**EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES**


**EXTERNAL QUALITY ASSESSMENT
IN VETERINARY MEDICINE**


**Definitive GLOBAL REPORT**

**Veterinary Medicine
Q-Fever
SURVEY 2021/12**

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A preliminary version of this report was submitted to the experts on: 06/01/2022

| | |
|---|---|
| **Authorization to release the report:** | By Bernard China (scheme coordinator) and Ynse Van de Maele (scheme coordinator in training) on 08/02/2022. |

All the reports are also available on our webpage:
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_nl/rapports_annee.htm
https://www.wiv-isp.be/QML/activities/external_quality/rapports/_fr/rapports_annee.htm

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PT VET Q-fever, definitive global report 2021/12. Date of publication: 08/02/2022.

FORM 43/124/E V14          3/11

## INTRODUCTION

This survey was dedicated to detect the agent of Q-Fever (*Coxiella burnetii*) by RT-qPCR in organ and milk samples.

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology, Scientific Directorate infectious diseases in animals, Sciensano.

For each reference sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference samples was based on (i) the historical background of the animals/farm and (ii) the results obtained by analytical tests (pre-verification before sending). The pre-verification tests consisted of an *in-house* extraction protocols, SOP/PRE/03, and a subsequent RT-qPCR assay using again an *in-house* method (SOP/BAC/ANA15).

Organs were obtained by spiking inactivated animal material free of *C.burnetii*. Samples were spiked with serial dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$) of confluent inactivated cell cultures infected with an avirulent (phase II) *C.burnetii* strain (RSA 439). The estimated concentrations of *C.burnetii* bacteria in the spiked samples were of 1.03E+05, 1.03E+04 and 1.03E+03 genomic equivalent (GE)/ml, respectively.

The reference milk samples were obtained from bulk tank milks of sheep/goat/cattle farms naturally infected or free of *C.burnetii*. All milks were inactivated (internal procedure) and lyophilized before sending.

## Organs

### Homogeneity

The homogeneity of the samples was tested by the NRL before the survey. The samples were considered homogeneous.

### The participants

Six laboratories participated to the Bacteriology survey: Sciensano, ARSIA, LNCR/ACSEDIATE, IDVET, Anses-SOPHIA ANTIPOLIS and LSI-Thermofisher Scientific.

### Target Values

The target values were determined by the NRL based on the homogeneity tests.
- PT2021QFVBACOP1, PT2021QFVBACOP2 and PT2021QFVBACOP3 are considered positive.
- PT2021QFVBACON1 and PT2021QFVBACON2 are considered negative.

### Stability

The samples were tested before and after the survey. The results were compared and the samples were considered as stable.

### Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

| Sample ID | 97504 | 97507 | 97510 |
|-----------|-------|-------|-------|
| QFO21-1 | PT2021QFVBACOP3 | PT2021QFVBACOP1 | PT2021QFVBACON2 |
| QFO21-2 | PT2021QFVBACON2 | PT2021QFVBACON1 | PT2021QFVBACOP2 |
| QFO21-3 | PT2021QFVBACOP1 | PT2021QFVBACOP2 | PT2021QFVBACON1 |
| QFO21-4 | PT2021QFVBACOP2 | PT2021QFVBACOP3 | PT2021QFVBACOP3 |
| QFO21-5 | PT2021QFVBACON1 | PT2021QFVBACON2 | PT2021QFVBACOP1 |
| **Sample ID** | **97522** | **97523** | **97534** |
| QFO21-1 | PT2021QFVBACOP3 | PT2021QFVBACOP3 | PT2021QFVBACOP2 |
| QFO21-2 | PT2021QFVBACOP1 | PT2021QFVBACON2 | PT2021QFVBACON1 |
| QFO21-3 | PT2021QFVBACON1 | PT2021QFVBACON1 | PT2021QFVBACON2 |
| QFO21-4 | PT2021QFVBACON2 | PT2021QFVBACOP1 | PT2021QFVBACOP1 |
| QFO21-5 | PT2021QFVBACOP2 | PT2021QFVBACOP2 | PT2021QFVBACOP3 |

*The panel consisted of 5 different samples.*

## Milk

### Homogeneity

The homogeneity of the samples was tested by the NRL before the survey. The samples were considered homogeneous.

### The participants

Six laboratories participated to the Bacteriology survey: Sciensano, ARSIA, LNCR/ACSEDIATE, IDVET, Anses-SOPHIA ANTIPOLIS and LSI-Thermofisher Scientific.

### Target Values

The target values were determined by the NRL based on the homogeneity tests.
- PT2021QFVBACMP1 and PT2021QFVBACMP2 are considered positive.
- PT2021QFVBACMN1 is considered negative.

### Stability

The samples were tested before and after the survey. The results were compared and the samples were considered as stable.

## Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

| Sample ID | 97504 | 97507 | 97510 |
|-----------|-------|-------|-------|
| QFM21-1 | PT2021QFVBACMP2 | PT2021QFVBACMP2 | PT2021QFVBACMP2 |
| QFM21-2 | PT2021QFVBACMP1 | PT2021QFVBACMP1 | PT2021QFVBACMP2 |
| QFM21-3 | PT2021QFVBACMN1 | PT2021QFVBACMP2 | PT2021QFVBACMN1 |
| QFM21-4 | PT2021QFVBACMP1 | PT2021QFVBACMP1 | PT2021QFVBACMP1 |
| QFM21-5 | PT2021QFVBACMP2 | PT2021QFVBACMN1 | PT2021QFVBACMP1 |
| **Sample ID** | **97522** | **97523** | **97534** |
| QFM21-1 | PT2021QFVBACMP2 | PT2021QFVBACMN1 | PT2021QFVBACMP2 |
| QFM21-2 | PT2021QFVBACMP2 | PT2021QFVBACMP2 | PT2021QFVBACMP1 |
| QFM21-3 | PT2021QFVBACMP1 | PT2021QFVBACMP1 | PT2021QFVBACMP2 |
| QFM21-4 | PT2021QFVBACMP1 | PT2021QFVBACMP2 | PT2021QFVBACMN1 |
| QFM21-5 | PT2021QFVBACMN1 | PT2021QFVBACMP1 | PT2021QFVBACMP1 |

*The panel consisted of 3 different samples.*

## Survey timeline

Transfer of the samples from NRL to QL: 12/10/2021
Randomization of the samples by QL: 14/10/2021
Sending samples (sent on dry ice) to participants: 18/10/2021
Deadline for the results encoding: 18/11/2021
Preliminary report: 06/01/2022

## RESULTS

### Organs

The panel consisted of 5 different samples: 3 positive and 2 negative samples.

### Results per sample

| Sample ID | Status | Number of repetitions (total results) | Observed result |
|-----------|--------|---------------------------------------|-----------------|
| OP1 | POS | 1 (6) | 6 POS |
| OP2 | POS | 1 (6) | 5 POS<br>1 false NEG |
| OP3 | POS | 1 (6) | 3 POS<br>3 false NEG |
| ON1 | NEG | 1 (6) | 6 NEG |
| ON2 | NEG | 1 (6) | 6 NEG |

Globally, on the 30 encoded results, 26/30 (86.7%) were correct.

### Used method

| Method | N | NR | NRC | % |
|--------|---|----|----|---|
| VetMAX™ C. burnetii Absolute Quant Kit | 2 | 10 | 8 | 80 |
| ID.VET - ID GENE® Q FEVER TRIPLEX | 1 | 5 | 5 | 100 |
| VetMAX™ C. burnetii Feces Kit | 1 | 5 | 5 | 100 |
| RT-qPCR home made | 2 | 10 | 8 | 80 |
| TOTAL | 6 | 30 | 26 | 86.7 |

*(NR = number of results; NCR = number of correct results).*

### Conclusion

The participants obtained a 86.7% of success in this survey.

**Milk**

The panel consisted of 5 samples, but samples MP1 and MP2 were repeated twice. Therefore, 4 positive and 1 negative samples were used.

### Results per sample

| Sample ID | Status | Number of repetitions (total results) | Observed result |
|-----------|--------|----------------------------------------|-----------------|
| MP1 | POS | 2 (12) | 12 POS |
| MP2 | POS | 2 (12) | 12 POS |
| MN1 | NEG | 1 (6) | 6 NEG |

Globally, on the 30 encoded results, 30/30 (100%) were correct.

### Used method

| Method | N | NR | NRC | % |
|--------|---|----|----|---|
| VetMAX™ C. burnetii Absolute Quant Kit | 2 | 10 | 10 | 100 |
| ID.VET - ID GENE® Q FEVER TRIPLEX | 1 | 5 | 5 | 100 |
| VetMAX™ C. burnetii Feces Kit | 1 | 5 | 5 | 100 |
| RT-qPCR home made | 2 | 10 | 10 | 100 |
| TOTAL | 6 | 30 | 30 | 100 |

*(NR = number of results; NCR = number of correct results).*

### Conclusion

The participants obtained a 100% of success in this survey.

# ANNEXES (NOT UNDER ACCREDITATION)

The boxplots were performed using the shiny.chemgrid.org/boxplotr/ software.

## Annex 1: Quantitative results

### Sample: PT2021QFVBACMP1

|         | 97504 | 97507 | 97510 | 97522 | 97523 | 97534 |
|---------|-------|-------|-------|-------|-------|-------|
| **Rep 1**   | 29.1  | 27.7  | 25.8  | 25.1  | 30.8  | 24.7  |
| **Rep 2**   | 29.2  | 26.4  | 26.5  | 25.3  | 31.2  | 26.6  |
| **Average** | 29.1  | 27.0  | 26.2  | 25.2  | 31.0  | 25.6  |
| **SD**      | 0.08  | 1.0   | 0.5   | 0.2   | 0.3   | 1.3   |
| **CV**      | 0%    | 4%    | 2%    | 1%    | 1%    | 5%    |
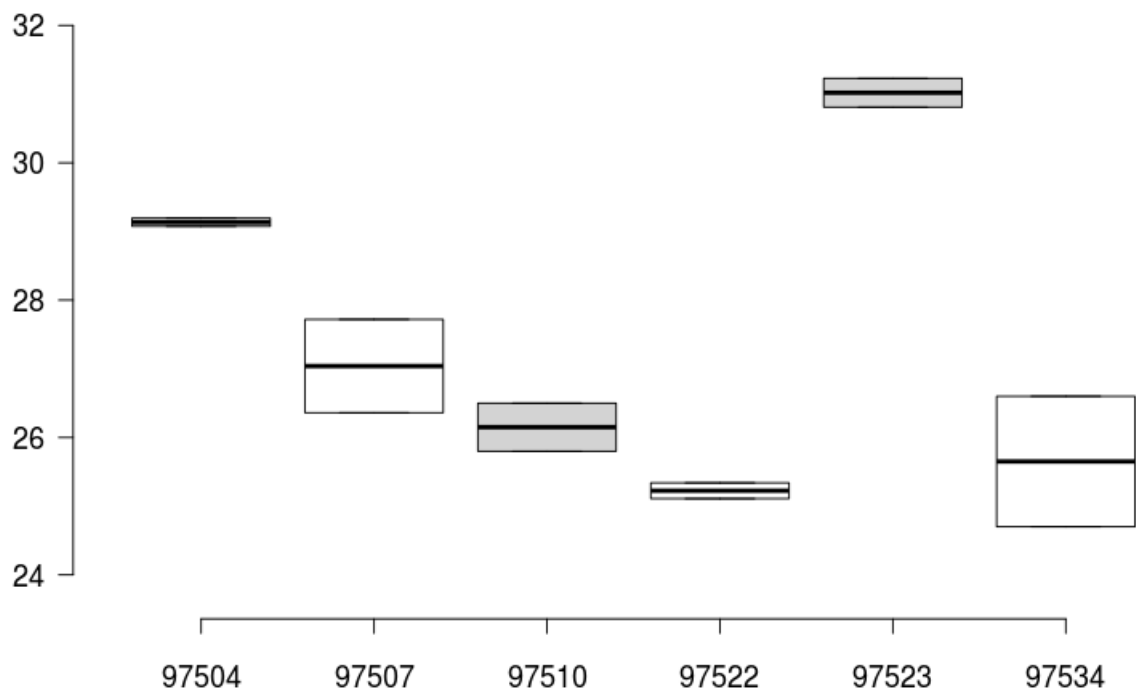


*Figure A1. Dispersion of the Ct values (box-plots) per laboratory.*

**Sample: PT2021QFVBACMP2**

| | 97504 | 97507 | 97510 | 97522 | 97523 | 97534 |
|---|---|---|---|---|---|---|
| **Rep 1** | 31.1 | 28.1 | 27.1 | 27.0 | 32.4 | 26.8 |
| **Rep 2** | 31.1 | 28.4 | 27.9 | 27.2 | 31.1 | 26.6 |
| **Average** | 31.1 | 28.2 | 27.5 | 27.1 | 32.3 | 26.7 |
| **SD** | 0.02 | 0.2 | 0.6 | 0.2 | 0.2 | 0.1 |
| **CV** | 0% | 1% | 2% | 1% | 1% | 1% |
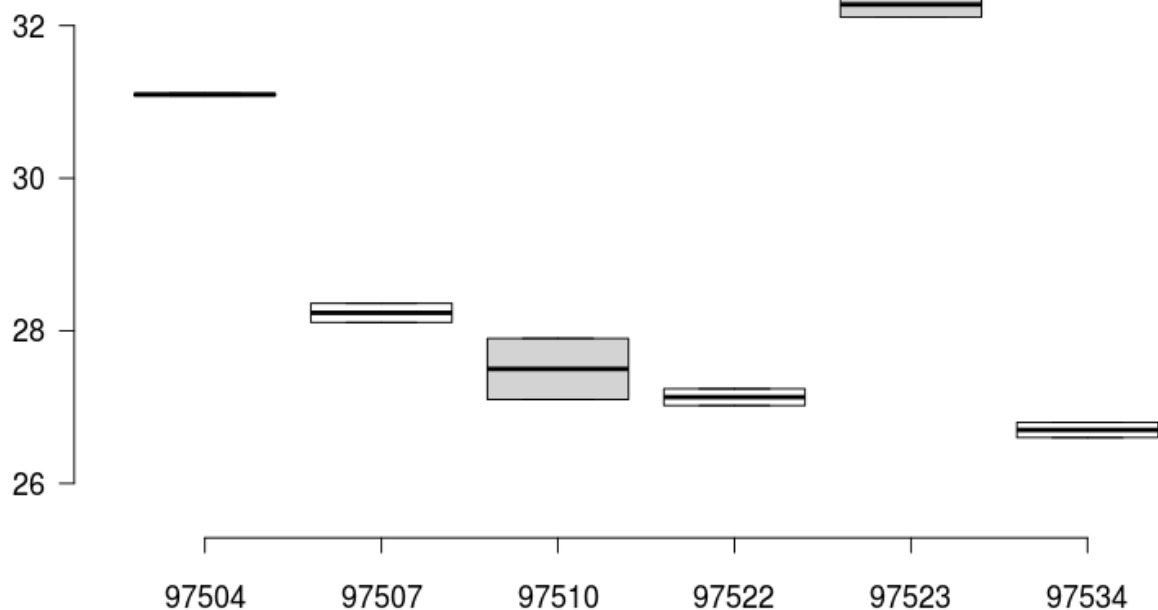


*Figure A2. Dispersion of the Ct values (box-plots) per laboratory.*

## Annex 2: Additional information

The preliminary report of this survey is available on our website via the following link: https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

The calendar for Proficiency Testing in Veterinary diagnosis is available on our website: https://www.wivisp.be/QML/activities/external_quality/calendar/calender_PT%20VET/_fr/Calendrier_2020-PT%20VET%202.htm

## Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 3 participants:

- A rectangle ranging from percentile 25 ($P_{25}$) to percentile 75 ($P_{75}$)
- A central line representing the median of the results ($P_{50}$)
- A lower limit showing the smallest value $x > P_{25} - 1.5 * (P_{75} - P_{25})$
- An upper limit representing the largest value $x < P_{75} + 1.5 * (P_{75} - P_{25})$
- All points outside this interval are represented by a dot
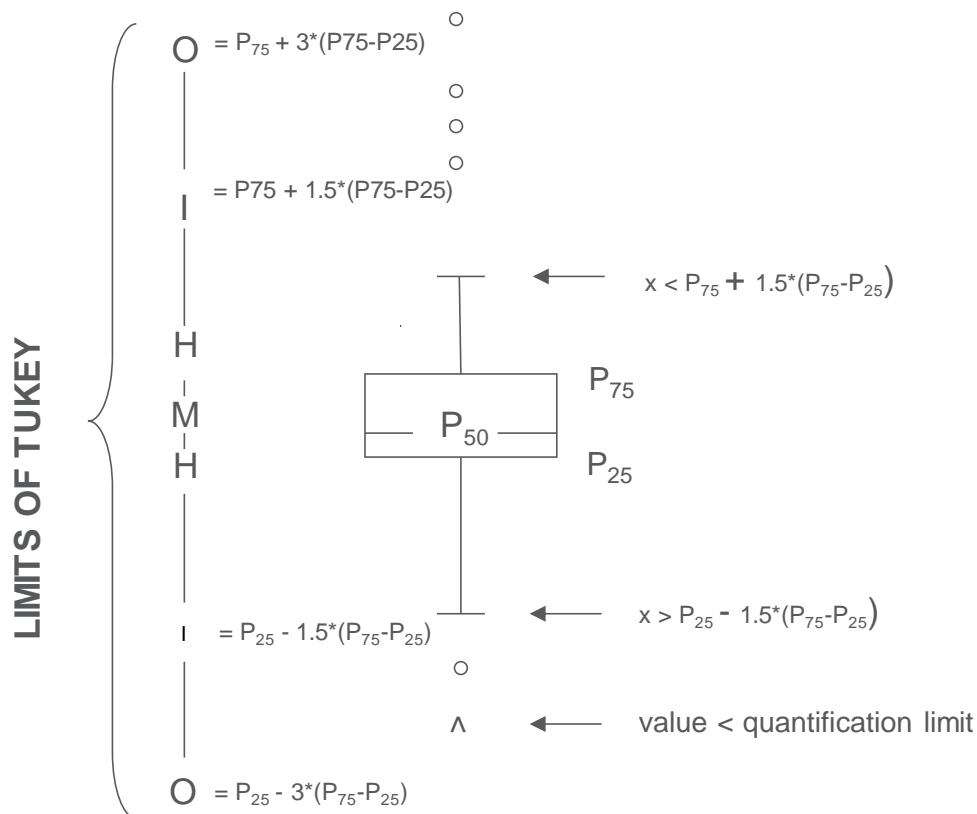


**Corresponding limits in case of normal distribution**

**END**