Euro Reference
journal of Reference

anses
French agency for food, environmental and occupational health & safety

Journal No. 8
Winter 2012

Methods

Adoption by a network's laboratories of a validated quantitative real-time PCR method for monitoring Q fever abortions in ruminant livestock

É. Rousset (elodie.rousset@anses.fr) (1), M. Prigent (myriam.prigent@anses.fr) (1), G. Ameziane (biomol@aveyron-labo.fr) (2), R. Brugidou (brugidou@aveyron-labo.fr) (2), I. Martel (isabelle.martel@cg13.fr) (3), A. Grob (anne.grob@cg13.fr) (3), G. Le Gall (ghislaine.le-gall@idhesa.fr) (4), S. Kerninon (sandrine.kerninon@idhesa.fr) (4), J. Delaval (jdelaval@cg37.fr) (5), A. Chassin (achassin@cg37.fr) (5), B. Vassiloglou (barbara.vassiloglou@cg42.fr) (6), S. Aulagnon (stephane.aulagnon@cg42.fr) (6), A. Valogne (aurele.valognes@cg53.fr) (7), M. Ogier (myriam.ogierdebaulny@cg53.fr) (7), C. Audeval (chantal.audeval@cg58.fr) (8), F. Colocci (francoise.colocci@cg58.fr) (8), S. Perennes (s.perennes@labos-pyrenees.com) (9), L. Cazalis (l.cazalis@labos-pyrenees.com) (9), P. Nicollet (philippe.nicollet@vendee.fr) (10), C. Maingourt (cyril.maingourd@lasat.fr) (10), Karim Sidi-Boumedine (karim.sidi-boumedine@anses.fr) (1)

(1) ANSES, National Reference Laboratory for Q fever, Sophia-Antipolis, France
(2) SEML AVEYRON LABO, Rodez, France
(3) Bouches-du-Rhône Departmental Analysis Laboratory, Marseille, France
(4) GIP IDHESA Bretagne Océane, Quimper, France
(5) Touraine Laboratory, Parcay-Meslay, France
(6) Loire Departmental Veterinary Laboratory, Montbrison, France
(7) Mayenne Departmental Analysis Laboratory, Laval, France
(8) Nièvre Departmental Analysis Laboratory, Nevers, France
(9) Pyrénées Laboratory, Lagor, France
(10) LASAT Niort site, Niort, France

E. Rousset, M. Prigent, G. Ameziane, R. Brugidou, I. Martel, A. GROB, G. Le Gall, S. Kerninon, José Delaval, A. Chassin, B. VASSILOGLOU, S. Aulagnon, A. Valogne, M. Ogier, C. Audeval, F. Colocci, S. Perennes, L. Cazalis, P. Nicollet, C. Maingourt, K. Sidi-Boumedine (2012). Adoption par un réseau de laboratoires d'une méthode de PCR temps réel quantitative validée pour conduire une surveillance des avortements dus à la fièvre Q en élevages de ruminants, N°8, ER08-12ME02 http://www.anses.fr/euroreference/numero8/

In France, Q fever is a disease of concern for the state, both in terms of animal and public health. However, there is as yet little knowledge of epidemiological situations or the exposure risks. To ensure the relevance of both risk assessment and risk management, it therefore seemed essential to implement a surveillance scheme for serial abortions due to Q fever in ruminant livestock.

A key feature of this scheme is the production of reliable, comparable data by a network of participating veterinary analysis laboratories. To achieve this, the PCR methods to be used were first validated in accordance with the new French standards XP-U47-600-1 and XP-U47-600-2. Also with regard to standards, the National Reference Laboratory (NRL) for Q fever organised the adoption of the PCR methods by the laboratories. This adoption phase involved testing to verify that the laboratories obtained the expected performance, as determined during the validation step with regard to limits of detection and accuracy of quantification.

All of the laboratories successfully adopted the methods. The overall analysis of these adoption results is presented to demonstrate for the first time the consistency of the laboratories network's PCR results.

Introduction

Q fever, caused by the bacterium *Coxiella burnetii*, is widespread both geographically (found throughout the world) and in terms of affected animal species (Rousset *et al.,* OIE 2010). In ruminants, the disease is characterised by abortions and can cause significant economic losses (Touratier *et al.,* 2012). In addition, *C. burnetii* is a zoonotic agent, whose transmission to humans occurs primarily by air. Infected animals may shed the bacterium and contaminate the environment. Bacteria can persist in the environment as pseudospores and then be disseminated. The occurrence of cases or outbreaks in the population appears to depend on a combination of factors favouring its airborne diffusion, such as a site's topography or weather conditions (Forland *et al.,* 2012). Nevertheless, the greatest risk of environmental contamination appears to be associated with abortion episodes in livestock, combining both a large number of shedding animals and shedded high individual loads (De Bruin *et al.,* 2012; De Crémoux *et al.,* 2012). Surveillance of farms affected with clinical Q fever has been considered, in order to gain a better understanding of the situation with this disease and its development in France, with a view to assessing the means of control. This surveillance is to be coordinated by the National Platform for Epidemiological Surveillance in Animal Health, created recently in response to the guidelines adopted in 2010 at the national consultation on the health sector (*Etats généraux du sanitaire*) organised by the French Minister for Agriculture.

Euro Reference
journal of Reference
anses
French agency for food, environmental and occupational health & safety

Journal No. 8
Winter 2012

Methods

The surveillance scheme will involve 10 *départements* for three years in order to apply the most rational optimisations with a view to standardising methods (sampling in farms, testing in laboratories and interpreting results). A protocol was therefore proposed for the detection of an episode of Q fever in livestock (Service Memo DGAL/SDSPA/N2012-8188 of 11 September 2012). This protocol is mainly inspired by the conclusions drawn in 2007 by a national working group on Q fever. It was also taken up at European level as a proposal for surveillance based on a passive system (Sidi-Boumedine *et al.,* 2010). PCR analysis performed in the laboratory is a crucial part of this diagnostic procedure. It enables the detection and quantification of bacteria in vaginal (sheep, goats), endocervical (cattle) or placental swabs (all ruminant species) from sampled females. The result is then interpreted in relation to a bacterial load threshold, determined according to expert opinion. The distributions of quantitative data from the surveillance protocol will be studied in order to adjust this clinical threshold, if necessary, depending on the animal species but also on the type of sample.

Therefore, based on the needs and objectives of the surveillance to be implemented, work was undertaken to harmonise and validate the quantitative real-time PCR (qRT-PCR) to be used for the molecular diagnosis of abortive Q fever. Two manufacturers (Adiagene and LSI) submitted their qRT-PCR kits for validation according to a standard procedure proposed by the NRL, and in keeping with the recommendations of the new French standard XP U47-600-2 relating to PCR in animal health, published by AFNOR in June 2011. The supplier reports were reviewed and validated by the NRL in December 2011 according to predetermined performance criteria. Meanwhile, a validation certificate was also issued to one of the network's laboratories that had developed its own method. These validated methods were then approved by the Ministry of Agriculture during accreditation of the laboratories.

Prior to the routine implementation of the method, the French standard recommends conducting adoption tests to verify that the user has been able to achieve the performance claimed by the supplier. Adopting a molecular diagnostic method involves confirming the performance of firstly the PCR step (recommendations in Chapter 11 of the Standard XP U47-600-1, pages 30-32) and secondly the complete analytical method (pages 32-33) in terms of limit of detection and accuracy of quantification. This adoption stage was not required for the one laboratory in the network that had validated its own in-house method. All the other laboratories in the network returned results that complied with the criteria required for the adoption testing.

The purpose here is to analyse all the results in order to obtain a preliminary assessment of the PCR method implemented within the network's laboratories in the context of the surveillance scheme. In addition, the description of this pioneering exercise, conducted under real conditions, will serve to facilitate assimilation of the standard's requirements by future adopters.

Table 1. Distribution of commercial reagents, matrices and devices based on the 11 data sets obtained

Set code	PCR kit manufacturer	Negative biological matrix	Real-time thermal cycler	DNA extraction kit
j	ADIAGENE	Bovine vaginal mucus	Stratagène MxPro 115108	Macherey Nagel Nucleospin Tissue
d	ADIAGENE	Caprine vaginal mucus	Applied Biosystems Abiprism 7500 SDS	Qiagen QIAamp DNA Mini kit
a	ADIAGENE	Bovine vaginal mucus	Applied Biosystems Abiprism 7500	Qiagen QIAamp DNA Mini kit
c	ADIAGENE	Bovine placental cotyledons	Applied Biosystems Abiprism (2*)	Qiagen QIAamp DNA Mini kit
b	LSI	Bovine vaginal mucus	Applied Biosystems Abiprism 7500	Macherey Nagel Nucleospin Tissue
e	LSI	Bovine vaginal mucus	Stratagène MxPro 115108	Macherey Nagel Nucleospin Tissue
k	LSI	Bovine vaginal mucus	Applied Biosystems Abiprism (2*)	Qiagen QIAamp DNA Mini kit
f	LSI	Bovine vaginal mucus	Biorad CFX96 and Biorad CHROMO4 (2*)	Macherey Nagel Nucleospin Tissue
h	LSI	Bovine vaginal mucus	Roche Light Cycler 480	Qiagen QIAamp DNA Mini kit
i	LSI	Bovine vaginal mucus	Applied Biosystems Abiprism 7500 (2*)	Qiagen QIAamp DNA Mini kit
g	LSI	Bovine vaginal mucus	Applied Biosystems Abiprism 7500 (2*)	Qiagen QIAamp DNA Mini kit

*2 different thermal cyclers tested

Methods

Materials and Methods, Results

Description of the quantitative real-time PCR (qRT-PCR) methods

Two commercial PCR methods were assessed in this interlaboratory adoption test: the ADIAVET® COX REALTIME kit, # ADI143 (Adiagene, France) and the TaqVet™ *Coxiella burnetii* - Absolute Quantification kit, # FQPAQ (LSI, France). The requirements of French standard XP U47-600-2, as well as the performance criteria predefined by the NRL, were applied to characterise and validate the methods required for the diagnosis of abortions. Each qRT-PCR kit was associated with two defined methods for extraction and purification of total DNAs using reagents on silica columns (Nucleospin Tissue, Macherey-Nagel, France, and QIAamp DNA mini kit, Qiagen, France). Validation focused on the endocervical, vaginal or placental matrices. As a minimum, one of the three biological matrices from one of the three animal species was to be assessed, since the inhibitory effect on the PCR is considered equivalent in these samples. The two kit manufacturers conducted validation experiments on the same thermal cycler model: ABI Prism 7500® from Applied Biosystems.

The two methods were validated to allow absolute quantification of *C. burnetii* between at least 1.10^3 and 1.10^6 genome equivalents (GEs) per mL of sample. The target is an IS*1111* insertion sequence specific to the genome of *C. burnetii* (Berri *et al.,* 2003). The plasmid standard for each kit, which enables a calibration curve to be constructed, was verified and linked to the genomic DNA standard of the NRL for Q fever. The result is given in GEs of the Nine Mile reference strain. The genome of this reference strain contains 20 copies of IS*1111*. Thus, the result is placed into perspective considering that each bacterium has an average of 20 copies of IS*1111*. Each of the two kits simultaneously detects a second target specific to the genome of cattle, sheep and goat cells in order to obtain information about the validity of the total DNA extraction and to check for possible inhibitory effects of the sample on the PCR. The manufacturer's instructions were drafted in consultation with the NRL to specify the detailed procedure and the validated performance characteristics of both the PCR and the complete method. They serve as the standard official protocol.

Number and distribution of "method adoption" data sets

In total, 11 data sets were obtained: seven with the LSI kit and four with the Adiagène kit (Table 1). Of the nine departmental laboratories in the network required to undergo adoption testing on the commercial methods, two laboratories performed the exercise on both the proposed kits. The laboratories used the qRT-PCR kits in combination with one or other of the two extraction methods (seven sets with the Qiagen kit and four sets with the Macherey Nagel kit).

In total, 16 different thermal cyclers were used (representing 5 models), with most laboratories being equipped with the Abiprism 7500® model. Five laboratories possessing more than one thermal cycler conducted checks on two different models with a view to their routine use.

PCR limit of detection (LD_{PCR})

The limits of detection for kits A and B respectively were defined as 1.5 and 1.0 GEs per PCR. These values correspond respectively to 300 and 200 GEs per mL that can be detected in 95% of cases.

A measured DNA reference material was provided by the NRL. Each laboratory performed two independent tests of three repetitions of a DNA level three times the LD_{PCR}. Detection of all six tests verified the expected LD_{PCR} performance (Table 2 A). The term fidelity (or intermediate fidelity), used hereafter, expresses repeatability and intra-laboratory reproducibility.

Operators obtained mean Ct values of 33.41 and 34.23, with kits A and B, respectively (Table 3). Standard deviations of fidelity did not exceed 0.50 Ct, and standard deviations of reproducibility were 1.32 Ct for the four laboratories that used kit A (24 measurements at the level 900 GE/mL) and 0.87 Ct for the seven tests performed with kit B (42 measurements at the

Table 2. Designs for the experiments conducted for the adoption of a validated PCR method for Q fever diagnosis in the context of abortion disease surveillance in ruminants

A. Limit of detection of PCR and the complete method (in the presence of measured reference material)

Adoption step	Levels tested	Number of independent tests*	Minimum number of operators	Number of replicas	Number of measurements	Acceptability
PCR	3 x LD_{PCR}	2	1	3	6	100% of results positive
Complete method	5 x LD_{Method}	2	1	2	4	100% of results positive

B. Scope and limit of quantification of PCR and the complete method (in the presence of measured reference material)

Adoption step	Levels tested	Number of independent tests*	Minimum number of operators	Number of replicas	Number of measurements	Acceptability
PCR	Range (5 points including LQ_{PCR})	1	1	5 range points and 5 replicas of LQ_{PCR}	10 (including 6 LQ_{PCR})	Bias below 0.5 $\log_{10}$ GEs/mL for each level
Complete method	5 x LQ_{Method}	2	1	2 (with two 5-point ranges)	14	100% of results positive and quantified with bias below 0.7 $\log_{10}$ GEs/mL

*1 for each thermal cycler
LD, limit of detection; LQ, limit of quantification

Methods

level 600 GE/mL). The coefficients of variation (CV) of fidelity were below 1.47%. The method yielded CV of reproducibility of 3.96% with kit A and 2.54% with kit B.

Limit of detection for the complete method (LD_{Method})

The complete method involves two distinct phases: extracting DNA from the biological sample, and then amplification by PCR of the target sequence on this extracted DNA. To date, the method's performance has been validated for two common methods for extracting total DNA on silica columns (Nucleospin Tissue, Macherey-Nagel and QIAamp DNA Mini kit, Qiagen) according to the instructions in Standard XP U47-600-2 (Chapter 7.3). It is interesting to note that the limits of detection were similar for PCR and the complete method with either kit: 300 GEs per mL with kit A and 200 with kit B.

Testing was also performed using another reference material provided by the NRL, this time consisting of quantified bacteria. Representative samples were prepared by adding a known number of *C. burnetii* bacteria to one of the biological matrices under study (Table 1). For these, the laboratories were asked to obtain the quantity of negative matrices needed for adoption testing: cell suspensions prepared from vaginal swabs for small ruminants, endocervical swabs for cattle, or placental cotyledon regardless of the ruminant species (1 swab being resuspended in 1 ml of PBS pH7.4). As a minimum, one of the three biological matrices from one of the three species has to be submitted for adoption.

Following a principle similar to the LDPCR adoption step, the LDMethod was confirmed at a concentration level corresponding to 5 times the LDMethod analysed in duplicate under intermediate fidelity conditions over the entire process of DNA extraction and PCR amplification of the target (Table 2 A). The Ct values obtained by the laboratories were extended from 31.09 to 34.49 for the level 1500 GEs per mL (deviation of 3.4 Ct over 16 measurements) with kit A and from 31.71 to 36.20 (deviation of 4.49 Ct over 28 measurements) for the level 1000 GEs per mL with kit B (Table 3). The dispersion of detailed results for each laboratory reached a deviation of 2.23 Ct but was mostly between 0.80 and 1.50 Ct (results not shown). The overall means were 32.71 and 34.30 with kits A and B respectively. The CVs of both fidelity and reproducibility were relatively close to those calculated for the LDPCR for kit A and slightly higher for kit B. The overall results showed a CV of reproducibility of less than 4%.

Quantification and limits of quantification: LQ_{PCR} and LQ_{Method}

In PCR, the limits (LQ_{PCR}) were determined at 400 and 300 GEs per mL with kit A and kit B respectively. Regardless of the PCR kit used, the validated limit of quantification was 2.70 $\log_{10}$ GEs/mL for the complete method, or 500 GEs/mL, bearing in mind that the diagnostic threshold is currently set (by expert opinion) at 4 $\log_{10}$ GEs/mL (Service Memo DGAL/SDSPA/N2012-8188 of 11 September 2012). In addition, the methods were validated to ensure linear quantification over 5 orders of magnitude to at least 1.106 GEs per mL (or bacteria per swab).

Confirmation of quantification performance was assessed, using the measured reference materials, by calculating the bias between the expected quantified value and that obtained, whether for the gene amplification step alone or for the complete method. The maximum bias permitted was set at 0.5 $\log_{10}$ for PCR and 0.7 $\log_{10}$ for the complete method (Table 2 B). Calculations had to be performed on data expressed in logarithm form.

For PCR, the experimental design followed involved conducting a test for the 5 levels of ten-fold serial dilution constituting the calibration range, including six copies of the last level representing the LQ_{PCR}. The bias was to be calculated for each level and for the five replicates of LQ_{PCR}.

For the complete method, two tests were performed for the quantification of a sample in duplicate. In total, four positive quantified results were obtained for the level of 5 times the LQ_{Method}, i.e. 3.40 $\log_{10}$ GEs per mL regardless of the kit used (2500 bacteria/swab).

The calibration curves were assessed after each test by visually examining the alignment and uniform distribution of

Table 3. Ct values obtained during testing to verify limits of detection

	A				B			
	Limit of detection		Limit of quantification		Limit of detection		Limit of quantification	
Levels verified Number of GEs in $\log_{10}$ / mL Number of GEs / mL	$3LD_{PCR}$ 2.95 900	$5LD_{Method}$ 3.18 1500	LQ_{PCR} 2.60 400	$5LQ_{Method}$ 3.40 2500	$3LD_{PCR}$ 2.78 600	$5LD_{Method}$ 3.00 1000	LQ_{PCR} 2.48 300	$5LQ_{Method}$ 3.40 2500
Number of laboratories	4	4	4	4	7	7	7	7
Number of repetitions	6	4	8	4	6	4	8	4
Number of measurements	24	16	32	16	42	28	56	28
Mean value obtained	33.41	32.71	33.72	32.01	34.23	34.30	35.06	32.93
Minimum/maximum value	32.03 / 35.90	31.09 / 34.49	32.09 / 36.54	29.60 / 33.66	32.67 / 36.10	31.71 / 36.20	33.65 / 36.66	30.81 / 34.50
Standard deviation of fidelity	0.46	0.55	0.54	0.40	0.50	0.80	0.50	0.65
Standard deviation of reproducibility	1.32	1.00	1.37	1.44	0.87	1.34	0.85	1.15
CV fidelity (%)	1.38	1.67	1.61	1.25	1.47	2.34	1.43	1.98
CV reproducibility (%)	3.96	3.49	4.05	4.49	2.54	3.92	2.43	3.50

CV, coefficient of variation; LD, limit of detection; LQ, limit of quantification

Methods

points, as well as the absence of outliers. In addition, two criteria documented in the kit instructions were verified: the value of the correlation coefficient R^2 greater than 0.90 and PCR efficiency between 85 and 115%. The calibration curves of 33 different PCR assays showed good reproducibility of the Ct values obtained as a function of concentration (Figure 1). Standard deviations of reproducibility varied from 0.42 to 1.31 Ct. The corresponding CVs were between 3.63 and 4.52% with kit A and between 1.91 and 3.00% with kit B. The overall means of 33 ranges (11 sets) found for the slope, y-intercept and efficiency were respectively -3.41, 43.33 and 97%. The network of laboratories involved in surveillance therefore seems capable of providing results with a good degree of consistency. The acceptance criteria to be complied with for the quantification curves, as well as the high number of points (5 points) in the range, help to ensure this reproducibility.

The standard deviations of both fidelity and reproducibility, from concentrations measured at the LQ_{PCR} and the LQ_{Method}, were entirely similar regardless of the kit (Table 4). The CVs of fidelity and reproducibility were higher for the concentration measurements obtained with the complete method than for PCR alone. As suspected, the DNA extraction step introduces factors that influence the result.

The laboratories provided measurements of concentrations that were within the predetermined bias limits. PCR alone clearly achieved greater precision than the complete method (Table 4). The maximum acceptability limit set at 0.7 $\log_{10}$ for the complete method means that variations up to a factor of 5 can be detected compared to the target level. In other words, a deviation of 2.33 Ct is permitted.

Taken together, the maximum bias for the nine laboratories was 0.67 (results not shown). Most of the measurements (96/132, or 73%) showed a bias of less than 0.25. However, with the complete method, fewer biases (18/44 or 41%) were less than 0.25. A bias of less than 0.25 $\log_{10}$ indicates that the measurements have a maximum variation of a factor of 1.78 compared to the expected concentration, or less than one Ct. A comparison between the laboratories of the measurements at the level of $5xLQ_{Method}$ (2500 bacteria/swab) showed that the minimum and maximum amounts measured ranged from 2.73 to 3.84 (seven sets) with kit B and from 2.72 to 3.93 (four sets) with kit A (Table 4). The precision of the measured concentrations shown in box plots appears similar between the two kits (Figure 2).

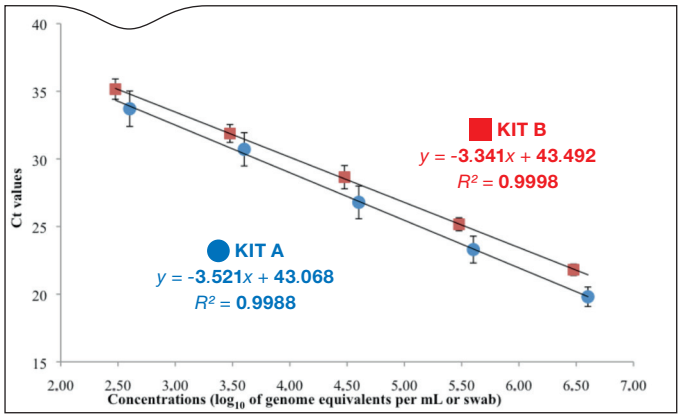

Figure 1. Calibration curves, between the Ct values and concentrations introduced, obtained with quantification standards of known DNA concentration, provided with each of the PCR kits.

The means and standard deviations of interlaboratory reproducibility were calculated from Ct values obtained from 12 ranges with the Adiagène kit (4 data sets) and 21 ranges with the LSI kit (7 data sets).

Table 4. Concentrations measured (in $\log_{10}$ / mL)

	A		B	
Levels expected Number of GEs in $\log_{10}$ / mL Number of GEs / mL	LQ_{PCR} 2,60 400	$5LQ_{Méthode}$ 3,40 2500	LQ_{PCR} 2,48 300	$5LQ_{Méthode}$ 3,40 2500
Number of laboratories	4	4	7	7
Number of repetitions	8	4	8	4
Number of measurements	32	16	56	28
Fidelity				
Mean concentration found	2.62	3.16	2.48	3.18
Minimum/maximum concentration	2.16 / 2.94	2.72 / 3.93	2.05 / 2.77	2.73 /3.84
Standard deviation of fidelity	0.15	0.24	0.15	0.21
Standard deviation of reproducibility	0.16	0.34	0.15	0.31
CV fidelity (%)	5.85	7.49	6.02	6.52
CV reproducibility (%)	6.23	10.79	6.17	9.63
Precision				
Mean bias in absolute value	0.13	0.35	0.12	0.31
Minimum bias in absolute value	0.01	0.03	0.00	0.02
Maximum bias in absolute value	0.44	0.67	0.42	0.66

CV, coefficient of variation; LD, limit of detection; LQ, limit of quantification

Methods

Conclusions and outlook

Surveillance of abortion diseases in ruminants is currently a priority issue in animal health in France. However, a recent inventory highlighted the heterogeneity of the diagnostic procedures used for abortions (Touratier *et al.,* 2012). Homogeneous differential diagnosis protocols are currently being proposed in collaboration with professional organisations. For Q fever, surveillance has been designed in conjunction with that for brucellosis, a disease that has now been eradicated and that therefore requires surveillance that can ensure a rapid response. Because of its pilot scale, this scheme should be flexible enough for all the adjustments needed in terms of feasibility and cost. The developments implemented should help to improve the procedures for other abortion diseases.
Because the PCR tool for the diagnosis of Q fever is a fundamental part of surveillance, a standardised validated method was essential: to enable surveillance data to be analysed, it is important to ensure that the results obtained are comparable. The method's performance characteristics are key components, not only for the analysis laboratory but also for the statistician analysing the surveillance data (Laurentie and Delmas, 2011). Two French standards relating to PCR in animal health were recently developed to assist with the production of reliable data (Standards XP U47-600-1 and -2). In accordance with these standards, work was undertaken to provide a standard method and determine its performance characteristics. The next necessary step was to verify that the network's laboratories were capable of achieving the performance specified under operational conditions for routine analysis. To our knowledge, this is the first interlaboratory adoption test for a PCR method involving peripheral laboratories and kit manufacturers. The results of the PCR method adoption exercise provided a first glimpse of the level of performance within the laboratories and served to verify the consistency of the results.
In a previous study to evaluate quantitative real-time PCR targeting the gene IS*1111*, by an interlaboratory test, the overall agreement of results in Ct values was deemed acceptable. Deviations in Ct obtained by the seven participating laboratories ranged from 4.0 to 7.2 depending on the seven positive DNA

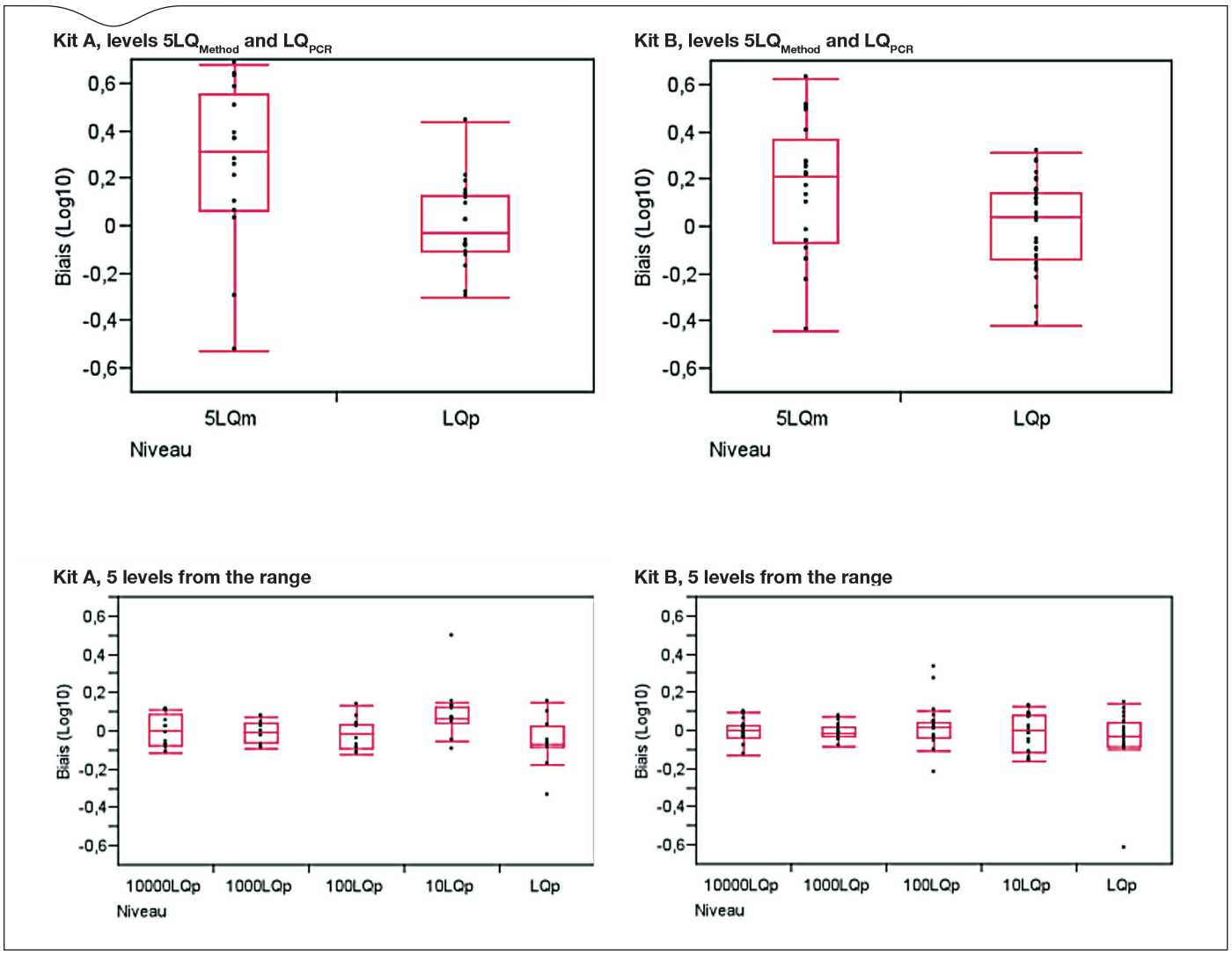

Figure 2. Box-plot representation of the precision of the measured concentrations (bias in $\log_{10}$ / mL)

Methods

samples tested (Jones *et al.,* 2011). In the adoption exercise presented here, involving nine laboratories, the quantitative results provided by the validated and adopted PCR method are all far more comparable. The interlaboratory Ct deviations ranged from 2.57 to 4.49 at low and medium levels ranging from 300 to 2500 GEs/mL (Table 3). The maximum CV of reproducibility was 4.49%. Standard deviations of fidelity for Ct values were generally between 0.40 and 0.80 Ct (CV between 1.25 and 2.34%).

This adoption test showed that for the expected concentration measurements, a similar level of accuracy (fidelity combined with precision) was obtained regardless of the kit used. For example, for the precision of the complete method, the measurements found for the tested level of 2500 bacteria per mL ($5xLQ_{Method}$) varied from 2.72 log_{10} (525) to 3.93 log_{10} (8511) bacteria per mL (Table 4). The mean absolute bias was 0.3 log_{10}, i.e. a factor of 2 between the expected and measured concentration. In terms of dispersion of measurements, the laboratories achieved CVs of fidelity below 8% and CVs of reproducibility below 11%.

Moreover, the bias values, like the coefficients of variation, can serve as initial baseline data for this method that has been officially defined, validated and shared by a network of laboratories. More substantiated interlaboratory reproducibility should be estimated by participation in regular interlaboratory tests. It is especially important to correctly define the level of the diagnostic threshold. It is also essential for each laboratory to produce a control chart on the critical point formed by the threshold. This involves incorporating a control determined to 4 log_{10} bacteria per ml in each extraction series. This internal reference material thereby serves as a method control. It also enables monitoring in the form of an intra-laboratory control chart (limit of bias acceptability set at less than 0.7 log_{10}). The measurement uncertainty should be taken into account in interpreting the results. A measurement at 2000 bacteria/swab will be interpreted as a highly positive result because it corresponds to the lower limit of the threshold level at 10,000 bacteria/swab.

Finally, information on the accuracy capabilities of the results around the threshold should help progress towards a simplified and less expensive method in the future (i.e. use of a threshold standard instead of a range of five quantification standards).

Acknowledgements:

The team at the NRL for Q fever would like to thank Maëlle LEBORGNE and Béatrice BLANCHARD from Adiagène, as well as Sandrine MOINE-BOULEY and Eric SELLAL from LSI for their constructive cooperation in conducting validations and preparation for adoption. They also wish to express their gratitude to Claire PELLETIER from LDA Saône-et-Loire for the relevant discussions held during the validation phase for their in-house method.

Bibliographical references:

Berri M, Arricau-Bouvery N, Rodolakis A. 2003. PCR-based detection of *Coxiella burnetii* from clinical samples. *In*: Methods in Molecular Biology. PCR Detection of Microbial Pathogens, 216:153-161.

de Bruin A, van der Plaats RQJ, de Heer L, Paauwe R, Schimmer B, Vellema P, van Rotterdam BJ, van Duynhoven YTHP. 2012. Detection of *Coxiella burnetii* DNA on small-ruminants farms during Q fever outbreak in The Netherlands. *Applied and Environmental Microbiology*, 78:1652–1657.

De Cremoux R, Rousset E, Touratier A, Audusseau G, Nicollet P, Ribaud D, David V, Le Pape M. 2012. *Coxiella burnetii* vaginal shedding and antibody responses in dairy goat herds in a context of clinical Q fever outbreaks. *FEMS Immunology & Medical Microbiology*, 64:120-122.

Forland F, de Carvalho Gomes H, Nokleby H, Escriva A, Coulombier D, Giesecke J, Jansen A. 2012. Applicability of evidence-based practice in public health: risk assessment on Q fever under an ongoing outbreak. *Eurosurveillance*, 17 (3). http://www.eurosurveillance.org/images/dynamic/EE/V17N03/art20060.pdf

Jones RM, Hertwig S, Pitman J, Vipond R, Aspán A, Bölske G, McCaughey C, McKenna JP, van Rotterdam BJ, de Bruin A, Ruuls R, Buijs R, Roest HJ, Sawyer J. 2011. Interlaboratory comparison of real-time polymerase chain reaction methods to detect *Coxiella burnetii*, the causative agent of Q fever. *Journal of Veterinary Diagnostic Investigation*. 23(1):108-11.

Laurentie M, Delmas JM. 2010. Le profil d'exactitude, un outil pour décider et diagnostiquer la validité d'une méthode analytique. *EuroReference*, 3:9-11. http://www.afssa.fr/euroreference/Documents/ER03-Rech-ProfilExa.pdf

Norme NF XP-U47-600-1. Juin 2011. Exigences et recommandations pour la mise en œuvre de la PCR en santé animale. *AFNOR*, 49 pp.

Norme NF XP-U47-600-2. Juin 2011. Exigences et recommandations pour le développement et la validation de la PCR en biologie vétérinaire. *AFNOR*, 40 pp.

Rousset E, Sidi-Boumedine K, Thiéry R. 2010. Chapter 2.1.12. Q fever. *In*: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). *7th ed., O.I.E*, 13 pp. http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/

Sidi-Boumedine K, Rousset E, Henning K, Ziller M, Niemczuck K, Roest H-J, Thiéry R. 2010. Development of harmonised schemes for the monitoring and reporting of Q-Fever in animals in the European Union. *EFSA Scientific report*, 48 pp. http://www.efsa.europa.eu/en/supporting/pub/48e.htm

Touratier A, De Cremoux R, Bronner A. 2012. Diagnostic différentiel des avortements infectieux chez les ruminants: état des lieux des actions conduites en France. *Bulletin GTV*, 63:99-104.

Euro Reference
journal of Reference

anses
French agency for food, environmental and occupational health & safety

Journal No. 8
Winter 2012

ISSN 2110-5294