Q fever case definition summary

Public Health Laboratory Network case definitions

PHLN0024
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1. Introduction

Q fever in its acute primary and various chronic forms is arguably the commonest and most serious zoonosis in Australia. It is probable that many mild acute cases are not recognised. The current serological tests for laboratory diagnosis present difficulties of interpretation for general practitioners and specialist physicians not working in the field of infectious diseases. Diagnostic approaches for the various chronic sequelae are still being refined and a consensus for the range of tests offered, particularly for serodiagnosis, is still lacking.

The causative organism of Q fever is *Coxiella 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 these animals, after an initial infection, the coxiella later recrudescence in the pregnant animal and multiply in the placenta at the end of parturition. They are 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 the coxiella 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 the coxiella is destroyed at temperatures between 60-70°C and pasteurisation of infected milk destroys the organism. The coxiella 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.

2. Laboratory diagnosis of Q fever

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. NB Coxiella are highly infectious and unfixed tissue samples for examination for coxiella should be handled in a biohazard cabinet until fixed with formalin, dehydrated methanol or acetone. Coxiella cultures are risk level 3 organisms and should only be handled by immune staff in a PC3 facility.

**Polymerase chain amplification**

Polymerase chain amplification of *C. burnetii* genomic sequences offers a highly sensitive and safe method of detection of coxiella (2). The initial extraction of the DNA involves procedures – proteinase K treatment, chloroform phenol extraction, heating over 90°C – which inactivates viable coxiella.

**Suitable Specimens**

Tissue, cell cultures, fixed wax embedded tissue blocks.

**Test Sensitivity and Specificity**

This method is very sensitive and it has been estimated that an optimised assay can detect as few as 1-2 targets (organisms). Both living and dead organisms can be detected.

**Suitable Test acceptance/validation criteria**

In run and positive and negative control (non template controls)

**Suitable internal controls**

Q fever vaccine (Qvax®, 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 (3)

**Suitable external QC program**

Not currently available

**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 (4). Examination of acute and convalescent sera fulfils the most reliable criteria for an acute infection – namely, a four-fold or greater rise of (CF) antibody (phase 2) between the paired sera. 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 (1). 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 (four fold titre changes between acute and convalescent samples are the “gold standard”).

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.

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 QC program

Not currently available

Enzyme linked immuno assay (ELISA) Testing

There is a commercial kit (Pan Bio Ltd Brisbane) which measures IgM and IgG antibody 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.

Suitable specimens

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

Test sensitivity and specificity

False positive and false negative results can occur. This test is not quantitative so should be used only as a screening test to select samples for the more labour intensive CFT or IFA testing (1).

Suitable test acceptance/validation criteria

The kit insert should be followed to ensure the correct test criteria are met

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**

Not currently available

**IFA testing**

IFA tests are of particular value for extended analysis, particularly on sera from “problem” cases. The technique involves titrating sera by immunofluorescence (IFA) on microdots of *C. burnetii* with conjugates directed against IgG, IgM and IgA immunoglobulin classes (5). 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

3. **Summary**

The diagnosis of Q fever is complicated by the presence of acute and chronic presentations and the different antibodies that can be found using different tests at different stages of the disease..

4. **PHLN laboratory definition**

4.1 **Conditions**

- Acute Q fever
• Chronic Q fever
• Q fever Chronic Fatigue syndrome
• Chronic granulomatous Q Fever
• Endocarditis

4.2 Tests

Definitive Criteria for acute Q fever
Seroconversion in paired sera tested in parallel by ELISA and CFT and/or
Detection of *Coxiella burnetii* by DNA sequence detection, usually after PCR, or by culture.

Definitive criteria for Chronic Q fever “syndromes”
IFA phase 1 and phase 2, IgM, IgG and/or IgA positive on more than one serum sample
and/or
Detection of *Coxiella burnetii* by DNA sequence detection, usually after PCR, or by culture

Suggestive Criteria Detection of *C burnetii* antibodies by more than one recognised test
method

(See attached table for titre ranges, tests and related conditions)

References

1. WilliamsJC, Thompson HA *Q fever the biology of Coxiella burnetii* CRC Press
   Boca Raton Flo USA 1991

2. Minnick MF, Heinzen RA, Frazier ME, Mallavia LP. Characterisation and expression

3. Harris R, Storm PA, Lloyd A, Arens M, Marmion BP. *Long term persistence of
   Coxiella burnetii in the host after primary Q fever*, Epidem Infect 2000; 124; 543-549


5. Worswick DA, Marmion BP. Antibody responses in acute and chronic Q fever and in

Summary Table for Q fever Diagnosis and Test Interpretation

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Acute primary Q fever</th>
<th>Persistent infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Persistent infection (or antigen) Post Q fever fatigue syndrome</td>
<td>Granulomata (testes, liver, lung, pleura, bone)</td>
</tr>
<tr>
<td>Direct Detection Microscopy</td>
<td>Light</td>
<td>-</td>
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<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>IFA</td>
<td>-</td>
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<tr>
<td>EM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCR</td>
<td>Variable, low level positives with acute phase blood terminated by antibody formation (scanty data)</td>
<td>Positive (bone marrow 65%) NB neg and pos controls</td>
</tr>
<tr>
<td>Isolation</td>
<td>Cell cultures</td>
<td>Acute phase blood positive; Medium sensitivity</td>
</tr>
<tr>
<td>Animals</td>
<td>Acute phase blood/urine; positive; Medium sensitivity</td>
<td></td>
</tr>
</tbody>
</table>
| Antibody responses        | CFT   | Ph1&2 ag, Acute and convalescent convalescent serum >=+->4x increase ab ph2 ag, neg or low ab v ph1 ag | Variable | Variable IgG-IgM ab =>640-5120 | Ph1&2 ag (>640-25605120+)
<p>|                           |      | Diagnostic power HIGH | HIGH diagnostic power | Diagnostic power |
|                           |      | Single Convalescent serum CF ab (=&gt;80-2560) v ph2ag | Not useful applicable | Not applicable Not useful |
|                           |      | Low to medium (variable titre) | | |
| IFA on C. burnetii microdots | Ac and conv sera; initial &gt; IgM to ph 2 ag (80-5120), then IgM to ph1ag (&gt;20-320); finally IgG ph2 ag (&gt;40-640) and later IgG ph1 (&gt;40-80) Approx 25% form IgA to ph2 ab | IgG ph1ag (&gt;160-640) IgG ph2ag (&gt;160-1280) IgM ph2ag (&lt;10-40) IgM ph1ag (&lt;10-40) | IgG ph1 &amp; ph2 (&gt;=1280-5120) IgM variable (40-160) | IgG ph1 &amp; ph2 ag (2560-10 240) IgM ph1&amp;ph2 ag (usually &lt;10) IgA ph1&amp;ph2 ag (=&gt;1280) (“Burnt out” cases may have lower IgG ph1&amp; ph2 ag &amp; no IgA ab) |
|                           |      | HIGH Diagnostic power | Medium–low Diagnostic power | Medium Diagnostic power |
|                           |      | Single convalescent | | | HIGH Diagnostic power |</p>
<table>
<thead>
<tr>
<th>Test</th>
<th>Medium Diagnostic Power</th>
<th>Low Diagnostic Power</th>
<th>Low Diagnostic Power</th>
<th>Low Diagnostic Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgM ph2 ab (&gt;80-2560)</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
<td></td>
</tr>
<tr>
<td>Serum IgM ph 1ab (&gt;40-320)</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
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<tr>
<td>Serum IgG ph2ag (&gt;40-2560)</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
<td>Low diagnostic power</td>
<td></td>
</tr>
<tr>
<td>EIA</td>
<td>Use as screening test only</td>
<td>Use as screening test only</td>
<td>Use as screening test only</td>
<td></td>
</tr>
<tr>
<td>Acute and convalescent serum IgG &amp; IgM ph2 ag First IgM ph2 ag then IgG</td>
<td>Use as screening test only</td>
<td>Use as screening test only</td>
<td>Use as screening test only</td>
<td></td>
</tr>
<tr>
<td>EIA HIGH diagnostic power</td>
<td>LOW diagnostic power</td>
<td>LOW diagnostic power</td>
<td>LOW diagnostic power</td>
<td></td>
</tr>
<tr>
<td>Single convalescent serum IgM and IgG ph2 ag present</td>
<td>LOW diagnostic power</td>
<td>LOW diagnostic power</td>
<td>LOW diagnostic power</td>
<td></td>
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</tbody>
</table>

(=>1280-5120 )= range of titres commonly observed

**Draft Algorithm for the Serological Diagnosis of Acute Q fever**
Serum sample from patient with suspected acute Q fever (e.g., pneumonitis, hepatitis, fatigue, fevers)

No evidence of recent or past exposure to Q fever suggest repeat in 10-14 days if clinically relevant

Screening test
EIA phase 2 IgG
EIA phase 2 IgM

Specific test
CFT phase 2
CFT phase 1

CFT phase 1 < 8
CFT phase 2 < 8
No serological evidence of acute Q fever

CFT phase 1 < 8
CFT phase 2 > 8
Seraological evidence suggestive of acute Q fever. Suggest repeat testing in 2-4 weeks to monitor titre

CFT phase 1 > 8
CFT phase 2 > 8
Seraological evidence consistent with acute Q fever. Suggest repeat testing in 1-2 months to monitor

EIA IgG positive
EIA IgM negative
Possible past exposure

EIA IgG positive
EIA IgM positive
Possible acute Q fever

EIA IgG negative
EIA IgM positive
Possible acute Q fever or false positive