limit of 2 Standard Deviation *Limite haute de 2 écart-types*	Repeatability *Répétabilité (%CV)*	Reproducibility *CV (%) de fidélité intermédiaire (FI)*
1/2	54	43	65	8.3	10.3
1/4	24	18	30	8.4	13.0

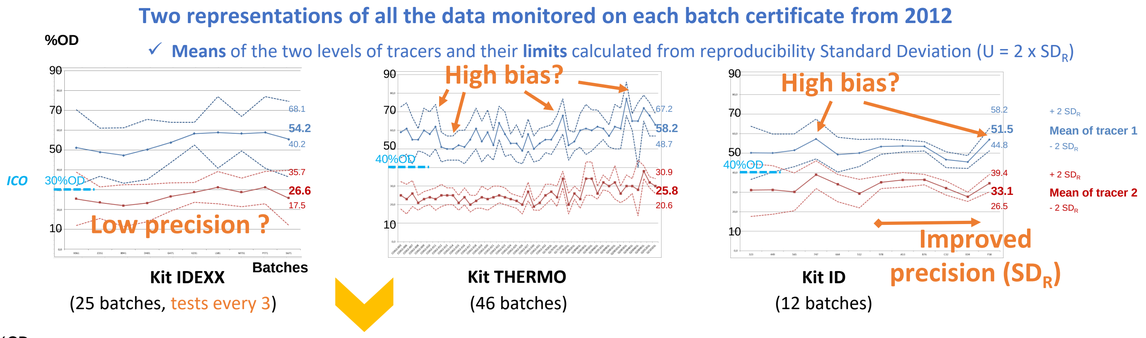
Monitoring of the two tracers around the ICO (each batch)

STEP 2 : to carry out a preliminary study, in order to acquire data on variability parameters from successive batches

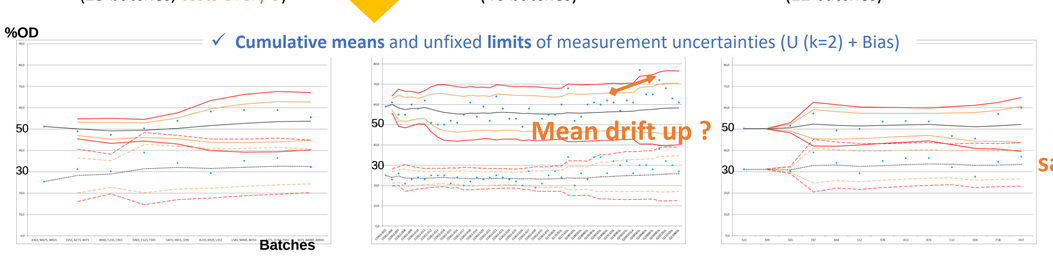
according to the experimental scheme defined in step 1

Two representations of all the data monitored on each batch certificate from 2012

✓ **Means** of the two levels of tracers and their **limits** calculated from reproducibility Standard Deviation (U = 2 x SD_R)



✓ **Cumulative means** and unfixed **limits** of measurement uncertainties (U (k=2) + Bias)



Global precision (SD_R) not satisfactory for the 3 kits ?

Variability parameters estimated via the collection of measures, of **two levels of tracers** prepared from a Reference Material, from **all batches of each kit produced over 10 years**

STEP 3 : to define calibration criteria to control the ICO area for each kit

√ Kit producers (data from batch certificates, i.e. idem step2)

Tracers (2 par kit)	Parameters	Kit IDEXX	Kit THERMO	Kit ID
RM 1/1	Mean (in %OD)	54,2		
RM 1/2		**26,6**	**58,2**	
RM 1/4			25,8	**51,5**
RM 1/8				33,1
RM 1/1	U (k=2) in %OD	14,0		
RM 1/2		**9,1**	**9,0**	
RM 1/4			5,2	**6,7**
RM 1/8				6,3
RM 1/1	CV_R (in %)	11,4		
RM 1/2		**10,4**	**7,6**	
RM 1/4			6,2	**7,2**
RM 1/8				10,8
Number of batches		25	45	12
Number of tests		10	45	12

√ Lab user (single tests per series of the NRL's own activity since 2012)

Tracers (2 par kit)	Parameters	Kit IDEXX	Kit THERMO	Kit ID
RM 1/1	Mean (in %OD)	54,3		
RM 1/2		**29,0**	**55,9**	
RM 1/4			25,6	**55,2**
RM 1/8				34,5
RM 1/1	U (k=2, +Bias) in %OD	17,1		
RM 1/2		**9,4**	**13,8**	
RM 1/4			7,8	**14,1**
RM 1/8				12,3
RM 1/1	CV_R (in %)	13,3		
RM 1/2		**12,9**	**9,4**	
RM 1/4			10,7	**10,6**
RM 1/8				13,2
Number of batches		9	21	9
Number of tests		193	407	199

Two data sources: common dilutions of the RM (1/1 to 1/8) distributed for calibration

√ Inter-laboratory Proficiency tests (focus on ICO area of ILPT samples)

ILPT samples	Parameters	Kit IDEXX	Kit THERMO	Kit ID
E4	Mean (in %OD)	84,7	61,7	86,8
E8		**55,7**	**35,5**	63,4
E16		34,1	18,6	**45,2**
E32		20,3	9,0	31,6
E4	U (SD_{PT} by robust statitics) in %OD	9,0	8,4	11,5
E8		**10,1**	**7,8**	10,3
E16		5,2	4,3	**7,8**
E32		5,2	3,1	5,6
E4	CV_{PT} (calculated by robust statitics) in %	10,6	13,6	13,2
E8		**18,1**	**22,0**	16,2
E16		15,2	23,1	**17,3**
E32		25,6	34,4	17,7
Number of batches		4	6	2
Number of tests		24	20	30

Third data source: some ILPT samples (E4 to E32 tested using kits batches in 2019)
Highest CV_R consistent with lab network tests

Correct consistency between the 2 data sources (different schemes for tests performed in one lab: "step 1" process by producers and control charts by a lab user)

The results allowed to **propose a calibration process** for each batch of the 3 kits →

Criteria	IDEXX	THERMO	*ID*
Dilution of RM (level in ICO area) Choice of a single tracer	**1 : 2**	**1 : 2**	*1 : 4*
Expected mean (reference value)	**26 %DO**	**58 %DO**	*51 %DO*
High and low limits set Measurement uncertainty (U=2xSD_R)	**16 - 36 %DO** ± 15 %DO	**43 - 73 %DO** ± 15 %DO	*36 - 66 %DO* *± 15 %DO*
CVR (reproducibility)	**< 15 %**	**< 15 %**	*< 10 % (?)*
Experimental scheme	**1 batch / 1 operator / 3 independent tests / 24 replicates per plate (72 measurements)**	**1 batch / 1 operator / 3 independent tests / 28 replicates per plate (84 measurements)**	*1 batch / 3? operator / 3 independent tests / 28 replicates per plate (84 measurements)*
ICO fixed by the kit producer	**30 %OD**	**40 %OD**	*40 %OD*

Conclusions and discussion

- A common process was defined to **standardize the analytical performances** of the three ELISA tests.
- The process is **currently being adopted by the three suppliers of the available kits (2 out of 3 have implemented it)**, it needs to be monitored and adjusted if necessary.
- The **known and maintained analytical uncertainty in the ICO area** will be useful for user labs to set modalities for acceptance of each new batch (initial control) and to **establish a single control chart for successive batches based on the assigned value of one tracer per kit** (improve the internal validity of the results as well as the external quality of ILPT).
- Once the kit is a standardized operating procedure (SOP), the **next step is to keep on improving the concordance rate between kits** (working hypothesis taking the species into account)

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Aknowledgements and statements

We thank all the kit producers for their responsible cooperation and their trust. The French Reference laboratory for Q fever acts, either singularly or in collaboration, with the commercial sector, when required, and with impartiality, to ensure new tests are correctly standardized or evaluated to benefit the continual improvement of diagnostic tools.