

SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES
FOR
SUSPECTED AGENTS OF BIOTERRORISM
AND
EMERGING INFECTIOUS DISEASES

Coxiella burnetii

American Society for Microbiology (ASM)

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PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE.

Coxiella burnetii is the etiologic agent of Q fever. It is a pleomorphic coccobacillus that is Gram-negative, obligately intracellular, and 0.3 to 0.7 μ m long. *C. burnetii* is phylogenetically related to *Pseudomonas*, *Francisella*, and *Legionella*, within the *Legionella* group of the γ -*Proteobacteria* subdivision. It is more distantly related to *Rickettsia* (1). There is phase variation, similar to that in *Salmonella*, in which the lipopolysaccharide (LPS) varies chemically as either the virulent, phase I “smooth” type LPS, or the phase II “rough” LPS, associated with avirulent *C. burnetii*. Q fever is a zoonotic disease, especially of parturient goats, sheep, or cattle and occasionally domestic cats. That the infectious dose is very low combined with properties of aerosolized contaminated dust particles being an efficient source of infection, and resistance to inactivation in the environment, make *C. burnetii* a potential agent of bioterrorism. It has historically been developed for such a purpose and, hence classified as a non-Tier 1 Select Agent by the Centers for Disease Control and Prevention, Department of Health and Human Services.

Note: sentinel clinical laboratories do not require registration with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier 1. Testing for Select Agents may be performed by laboratories as long as the laboratory destroys any residual specimen and destroys or transfers the confirmed select agent within 7 days of identification. Reporting of all identified Select Agents is still required; laboratories will need to complete Form 4. If the organism is transferred following confirmatory identification, then the laboratory must also complete Form 2. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov.

Q fever persists from a reservoir among wildlife, including mammals, birds, and arthropods (2). Ticks may be infected with *C. burnetii*; however, they do not represent a major vector of human disease. Human-to-human transmission is rare. Infection may be acquired through ingestion of contaminated milk [bulk milk supplies commonly contain *C. burnetii* (3)] resulting in seroconversion, although the disease acquired is usually subclinical.

Until 2008, active surveillance of Q fever was inconsistent, but where there has been active surveillance for the disease, it has been found (4, 5, 6). On a worldwide basis, *C. burnetii* is the leading cause of overt laboratory-acquired infections among viral, rickettsial, and chlamydial agents. Most of these have involved work with pregnant sheep in research laboratories (7). Geographic distribution of Q fever cases in the U.S is most densely located in areas associated with sheep, goat, or cattle ranching. States representing the majority of human Q fever cases include California, Colorado, Illinois, Kentucky, Missouri, Tennessee, and Texas (<http://www.cdc.gov/qfever>).

The symptoms of Q fever are generally nonspecific. There are multiple presentations, and two stages of disease, **acute** and **chronic** Q fever. The three most common presentations are **pneumonia** (47 to 63%), **hepatitis** (60%), or **fever only** (14%). (11, 14, 15) It is estimated that self-limited febrile illness, in fact, may be the most common form of the disease. The incubation period is 2 to 3 weeks. The organisms proliferate in the lung following inhalation of contaminated aerosols and then invade the bloodstream. There is a spore like form, the small cell variant, which is remarkably stable in extracellular environments. A large cell variant also exists that is the vegetative, metabolically active form. Mixtures of both forms are found in phagolysosomes. Acute Q fever is characterized by sudden onset of high fever, headache, myalgias, arthralgias, cough, and, less frequently, rash or a meningeal syndrome. In addition to radiographic manifestations of pneumonia, patients often have elevated liver enzyme levels and erythrocyte sedimentation rates and thrombocytopenia. Development of chronic Q fever is a more serious disease, which can occur up to 20 years after the initial infection. The major complication of chronic Q fever is endocarditis. Overall, the mortality rate of Q fever is low, approximately 2.4% (8), but it may be as high as 65% among those with chronic Q fever (9). Q fever-associated abdominal aortic aneurysms with or without simultaneous endocarditis have been reported in patients with *C. burnetii* infection (10).

Several potential bioterrorism agents could present as community-acquired pneumonias, including those causing tularemia, plague, anthrax, or Q fever (11). The milder forms of pneumonic tularemia would be the most likely to be clinically indistinguishable from Q fever, whereas plague or anthrax would typically follow a more rapidly fulminant course. In addition to atypical pneumonia as part of a clinical algorithm leading to consideration of Q fever, the following should suggest Q fever (12): influenza-like illness during periods of low influenza activity; hepatitis without markers for hepatitis A, B, or C; and fever of unknown origin in children.

II. SAFETY CONSIDERATIONS.

A. Handling of samples for isolation and identification or those from environmental or animal sources.

1. Because of the highly infectious nature of this organism (Biosafety Level 3 [BSL-3]), specimens from suspected cases of Q fever should be immediately forwarded to a Local or State Health Department identified as your Laboratory Response Network (LRN) reference laboratory.
2. Surface decontamination requires use of 95% alcohol.

B. Precautions.

1. Due to the extreme infectivity of *C. burnetii*, Sentinel Laboratories should not attempt to culture this organism, but should be aware of the potential for inadvertent isolation of *C. burnetii* in cell culture systems designed for virus isolation. *C. burnetii* can be inadvertently isolated in conventional cell cultures in a wide variety of cell lines, including all fibroblast cell lines. After an incubation period of 5 to 15 days, *C. burnetii*-infected cells are detectable as cytoplasmic inclusions (Fig. 1).
2. Biohazardous waste should be decontaminated by autoclaving. Contaminated equipment or instruments can be decontaminated with

approved disinfectants. The spore-like form of *C burnetii* may be resistant to disinfection using dilute bleach, ultraviolet radiation, or heat achieved by autoclaving or boiling for less than 10 min. Special decontamination procedures are necessary for surfaces potentially contaminated with *C. burnetii*. Household bleach solutions may be ineffective. Minor spills should be covered with absorbent paper, such as paper towels, and then flooded with 70-95% alcohol or 5% MicroChem-Plus (a dual quaternary ammonium compound), which should be allowed to act for 30 min before cleanup. Spills that involve samples with high concentrations of organisms, involve organic matter, or occur in areas of lower temperatures (e.g., refrigerators or freezers), should be exposed to disinfectant solution for 1 h before cleanup.

III. SPECIMEN COLLECTION.

A. Collection of Clinical Specimens for Transport to Reference Laboratory	
Whole blood	<ul style="list-style-type: none"> • Collect blood in EDTA (lavender) or sodium citrate (blue) top tube. • Maintain at 4°C for storage and shipping for PCR or special cultures. • Collect specimens prior to antimicrobial therapy, if possible.
Tissue, body fluids, and others, including cell cultures and cell supernatants.	<ul style="list-style-type: none"> • Maintain at 2 to 8°C and transport within 24 h. • Store biopsies for PCR or immunohistochemical staining frozen at minus 70° C or on dry ice.
Serum	<ul style="list-style-type: none"> • Acute phase: Collect serum (red-top or serum separator tube [SST], tiger-top tube) as soon as possible after onset of symptoms. • Convalescent phase: Collect a follow-up specimen at ≥14 days. • Separate and store serum frozen until testing.

B. Rejection criteria.

1. Incomplete documentation; all specimens must include the sender's name and telephone number to contact for the preliminary report and additional information.
2. Improper packaging/shipping.
3. Lack of prior approval. Do not ship specimens to LRN Reference laboratories without preliminary arrangements.

IV. SPECIMEN PROCESSING.

A. Serologic.

1. Since the laboratory diagnosis of Q fever is based mainly on serologic testing, serum samples should be submitted for measurement of antibody responses against phase I and II antigens of *C. burnetii*. Patients with acute Q fever typically produce an antibody response primarily to *C. burnetii*

phase II antigen, while chronic *C. burnetii* infections typically elicit a higher antibody response to phase I antigen.

B. Culture.

1. *C. burnetii* cannot be cultured on routine bacteriologic media. Special culture procedures must be performed in a laboratory with full biosafety level 3 capability. *C. burnetii* can be cultured in various cells, including human embryo fibroblast cells, L cells, and green-monkey kidney cells (13). Recently, a method for cell-free cultivation of *C. burnetii* has been described (14).

ANALYTICAL CONSIDERATIONS

V. QUALITY CONTROL.

Commercial reference laboratories offer serologic testing; PCR, immunostain procedures and culture are generally performed only in research laboratories.

VI. IDENTIFICATION.

If your laboratory uses Mass Spectrometry (MALDI-TOF or bioMerieux) for bacterial identification and if the manufacturer provides your facility with an alternate tube extraction method, it is recommended that the resulting extract be filtered using a 0.2 μ (or less) filter. This additional step is recommended to reduce the risk of laboratory contamination with viable bacteria and spores.

Using automated systems, including Mass Spectrometry (MALDI-TOF, bioMerieux) technology may result in exposure to dangerous pathogens, and could result in erroneous identification, Ex: *Bacillus anthracis* misidentified as *B. cereus*; *Yersinia pestis* misidentified as *Y. pseudotuberculosis*.

A. Confirmatory diagnosis.

1. Acute Q fever: demonstration of fourfold or greater changes in antibody titer between paired acute- and convalescent-phase serum samples by immunofluorescence antibody (IFA) testing. Comparable sensitivity and specificity can be achieved with an enzyme-linked immunosorbent assay (ELISA) when testing for IgG (15), however the CDC case definition (<https://www.cdc.gov/mndss/conditions/q-fever/case-definition/2009/>) is based on IFA testing, or
 - Detection of *C. burnetii* by polymerase chain reaction (PCR). Real-time PCR assays have been developed that are sensitive and specific, based on targets of the IS1111 sequence present in multiple copies in the *C. burnetii* genome (16, 17), or
 - Immunohistochemical staining of biopsy material from affected organs (18), or
 - Isolation of *C. burnetii* in culture of biopsy or blood samples (12, 13).

2. Chronic Q fever: IFA titer to phase I antigen $\geq 1:800$ with phase I titer being higher than the phase II titer, or
 - Detection of *C. burnetii* target in affected tissue by PCR, or
 - Immunohistochemical detection of *C. burnetii*, or
 - Isolation of *C. burnetii* in culture.

B. Presumptive diagnosis.

1. Acute Q fever: supported by a single positive IFA IgG titer $\geq 1:128$ (CDC cutoff value) to phase II antigen (phase I titers may be elevated as well), or elevated (as defined by the testing laboratory) phase II IgG or IgM antibody reactive with *C. burnetii* antigen by ELISA. It is not possible to determine a standard cutoff value of ELISA or IFA applicable to all laboratories, and equivocal results may occur in some cases (19).
2. Chronic Q fever: supported by IgG antibody titer to *C. burnetii* phase I antigen $\geq 1:128$ and $< 1:800$ by IFA.

C. Clues from ancillary tests suggestive of Q fever.

1. Culture-negative endocarditis may occur in conjunction with the diagnosis of chronic Q fever.
2. A histological appearance of bone marrow or liver in which a fibrin ring is associated with granuloma formation (doughnut granulomas) is a clue to the presence of *C. burnetii* (12, 18), although this finding also is associated with other agents such as *Francisella tularensis* (Fig. 2).

POSTANALYTICAL CONSIDERATIONS

VII. SUMMARY/SPECIAL CONSIDERATIONS.

A. Serologic Results.

1. Interpretive criteria typically used by laboratories are shown in Table 1.
2. Assistance with interpretation as to the significance of serologic results in the context of a diagnosis of Q fever should be provided.
3. The interpretation should indicate if the results are supportive of either acute or chronic Q fever, and if indicated the recommendation to submit a second serum sample for determination of a fourfold rise in titer.

B. Antimicrobial Therapy

1. Acute Q fever: the first line of treatment for all adults and those children with severe illness is doxycycline. Co-trimoxazole may be used in children with mild disease who are less than 8 years old.
2. Chronic Q fever: Doxycycline and hydroxychloroquine. The duration of treatment for chronic Q fever is typically 18 months.
(<http://www.cdc.gov/qfever/symptoms/index.html#treatment>)

C. Select Agent reporting and compliance.

1. Reporting of all identified Select Agents is still required, even though Sentinel laboratories are not required to register under the Select Agent Rule.
2. The laboratory must complete Form 2 within one week (7 days) following notification of the confirmed identification. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov
3. Reporting all identified Select Agents is required by completing Form 4 A within 7 days of confirmed identification. If the isolate is from a Proficiency test sample, Form 4 B is to be completed within 90 days of receipt of the sample.
4. Your designated LRN Reference Laboratory will advise you with completion of required forms (e.g., Forms 2, 3, and 4). Always refer to www.selectagents.gov for the latest guidance and versions of these forms.

D. Destruction.

1. Once the identification of a Select Agent is confirmed, the regulations require that the residual specimen and cultures of the isolate be destroyed or transferred to an approved Select Agent entity within 7 days of confirmed identification. Your designated LRN Reference Laboratory must advise you on destruction or transfer of isolates.
2. In the case of *C. burnetii* this is usually applicable only to clinical material. Specimens containing the organism should be autoclaved, incinerated on-site or submitted to the designated LRN Reference Laboratory for disposal. If no autoclave is available, contaminated items should be soaked in 95% alcohol, 10% bleach, or 10% formalin for 24 hours.

E. Limitations.

1. Laboratory diagnosis is often difficult due to lack of specific criteria leading to the suspected clinical diagnosis of Q fever.
2. Cultures are difficult and hazardous to perform, leading to reliance on serologic means of diagnosis in most cases of Q fever.

F. Packing and shipping.

1. Refer to the ASM Packing and Shipping Sentinel Guidelines.

2. All materials sent to your designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations.

G. Notifications.

1. Sentinel clinical laboratories should consult with their LRN reference laboratory prior to or concurrent with testing if *C. burnetii* is suspected by the attending physician.
2. The results of serology and other Q fever diagnostic testing obtained through commercial reference or public health laboratories should be entered into the patient's medical record. Report positive results to hospital infection control and public health officials, as well as to the patient's physician.

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Figure 1. **Fibroblast L929 cell line infected with *Coxiella burnetii*. (courtesy of Didier Raoult)**

Figure 3. Small granulomas exhibiting doughnut appearance.

Table 1. Typical Q fever diagnostic titers with indirect immunofluorescence (2)

Stage of disease	Phase I antibodies (IgG)	Phase II antibodies (IgG)
Acute	Positive but lower than titers to phase II <i>C. burnetii</i>	$\geq 1:128$ *
Chronic	$\geq 1:800$	Positive but same or lower than titers to phase I <i>C. burnetii</i>

* Four-fold rise in antibody titer between acute and convalescent samples is the strongest predictor of acute Q fever.