Detection of Selected Fastidious Bacteria

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The intent of this article is to describe the optimal methods for culture recovery of 7 fastidious bacteria: *Legionella* species, *Brucella* species, *Francisella tularensis*, *Leptospira* species, *Borrelia burgdorferi*, *Bartonella* species, and *Bordetella* species. These organisms share much in common beyond the fact that their genus names all end in the letter “a.” Culture recovery of these organisms, even from adequate clinical specimens, is logistically demanding, often costly, and lacking in both timeliness and sensitivity. In addition, there is generally no need to recover culture isolates on which to perform antimicrobial susceptibility tests because these 7 bacteria are nearly uniformly susceptible to specific, clinically useful antimicrobial agents and because, for some of them, susceptibility tests of proven reliability have not yet been devised. Perhaps for these reasons, alternative, more rapid, direct diagnostic approaches have been developed that are based on either immunochemical or nucleic-acid detection methods. These methods have generally served to supplant culture as a primary diagnostic modality. Situations exist, however, in which culture may be desirable, if not necessary, to establish a definitive diagnosis of infection with these 7 organisms. This review attempts to summarize how best to proceed in those cases.

Determining the precise etiology of infection in individual patients aids in management decisions, is of prognostic and epidemiological consequence, and may have profound public health and infection control ramifications. Culture techniques traditionally have formed the cornerstone of establishing an etiologic diagnosis of infection in patients whose disease is due to bacteria, fungi, mycobacteria, and, in some cases, viruses and parasites. During the past decade, first immunochemical techniques that detect microbial antigens and then molecular methods that detect microbial nucleic-acid sequences have been developed for infectious disease diagnosis.

By use of these methods, either specific antigens or nucleic acid segments are detected directly in clinical specimens. These methods, together with microscopic visualization, serve as adjuncts and, in selected instances, as surrogates to culture methods for establishing the etiology of infection. These methods have become increasingly popular because of the recognized limitations of many culture techniques (e.g., technical complexity, lack of timeliness, expense, and relative insensitivity with certain organisms).

Culture, however, retains 2 distinct advantages over non-culture-based direct detection methods. First, a microorganism is made available on which to perform antimicrobial susceptibility testing. Second, once a microorganism has been recovered in culture and accurately identified, there can be no mistaking the correctness of the finding; to wit, a positive culture has absolute specificity.

Simply recovering a microorganism from a high-quality specimen does not necessarily prove that the microorganism is unequivocally responsible for disease. One can conclude with certainty only that the microorganism is present; its clinical significance is ascertained by taking into account additional information. In certain cases, however, the mere recovery of certain microorganisms from a patient by means of culture nearly always identifies it as the etiology of disease in that patient. This is true of agents that rarely, if ever, colonize patients asymptotically.

This axiom applies to the microorganisms under discussion here, including *Legionella* species, *Brucella* species, *Francisella tularensis*, *Leptospira* species, *Borrelia burgdorferi*, *Bartonella* species, and, usually, *Bordetella pertussis*. All 7 of these organisms have in common the fact that they are generally slow-growth, fastidious bacteria for which culture methods are logistically complex. In addition, these bacteria are relatively infrequent causes of disease.

Because of the low prevalence of infection due to these bacteria, diagnostic specificity is absolutely mandatory. The predictive value of a positive test result with these organisms becomes unacceptably low if the test that is used for diagnosis yields even small numbers of false-positive results. For these reasons, culture remains a useful tool in establishing an etiologic diagnosis of infection due to these agents. The intent of this article is to discuss the optimal approach to the laboratory diagnosis of infections due to *Legionella* species, *Brucella* species, *F. tularensis*, *B. burgdorferi*, *Leptospira* species, *Bartonella* species, and *B. pertussis*.
**Legionella Species**

*Legionella*, now recognized as an important cause of pneumonia, is a facultative, highly fastidious, gram-negative bacillus widely distributed in nature, primarily in association with aquatic environments. Humans become infected after exposure to airborne droplets and potable water containing the organism [1]. At least 40 different species have been recognized; however, 1 species, *Legionella pneumophila*, accounts for the vast majority of human infections [2].

Culture recovery of *Legionella* species from patients with pneumonia is best accomplished by use of bronchoscopy or lung biopsy specimens [3–5]. Expectorated sputa or suctioned specimens from patients who are intubated should be considered inferior to specimens collected by more invasive means. Perhaps this is because of the ability of *Legionella* species to survive intracellularly and their tendency to cause an interstitial pulmonary process. Extracellular organisms free in the lower airways are probably uncommon. Pleural fluid is also a satisfactory specimen.

Several characteristics of *Legionella* species are relevant to their recovery in culture [6]. Organisms of this genus require L-cysteine for growth. In addition, optimum growth occurs only over a narrow range of pH and is enhanced by iron salts. Growth is impeded by small amounts of toxic substances, which may be present in respiratory tract specimens, and the organism is intrinsically resistant to several antimicrobial agents. In view of these characteristics, it is not surprising that *Legionella* species do not grow on standard supplemented media such as enriched chocolate agar, notwithstanding the published early finding of growth on Mueller-Hinton agar with IsoVitaleX (Becton Dickinson Microbiology Systems, Cockeysville, MD) [7].

A variety of observations [8–12] together define the optimal medium for culture recovery of *Legionella* species, namely, ACES-buffered charcoal yeast extract agar supplemented with L-cysteine, alpha-ketoglutarate, and iron (BCYE). When this medium is used for respiratory tract specimens that are probably contaminated with oropharyngeal bacterial flora, a selective version of BCYE should be used. Antibiotics to which *Legionella* species are usually resistant (i.e., anisomycin, polymyxin B, cefamandole, and vancomycin) are added to BCYE to inhibit growth of commensal bacteria [13].

Plates should be incubated in a humid environment at 35°C in 3%–5% CO₂ for at least 5 days [4, 6, 13]. Colonies typically become apparent on the second or third day of incubation. Blood cultures need not be performed, except in rare cases of culture-negative prosthetic valve endocarditis [14]. The fact that the nature and quality of specimens play a major role in the sensitivity of culture has been demonstrated by the finding that, in general, cultures for <50% of patients with legionellosis will yield positive results, even when adequate methods and media are employed [6]. Nonculture-based diagnostic methods have been reviewed in a prior article in this series [15].

**Brucella Species**

*Brucella* species are associated with a febrile illness in humans acquired by direct exposure to infected animals or, more commonly today, consumption of contaminated dairy products, especially fresh goat cheeses [16, 17]. Acquisition of this microorganism by laboratory workers as a result of laboratory accidents is also common. Four species are most commonly recognized as causes of human infections: *Brucella abortus*, *Brucella canis*, *Brucella melitensis*, and *Brucella suis* [17]. The usual animal reservoirs for these species are cattle, dogs, sheep and goats, and swine, respectively [17].

The specimens that yield *Brucella* species most often are blood and bone marrow from infected patients [18, 19]. In selected cases, organisms may also be recovered from biopsy specimens of the liver and lymph nodes [20]. Rarely, normally sterile body fluids such as CSF and peritoneal fluid have yielded the organism, as have soft-tissue biopsy specimens and urine [21].

Although the optimal blood culture method for detecting brucella bacteremia has not been defined, methods are evolving and have improved. Traditional methods that employ biphasic blood culture bottles with brain-heart infusion broth and agar media, vented and incubated in 5%–7% CO₂ at 35°C for 30 days [21], are effective but no longer necessary [22]. If used, however, biphasic bottles should be inverted to inoculate the agar surface once daily after careful macroscopic examination [21].

*Brucella* species have also been recovered with the most widely used continuous-monitoring automated blood culture systems [23–27], including the BACTEC NR660 and 9240 (Becton Dickinson Microbiology Systems) and the BacT/Alert (Organon Teknika, Durham, NC) blood culture systems. Moreover, published results, mostly concerning *B. melitensis*, show that ≥95% of isolates are recovered within 2–6 days with the most recent media and reports [23, 26, 27]. A terminal blind subculture of instrument-negative bottles is warranted for patients whose epidemiological and clinical circumstances suggest a high probability of brucellosis.

A third approach to detect *Brucella* species in blood is use of lysis-centrifugation (Isolator; Wampole Laboratories, Cranberry, NJ) [25, 27]. Comparative data concerning centrifugation-lysis versus automated blood systems are sparse; however, in 1 study the BACTEC 9240 enabled recovery of more *B. melitensis* strains (28 vs. 22) than did centrifugation-lysis, even though the numbers were insufficient for rigorous statistical conclusions.

During the acute stages of the disease, brucella bacteremia is typically continuous. Therefore, 2–3 blood cultures, each consisting of 10–20 mL of blood, should be sufficient to confirm the presence of bacteremia. For patients with chronic brucellosis, >2 or 3 blood cultures may be necessary to document bacteremia.

Fluid specimens, such as bone marrow aspirates, CSF, and
peritoneal fluid, should be cultured in biphasic blood culture bottles, in continuous-monitoring blood culture devices, or by the lysis-centrifugation method, as described above for blood specimens. Biopsy specimens and exudates may be cultured on standard agar media, such as 5% defibrinated sheep blood agar and enriched chocolate agar plates [21]. As was the case with subcultures of blood specimens processed by lysis-centrifugation, because of the slow-growth nature of Brucella species, plates should be incubated for up to 14 days in 5%-7% CO₂ at 35°C [20].

**Francisella tularensis**

*F. tularensis*, an infrequent cause of sporadic cases of the zoonotic infection tularemia, is a fastidious gram-negative bacillus found in nature in association with a wide variety of animals and birds [28]. Numerous arthropod vectors play an important role in maintaining the organism in mammalian and avian reservoirs [29]. Humans acquire infection by direct contact with infected animals or when bitten by an insect vector [30]. Lagomorphs represent the most common animal source for human infection [28]; ticks and (less commonly) deerflies are the most important arthropod vectors for human disease [29].

The most definitive specimens for recovery of *F. tularensis* are biopsy specimens of infected soft-tissue or lymph nodes. In addition, blood cultures should be performed, especially when the septicemic form of tularemia is suspected. *F. tularensis* is extremely fastidious and dies rapidly unless specimens are processed expeditiously. *F. tularensis* usually requires both cysteine and glucose for growth [29]. Recently, however, clinical isolates of this organism that lacked the cysteine growth requirement have been described elsewhere [31].

Traditionally, agar media supplemented with cysteine and glucose have been used to recover *F. tularensis* in the laboratory [29]. However, it has recently been found that enriched chocolate agar and nonselective BCYE adequately support the growth of *F. tularensis* and can therefore be recommended for use in isolating this organism from clinical specimens [32–34]. Plates should be incubated for at least 5 days in 5%-7% CO₂ at 35°C–37°C [29]. Most isolates appear after 2–4 days of incubation [29].

Even when adequate specimens are processed under optimal culture conditions, recovery of *F. tularensis* is problematic. As few as 10% of cases yield the organism [35]. When it is recovered in the laboratory, however, extreme care should be exercised in handling *F. tularensis*. It is a common cause of laboratory-acquired infection, notwithstanding its very infrequent isolation. Biosafety level 2 precautions should be employed [36].

In addition to biopsy-specimen cultures, blood cultures, especially for patients with the septicemic form of tularemia, may be appropriate even though they are rarely positive and the optimal detection system has not been delineated. As noted previously, current blood culture practices frequently involve use of a continuous-monitoring device. The media employed with these systems are typically enriched and probably support growth of *F. tularensis*. Indeed, recovery of *F. tularensis* by means of instrument-based blood culture methods has been described in the literature [37–40]. Incubation of blood culture bottles beyond the usual 5 to 7-day cycle recommended with these systems may be required; alternatively, a terminal gram stain and subculture could be done when the index of suspicion is high.

**Borrelia burgdorferi**

*B. burgdorferi* is the etiologic agent of Lyme borreliosis [41, 42]. Attempts to culture this organism are rarely necessary; however, if culture is to be performed, it should be restricted to the acute, primary stage of infection. The specimens of choice are skin lesion biopsy specimens, blood, and CSF from patients with clinical evidence of meningeal involvement [43]. Optimal sample sizes and transport conditions have not been defined. In light of the absence of such information, transport of specimens directly to the laboratory, followed by immediate inoculation and incubation of media, are recommended.

Several different media have been proposed for culturing *B. burgdorferi* from clinical specimens. All are derived from Kelly’s medium, originally described in 1971 as a means for propagating *Borrelia hermsii*, the cause of tick-borne relapsing fever in North America [44]. Stoesser’s modification, referred to as “fortified Kelly’s medium” was described in 1982 [45] and modified by Barbour, giving rise to BSK-I (Barbour-Stoenner-Kelly) medium [46] and later BSK-II medium, a semisolid liquid medium [47]. The principal ingredients of BSK-II medium are N-acetylglucosamine, peptone, bovine serum albumin, yeast extract, a supplement (CMRL, consisting of amino acids, vitamins, nucleotides, and other growth factors), glucose, rabbit serum, gelatin, and HEPES buffer [47].

Several additional modifications of BSK-II medium have subsequently been described [48–50], but use of BSK-II medium remains the cornerstone of efforts to recover *B. burgdorferi* from clinical specimens. Supplementation of BSK-II medium with various antimicrobial agents has been recommended as a means of enhancing recovery of *B. burgdorferi* from specimens such as skin biopsy specimens, which are potentially contaminated with nonfastidious bacteria [43, 51–54].

Tubes containing BSK-II medium with or without antimicrobial agents should be tightly closed after specimen inoculation and then incubated at 32°C–34°C in ambient air for up to 6 weeks before being considered negative and discarded. Cultures should be examined visually every 2–3 days for macroscopic evidence of growth (e.g., turbidity, often occurring near the bottom of the medium because of the microaerophilic nature of *B. burgdorferi*). If growth is observed, a drop of turbid medium should be removed and examined for the presence of...
organisms morphologically compatible with *B. burgdorferi* (i.e., spirochetes 10 to 30-μm long, with loose, irregular coils). Either phase-contrast or dark-field microscopy is the preferred method of visualizing *B. burgdorferi* microscopically.

*B. burgdorferi* stains inconsistently in gram preparations. Giemsa or silver stains are preferred but often are not available in clinical microbiology laboratories. At least once weekly in macroscopically negative cultures, a drop of medium from the bottom of culture tubes should be stained blindly for the presence of organisms morphologically compatible with *B. burgdorferi*.

The likelihood of recovering *B. burgdorferi* from human clinical specimens depends on the quality and nature of the specimen, the stage of the disease, and the expertise of the laboratory. Recovery from blood has been reported for only 3.1% and 5.6% of patients in 2 large studies [55, 56]. In contrast, up to 45% recovery rates have been reported for skin biopsies in patients with erythema chronicum migrans [48, 55, 57, 58]. Obviously, isolation rates are variable and tend to be low even when care is taken to maximize recovery. Irrespective of the specimen processed, recovery of *B. burgdorferi* probably occurs during the early stages of the disease, especially before antibiotic therapy is initiated.

**Leptospira Species**

The Leptospiraceae family is generally divided into 2 species, *Leptospira interrogans* and *Leptospira biflexa*, the latter species encompassing free-living, nonpathogenic forms [59]. More than 200 serovars of *L. interrogans* have been recognized and, in turn, are categorized into about 19 serogroups based on cross-reacting antigens. Organisms in the *L. interrogans* group are responsible for human infections. *Leptospira* species are harbored by numerous wild and domesticated animals [60] and are excreted in the urine.

The diagnosis of leptospirosis usually is based on the results of serological tests. Recovery of the organism in culture is largely restricted to reference and public health laboratories. However, occasionally, general clinical microbiology laboratories are justified in attempting to culture *Leptospira* species from human specimens. Blood should be cultured during the acute stage of the disease, CSF and urine later in the illness.

Numerous different semisolid media, dispensed in 5 to 10-mL aliquots in sterile screw-capped tubes, may be used to propagate *Leptospira* species. These include Ellinghausen-McCullough medium as modified by Johnson and Harrison (EMJH); supplemented with bovine serum albumin and polysorbate 80; and Fletcher’s, Stuart’s, and Korthof’s media [59–63], the latter 3 being supplemented with rabbit serum. The optimal medium for culture of *Leptospira* species has not been defined; it may be appropriate when performing *Leptospira* cultures to inoculate >1 medium.

Blood is cultured by placing 1–4 drops of specimen into each of 3–5 culture tubes. With CSF specimens, 0.5 mL of fluid is cultured per tube. Because of the possibility of inhibitory substances and bacterial contamination, urine specimens should be cultured undiluted and in serial 10-fold dilutions up to 10⁻³ or 10⁻⁴, 1 drop per culture tube. Five-fluorouracil (200 μg/mL) [63] or neomycin (6-mm disk containing 30 μg) [59] can be added to leptospiral culture media to suppress growth of contaminants present in urine specimens. Whenever an antibiotic-containing medium is inoculated, a companion tube lacking antibiotic should also be inoculated.

Culture tubes should be incubated at 30°C in ambient air in the dark for up to 4 months [59] and examined once weekly for evidence of growth of leptospiras. By use of aseptic technique, a drop of medium 1–3 cm below the surface is aspirated and examined with a dark-field or phase-contrast microscope for the presence of spirochetes with characteristic morphology and motility. *Leptospira* species organisms are typically ~0.1 μm in diameter and 6–12 μm in length. Organisms are tightly coiled (>18 coils per cell) and have conspicuous hooks at one or both ends.

A positive control culture should be inoculated with a known viable stock culture of *Leptospira* species at the time all clinical specimens are processed. This process attests to the adequacy of what is invariably a little-used culture routine and serves as a source of organisms from which microscopic comparisons can be made. Alternative leptospiral culture methods have been described elsewhere but remain investigational [64, 65].

Given the technical complexity and difficulties in culturing *Leptospira*, one can understand why such requests should virtually always be referred to public health or reference laboratories.

**Bartonella Species**

*Bartonella* species are small, curved, highly fastidious gram-negative bacilli. Five species have been delineated: *Bartonella bacilliformis, Bartonella vinsonii, Bartonella quintana, Bartonella henselae, and Bartonella elizabethae* [66, 67]. *B. bacilliformis* is the causative agent of a life-threatening bacteremic illness known as Oroya fever, which occurs in the Andean mountain regions of Colombia, Equador, and Peru. *B. vinsonii* is not known to cause human infection. *B. quintana* is the principal etiologic agent of louse-borne trench fever.

*B. henselae* is recognized as a cause of bacillary angiomatosis, parenchymal bacillary peliosis, endocarditis, and fever with bacteremia, all of which occur most commonly but not exclusively in patients who are infected with HIV [68–74]. Uncommonly, *B. quintana* and the most recently described species of *Bartonella, B. elizabethae* [67], may be associated with some of these same infections. In addition, *B. henselae* is now thought to be the principal etiologic agent of cat-scratch disease, in some cases presumably in conjunction with a related organism, *Afiopia felis* [73].
For patients suspected of having bartonella infections, blood or biopsy specimens of involved tissue offer the best opportunity for culture recovery of the organism [66]. Most recent experience with isolation of Bartonella species in the laboratory has been with B. henselae. Although B. henselae has been propagated on standard media, such as 5% sheep blood and enriched chocolate agar, the optimal solid culture medium for growth of this organism appears to be freshly prepared heart infusion agar containing 5%–10% defibrinated rabbit or horse blood [66]. Recently, a chemically defined liquid medium has been described that yielded excellent growth of several clinical isolates of B. henselae [75]. The utility of this liquid medium for processing clinical specimens from patients with bartonella infections needs to be further explored.

Tissue specimens should be transported to the laboratory expeditiously and then, after homogenization, inoculated directly onto solid medium. Blood specimens should be collected in lysis-centrifugation (Isolator) tubes and transported directly to the laboratory, and concentrates should be subcultured promptly to solid media [66]. The optimal volume of blood per culture, the preferred number of cultures, and the timing of collection for maximum recovery of Bartonella species have not been defined.

Similarly, the optimal subculture routine is unknown. Plates, whether inoculated with tissue specimens or lysis-centrifugation concentrates, should be incubated at 35°C–37°C in a humidified atmosphere of 5%–10% CO₂ for up to 4 weeks before they are considered negative and discarded. Recovery of Bartonella species from instrument-based broth blood cultures has been described elsewhere [76, 77]; however, the optimal system has not been defined.

The culture routines described above are probably also applicable to non-henselae Bartonella species, in particular, B. quintana and B. elizabethae. Growth of B. bacilliformis and A. felis is facilitated at lower temperatures of incubation (25°C–30°C).

The yield from cultures for Bartonella species is unknown. Even when cultures are performed under optimal conditions, isolation rates are very low. As a result, serology [66] and non-culture-based molecular detection methods such as PCR [78] are important adjuncts to establishing an etiologic diagnosis of bartonella infections.

Bordetella pertussis

Because of the resurgence of pertussis as a clinical problem in the United States [79], there is renewed interest in the recovery of B. pertussis in culture. Bordetella parapertussis may cause a similar albeit less-severe illness and may not have the same epidemiological implications as B. pertussis. This discussion will focus on the culture recovery of B. pertussis; however, the recommendations stated below may also be considered applicable to B. parapertussis.

Two other species within this genus, Bordetella bronchiseptica and Bordetella avium, pathogens of dogs and turkeys, respectively, have only rarely been isolated from humans and will not be considered herein.

B. pertussis is an extremely fastidious gram-negative bacillus that typically fails to grow even on enriched chocolate agar, at least on primary isolation. Optimal recovery is achieved by obtaining samples from the nasopharynx, either by swabbing with calcium alginate or synthetic-polyester swabs on a flexible wire or by aspiration [80–86]. Pharyngeal swab specimens should be avoided [83]. Cotton swabs should not be used because they contain toxic substances such as fatty acids on the cotton fibers. The swab should be inserted well into the nasopharynx, rotated several times, and left in place for 30–60 s [81]. Upon removal, nasopharyngeal swabs should be immediately inoculated to suitable agar medium at the patient’s location or placed directly into transport medium.

Numerous different transport media have been recommended [81, 86–90], but Regan-Lowe transport medium containing half-strength charcoal agar, 10% defibrinated horse blood, and cephalexin (40 μg/mL) is probably the most useful [81, 91] because of its long shelf-life, its commercial availability, and the extensive experience with its use. In circumstances where transport of nasopharyngeal swab specimens to the laboratory will be delayed, several studies have suggested that incubation of Regan-Lowe transport media at the collection site for 1–2 days prior to transport will increase culture recovery, presumably owing to inhibition of contaminants present in the specimen with simultaneous initial growth of B. pertussis [85, 87, 91, 92]. In addition, maintaining specimens at 4°C rather than at 25°C prior to and during transport may enhance recovery [86, 93]. When transport to the laboratory can be accomplished within a few hours or less, swabs in transport media should be transported immediately at room temperature.

Numerous different media have been advocated for use in the culture recovery of B. pertussis from clinical specimens [81, 82, 89, 92, 94–100]. Bordet-Gengou medium, consisting of starch, glycerol, NaCl, and 5%–20% defibrinated sheep or horse blood, supports luxuriant growth of B. pertussis, but it has a very short shelf-life and does not effectively suppress growth of contaminants [98]. As a result, the presence of B. pertussis, a slow-growth organism, can be obscured by contaminants. Addition of antimicrobials to Bordet-Gengou agar limits this problem.

In 1953 Mishulow et al. recommended use of charcoal agar for removing toxic substances from clinical specimens that interfere with growth of B. pertussis [99]. This substitution also substantially lengthened the shelf-life of the medium. Further modifications included the addition of penicillin at a concentration of 0.3 μg/mL [94] and later cephalexin (40 μg/mL) [96] to suppress contaminating flora, as well as 10% defibrinated horse blood to encourage growth [96]. It is this medium, often
referred to as charcoal–horse blood agar or Regan-Lowe agar, that is most commonly used today.

Because of the possibility of inhibition of the growth of some strains of *B. pertussis* by the high concentration of cephalexin in charcoal–horse blood agar, it has been recommended that a plate lacking cephalexin or containing different antimicrobial agent(s) be inoculated along with the cephalexin-containing plate [93]. Assuming fresh medium is used, this very conservative culture approach appears to be unnecessary in most cases [81, 101].

Plates should be incubated in a humidified environment for 7–10 days in ambient atmospheric air [101] at 35°C [81] and examined daily for the appearance of colony growth morphologically consistent with *B. pertussis*. Many laboratories have traditionally incubated *B. pertussis* cultures in an elevated CO₂ atmosphere of 5%±10%. There is now evidence, however, that ambient atmospheric air is superior to 5%–10% CO₂ incubation [101]. The use of broth enrichment prior to culture is of no definable value [102].

The rate of culture-positivity among patients with pertussis has been shown to vary markedly (e.g., 20%–83% [81, 103–105]). This wide variation undoubtedly is due to differences in patient ages; antibiotic administration; organism load at the time specimens were collected; adequacy of the specimens; incubation conditions; and the expertise and familiarity of laboratory personnel with *B. pertussis* cultures. Notwithstanding these considerations, culture remains an important vehicle for establishing an etiologic diagnosis of pertussis. The significance of culture recovery of *B. pertussis* is underscored by the recent recognition of resistance to erythromycin in a clinical isolate of this organism [106].

Conclusions

This discussion has centered around optimizing culture routines for recovery of 7 fastidious bacteria. As noted in the introductory paragraphs, assuming representative clinical specimens have been submitted, the culture recovery of *Legionella* species, *Brucella* species, *F. tularensis*, *B. burgdorferi*, *Leptospira* species, or *Bartonella* species unequivocally defines a patient as having disease due to that organism. In most cases, the same conclusion can be drawn from the culture recovery of *B. pertussis*. Furthermore, the prevalence of infection due to all of these organisms, again with the possible exception of *B. pertussis*, is also very low. As a result, many clinical laboratories often do not have the requisite expertise for performing cultures, and the cost of performing cultures may be prohibitive because the necessary media and supplies become outdated before they can be used.

For these reasons, cultures for *Legionella* species, *Brucella* species, *F. tularensis*, *B. burgdorferi*, *Leptospira* species, and *Bartonella* species, except in cases in which blood cultures are indicated and can be performed by use of a continuous-monitoring device, should probably be restricted to clinical microbiology laboratories that handle a large amount of referral testing.

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