Chapter 14
Coxiella burnetii

John Stenos, Stephen Graves, and Michelle Lockhart

14.1 Introduction

Q fever (infection with *Coxiella burnetii*) is usually a difficult disease to diagnose as it has no pathognomic symptoms or signs. The main features are fever, myalgia and fatigue, often with associated respiratory symptoms (similar to influenza or legionnaires disease) or hepatitis. The patient history often reveals animal contact (mainly cattle, sheep and goats in Australia), as Q fever is an occupational disease of unvaccinated workers in the meat industry, shearers or farmers. Occasionally a tick-bite is recalled. However, most patients have not had close contact with animals and have been infected by the air-borne route. A chest x-ray may show an interstitial pneumonia. A post Q fever fatigue syndrome is recognised in some patients. Laboratory tests often demonstrate a hepatitic pattern of hepatitis with raised transaminases, a normocytic leucocyte count but with lymphopaenia and thrombocytopaenia and significantly raised CRP (>100).

While serology is the standard method for diagnosing Q fever, antibodies are only detectable 7–10 days into the illness and during the acute illness the patient may be seronegative. During this time nucleic acid amplification is the diagnostic test of choice. More laboratories are now offering this test (public and private). A positive result is consistent with acute Q fever (or chronic Q fever if the patient has been unwell for some months). A serum sample should also be sent to the laboratory for Q fever serology.

Q fever is usually treated with doxycycline (or cotrimoxazole in pregnant patients and children less than 7 years).

J. Stenos (✉)
Australian Rickettsial Reference Laboratory, Barwon Biomedical Research, The Geelong Hospital, Bellarine Street, Geelong, VIC 3220, Australia; Division of Verterinary and Biomedical Science, Murdoch University, Murdoch, WA 6150, Australia
e-mail: jstenos@unimelb.edu.au

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14.2 Assay Description

The real time assay was designed as a duplex PCR, targeting the Com 1 and IS1111a sites. Although these targets can be amplified individually using the described conditions the assay was optimised for the two targets. Primers and probes were designed using Primer Express (Applied Biosystems) (Table 14.1). These conserved regions appear to amplify all of the Coxiella isolates tested to date. This assay is suitable for any open real time PCR platform, however this assay was optimised on a Rotorgene 3000 (Corbett Research). Extracted DNA from a cultured strain of *C. burnetii* is used as a positive control. Negative controls, including a non-template and extraction controls are incorporated in this assay. We have found that the sensitivity of this assay is between 1 and 10 copy numbers and the sensitivity is relatively high given the two gene targets.

Table 14.1 Master mix reagents for Q fever qPCR

Reagent	Stock	Final concentration	Amount
UDG master mix	2X	1X	12.5 μl
Com1 forward	10 μM	400 nM	1 μl
Com1 reverse	10 μM	400 nM	1 μl
Com1 probe	5 μM	200 nM	1 μl
IS1111a forward	5 μM	200 nM	1 μl
IS1111a reverse	5 μM	200 nM	1 μl
IS1111a probe	2.5 μM	100 nM	1 μl
MgCl	50 mM	1.5 mM	0.75 μl
H_2O			0.75 μl
DNA			5 μl

14.3 Acceptable Specimens

A variety of clinical samples can be used such as blood, serum, bone marrow, biopsies (including those imbedded in paraffin) and cerebrospinal fluid. However this assay has been optimised and validated using uncoagulated blood collected in an EDTA tube.

14.4 Sample Extraction

Buffy coat preparation: Blood samples were spun and the buffy coat transferred to a fresh 10 ml tube. A volume of 2.5 ml of red blood cell lysis solution (Qiagen) was added to each 0.5 ml of buffy coat. This was incubated at 35°C for 5–10 min before centrifugation at 4,500×g for 5 min. The resultant white blood cell pellet

was washed with 5–10 ml sterile phosphate buffered saline (PBS) and resuspended in a final volume of 0.2 ml PBS.

DNA was extracted using a blood mini Kit spin column (Qiagen). Samples such as biopsies (including those embedded in paraffin wax) can be prepared for DNA extraction as described by the handbook supplied with the kit. Buffer AL (200 μl) and 20 μl of proteinase K was added directly to the buffy coat and incubated with shaking at 56°C for 10 min followed by a pulse centrifuge. Two hundred microliters of 100% ethanol was added, mixed by vortex and pulse centrifuged. The solution was then transferred to a column and centrifuged at 8,000×*g* for 1 min. The column was placed into a fresh collection tube to which 500 μl of WB1 buffer was added, followed by centrifugation at 8,000×*g* for 1 min. The column was then transferred to a fresh collection tube and 500 μl WB2 buffer added, followed by centrifugation at 14,000×*g* for 3 min. The collected liquid was decanted and the column centrifuged at 14,000×*g* for 1 min. The column was placed in a fresh sample tube and 50 μl of EB buffer added. This was allowed to stand at room temperature for 1 min followed with centrifugation at 8,000×*g* for 1 min. The extracted DNA was kept at 4°C until tested.

14.5 Primer and Probe Sequences

The primers and probes for the Com1 OMP gene are:

Com1-F (5′- AAA ACC TCC GCG TTG TCT TCA-3′)
Com1-R (5′- GCT AAT GAT ACT TTG GCA GCG TAT TG-3′)
Com1 probe (5′- FAM AGA ACT GCC CAT TTT TGG CGG CCA BHQ1-3′)
The primers and probes for the IS1111a gene are:
IS1111a-F (5′- GTT TCA TCC GCG GTG TTA AT-3′)
IS1111a-R (5′- TGC AAG AAT ACG GAC TCA CG-3′)
IS1111a probe (5′- QUASAR 670 CCC ACC GCT TCG CTC GCT AA BHQ2-3′).

14.6 PCR Amplification and Analysis

The components of the PCR mixture are listed below. A UDG Master mix (Invitrogen) was utilised in this assay in order to minimise amplicon contamination in subsequent runs.

Amplification is performed on a Rotorgene 3000 (Corbett Research) under the following conditions: 50°C for 3 min followed by 95°C for 5 min and then 65 cycles of 95°C for 20 s and 60°C for 40 s. Fluorescence is recorded at the end of the annealing/extension step. A characteristic sigmoid amplification is observed with each respective channel. Confirmation of a successful run is given with the

amplification of a chromosomal positive control and the absence of amplicons in the negative controls. Samples are considered positive if they amplify in both assays.

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