



# Q fever (*Coxiella burnetii*)

## Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *coxiella burnetii*.

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## 1 PHLN Summary Laboratory Definition

### 1.1 Condition:

Acute Q Fever

#### 1.1.1 Laboratory Definitive Criteria

- Isolation of *C. burnetii*; OR
- Detection of *C. burnetii* by nucleic acid amplification test (NAAT); OR
- Seroconversion or 4-fold or greater increase in *C. burnetii* antibody titre to phase II antigen by complement fixation (CF) assay or by indirect immunofluorescent (IFA) IgG antibody assay in paired sera.

#### 1.1.2 Laboratory Suggestive Criteria

- IgM antibody to *C. burnetii* phase II antigen
- Single convalescent IgG antibody to phase II antigen > 1:128 by IFA assay

#### 1.1.3 Special Considerations / Guide for Use

- Detection by NAAT with negative serology results confirm acute Q fever, however serial serological testing is required to monitor for chronic infection.

### 1.2 Condition:

Chronic Q Fever - *Coxiella burnetii*

### 1.2.1 Laboratory Definitive Criteria

- Isolation of *C. burnetii* in heart tissue, bone or vascular graft; OR
- Detection of *C. burnetii* by nucleic acid amplification test (NAAT) of heart tissue, bone or vascular graft.

### 1.2.2 Laboratory Suggestive Criteria

- IgG antibody to *C. burnetii* phase I antigen >1:1024 by IFA assay
- Isolation of *C. burnetii* in blood
- Detection of *C. burnetii* by nucleic acid amplification test (NAAT) in blood

## 2. Introduction

Q fever in its acute primary and various chronic forms is the commonest and most serious zoonosis in Australia with an annual notification rate of 2.1 cases per 100,000 population<sup>1</sup>. It is probable that many mild acute cases are not recognised.

The causative organism of Q fever is *C. burnetii*. It has a world-wide distribution. There are reservoirs of infection in wild animals and their ticks, but these are mostly of little significance for humans. Domestic ruminants are the principal source of human infection. In female animals, after an initial infection, *C. burnetii* later recrudesces during pregnancy and multiplies in the placenta at the end of parturition. It is then shed in large numbers in the products of conception. Organisms may also be excreted in the milk for a long period. Companion animals such as cats and dogs may sometimes be infected and also shed the organism at parturition. The epidemiology and occupational prevalence of the disease in meat and livestock workers and subsidiary trades reflects this natural history of infection in cattle, sheep and goats. However, Q fever is by no means confined to occupationally exposed groups. The compact extracellular form of *C. burnetii* is highly resistant to drying and sunlight, and survives well in dust and aerosols. Contaminated dust may be carried on fomites (eg., straw, work clothing) and liberated in microenvironments at a distance from its animal source. In addition, there are many recorded examples of direct dust-borne spread to residents in urban areas near abattoirs, feed lots or pastures with numerous parturient animals. Despite its environmental stability *C. burnetii* is destroyed at temperatures between 60-70°C and pasteurisation of infected milk destroys the organism. *C. burnetii* is also inactivated by phenol, cresol mixtures, 70% ethanol-water mixtures and 5% chloroform. Although there has been a single report of formalin resistance, this has not been confirmed. Low concentrations of formalin consistently inactivate the organism during the preparation of Q fever vaccine. *C. burnetii* is a facultative intracellular parasite that can be isolated by animal inoculation or by inoculation of tissue culture cells<sup>2</sup>. It can also be grown in specialized cell-free media although this technique is not used for diagnosis<sup>3</sup>. Serological diagnosis of Q fever depends on the detection of antibodies to cell wall lipopolysaccharide (LPS<sup>4</sup>). Expression of *C. burnetii* LPS is subject to phase variation; when first harvested from the spleens of infected animals phase I antigen is expressed while hen egg yolk sac passage results in expression of phase II antigen<sup>5</sup>. Paradoxically, antibodies to phase II antigen are detected early in infection whereas those to phase I are detected later.

## 3. Laboratory diagnosis of Q fever

### 3.1 Direct detection of *C. burnetii*

Stains and direct immunofluorescence for light microscopy and electron microscopy are not performed routinely and will not be discussed further. *C. burnetii* are highly infectious and unfixed tissue samples for examination for *C. burnetii* should be handled in a biohazard cabinet until fixed with formalin, dehydrated methanol or acetone. *C. burnetii* cultures are risk level 3 organisms and should only be handled by immune staff in a PC3 facility.

### 3.2 Polymerase chain amplification

Polymerase chain amplification of *C. burnetii* genomic sequences in whole blood or buffy coat specimens offers a sensitive and safe method of detection<sup>2</sup>. The initial extraction of the DNA involves procedures – proteinase K treatment, chloroform phenol extraction, heating over 900 C – which inactivate viable *C. burnetii*.

#### Suitable Specimens

Tissue, whole blood, serum.

#### Test Sensitivity and Specificity

This method has >90% sensitivity compared with serodiagnosis early in infection. It is highly specific. Sensitivity decreases with progression of the illness with no detection after day 17<sup>6</sup>. However, sensitivity in chronic Q fever is only 50-60%<sup>7,8</sup>.

#### Suitable Test acceptance/validation criteria

In run and positive and negative control (non-template controls), extraction control.

#### Suitable internal controls

Q fever vaccine (Qvax<sup>®</sup>, CSL Ltd, Melbourne) suspension or preferably an “artificial target” – a construct with the same primer and probe sites but with an added restriction enzyme site not present in the native DNA amplicon. Use of the uridine-UNG system gives an additional way of detecting specimen or amplicon contamination<sup>3</sup>.

#### Suitable external QA program

Not currently available

### 3.3 Serological diagnosis

#### Complement Fixation Tests

Historically, the most frequently used technique was complement fixation with *C. burnetii* Phase 2 antigen, and later, in addition, with Phase 1 antigen. Phase 2 antibody is the first to be detected and phase 1 antibody is invariably present in chronic Q fever infection, including endocarditis, with or without phase 2 antibody<sup>9,10</sup>. Examination of acute and convalescent sera fulfils the most reliable

criteria for an acute infection: a four-fold or greater rise of (CF) antibody (phase 2) between the paired sera is diagnostic. The technique has the limitations that it is labour intensive and does not measure IgM so that a positive result may not be obtained until late in the disease<sup>1</sup>. Also, it does not measure IgA antibody which may be important in Q fever endocarditis.

### Suitable specimens

Clotted blood. Ideally acute and convalescent samples should be provided, especially in acute Q fever, to monitor titre changes.

### Test Sensitivity and specificity

CFT measures mainly IgG so may not be positive early in acute Q fever when the patient first presents and only IgM antibody may be present. The test should be repeated in 2-4 weeks and run in parallel with the first sample to determine titre changes. Sensitivity of a four-fold rise in titre has been estimated in two studies as 73% and 77.8% respectively and specificity has been estimated as 90%<sup>11,12</sup>.

### Suitable test acceptance/validation criteria

Use of internal and external controls and test performance should comply with method.

### Suitable internal controls

Known positive serum sample tested with each batch; result should be within one dilution of known titre.

### Suitable external QA program

RCPA QAP

## 3.4 Enzyme immunoassay (EIA) Testing

Commercial kits are available to measure IgM and IgG antibodies to phase 2 antigen. If only a single convalescent serum is available it may be difficult to distinguish persistent IgM from a past infection from that of a current infection, as not all Q fever cases make the IgM to IgG switch. IgM has been observed to persist for over 600 days after an acute Q fever infection. While acute primary Q fever is confirmed with reasonable efficiency with the EIA test it is unwise to rely solely on this method if chronic Q fever infection is suspected and where interpretation has to be based on the height of titres to Phase 1 and 2 antigens in the IgG and IgA classes. These tests are not quantitative so should be used only as a screening test to select samples for the more labour intensive CFT or IFA testing<sup>1</sup>.

### Suitable specimens

Clotted blood. Ideally acute and convalescent samples should be collected and tested in parallel.

### Test sensitivity and specificity

Published data suggest that EIA is highly sensitive and specific<sup>13,14</sup>. Data regarding commercial assays should be sought from the manufacturer.

## Suitable test acceptance/validation criteria

Follow manufacturer's instructions.

## Suitable internal controls

Known positive serum sample and the kit controls should be tested with each batch; results should be within one dilution of the known titre.

## Suitable external QC program

RCPA QAP.

## 3.5 IFA testing

IFA tests are of particular value for confirmation of acute infection and for diagnosis of chronic infection. The technique involves titrating sera by immunofluorescence (IFA) on microdots of *C. burnetii* expressing phase 1 or phase 2 antigens with conjugates directed against IgG, IgM and IgA immunoglobulin classes<sup>5</sup>. Commercial IFA slides are available for IgM and IgG detection (BioMerieux SpotIF and Fluoline-M &G immunofluorescent stain). IgA slides can be made in house with cultured coxiella (using PC3 facilities for preparation ex IMVS).

## Suitable Specimens

Clotted blood. Sequential samples should be tested in parallel.

## Test Sensitivity and Specificity

The microdot phase 1 and 2 IgM, and IgG and the IgA tests are highly sensitive and specific for the detection of Q fever antibodies.

## Suitable test acceptance/validation criteria

As determined by the method requirements. >1+ fluorescence is preferred to determine positivity.

## Suitable internal controls

Previous known positive of known titre to the specific antibody being sought.

## Suitable external QC program

Not currently available.

## 4. Summary

The diagnosis of Q fever is complicated by the presence of acute and chronic presentations and the differing kinetics of class specific antibody responses to phase 1 and phase 2 antigens in these infections.

## References

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