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Limited Applicability of Direct Fluorescent-Antibody Testing for *Bordetella* sp. and *Legionella* sp. Specimens for the Clinical Microbiology Laboratory▽

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The rapid diagnosis of infections with *Bordetella* and *Legionella* species is important for patient management. With observed increases in direct fluorescent-antibody (DFA) testing volumes, we retrospectively compared the performance characteristics of DFA testing to those of culture and PCR. For *Bordetella* sp., samples were classified as positive by DFA testing (184 [3%] of 6,195 samples) and culture (150 [2%] of 6,251 samples) significantly less often than by PCR (2,557 [10%] of 26,929 samples). Of 360 samples tested by both DFA and PCR methods, 81 (16 by DFA testing and 79 by PCR) were determined to be positive for *Bordetella*, with a sensitivity and specificity of DFA testing of 18% and 99%, respectively. Of 1,426 samples tested by both DFA and culture methods, 48 (44 by DFA testing and 15 by culture) were determined to be positive for *Bordetella*, with a sensitivity and specificity of DFA testing of 73% and 98%, respectively. For *Legionella* sp., samples were identified as positive by DFA testing (31 [0.25%] of 12,597 samples) and culture (85 [0.6%] of 13,572 samples) significantly less often than by PCR (27 [4%] of 716 samples). Of 62 samples tested by both DFA and PCR methods, none were positive for *Legionella* sp. by DFA testing and 3 were positive by PCR. Of 3,923 samples tested by both DFA and culture methods, 22 (3 by DFA testing and 21 by culture) were positive for *Legionella* sp., with a sensitivity and specificity of DFA testing of 9.5% and 100%. Overall, DFA testing for *Bordetella* sp. and *Legionella* sp. is an insensitive method, and despite its continued popularity, clinical microbiology laboratories should not offer it when more sensitive tests like PCR are available.

Prompt recognition of *Bordetella pertussis* and *Legionella* sp. infections is important for the initiation of appropriate antibacterial therapy and the implementation of infection control measures or epidemiological investigations. Aware of this need, clinicians are faced with a menu of testing options, including culture, nucleic acid amplification-based methods, direct fluorescent-antibody (DFA) testing, and serology, to diagnose these infections. Numerous studies have evaluated the role of these diagnostic tests (8–11, 15, 16), finding that while culture is the mainstay for diagnosis, nucleic acid-based amplification methods (e.g., PCR) have emerged as more reliable, faster alternatives (3). In fact, the Centers for Disease Control and Prevention recommends culture and PCR for *B. pertussis* rather than DFA testing as preferred detection methods (13). Despite these data, over the last 3 years, we have seen an 88% increase in requests for DFA testing for *B. pertussis* and a 50% increase in requests for DFA testing for *Legionella*. With the increasing incidence of *Bordetella* sp. infections and greater numbers of the population more susceptible to *Legionella* sp. infections, we revisited the performance characteristics of DFA testing by comparing the results of the DFA method to those of culture and PCR.

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MATERIALS AND METHODS

Clinical specimens. The results of DFA testing, culture, and PCR testing for *Bordetella* sp. from October 2002 to December 2005 and for *Legionella* sp. from January 1997 to December 2005 performed at the Associated Regional University Pathologists Laboratories, Salt Lake City, UT, were retrospectively reviewed. *Bordetella* sp. specimens consisted of nasopharyngeal swabs or nasopharyngeal aspirates. Testing for *Legionella* sp. was performed primarily with respiratory specimens (e.g., sputum, lung tissue, tracheal and bronchial fluids, and pleural fluid) and, in rare cases, specimens from other body sites.

DFA testing. Client-prepared slides or moist swabs in bacterial transport media were received for testing. DFA testing for *B. pertussis* was performed with a commercial kit (BD Difco fluorescent-antibody *B. pertussis* kit) according to the protocol of the manufacturer. DFA testing for *Legionella* sp. was performed with the MonoFluo *Legionella pneumophila* immunofluorescence assay kit according to the protocol of the manufacturer (Bio-Rad).

Culture. *B. pertussis* specimens were cultured on Regan-Lowe medium (Becton Dickinson Microbiology Systems, Sparks, MD). *Legionella* sp. specimens were plated onto buffered charcoal-yeast agar and buffered charcoal-yeast agar with polymyxin B, anisomycin, and vancomycin (Remel, Lenexa, KS). Plates were incubated at 35°C and examined daily for 7 days. Identification by characteristic colony growth was confirmed with Gram staining and DFA testing.

PCR. DNA was extracted from clinical specimens using the QIAamp DNA mini kit (QIAGEN, Valencia, CA). PCR for *B. pertussis* was performed as previously described using the LightCycler *Bordetella* commercial assay (Roche Molecular Diagnostics, Indianapolis, IN) (3). PCR for *Legionella* was performed with a GeneAmp PCR system (PE Applied Biosystems, Foster City, CA) and agar gel electrophoresis for detection; samples with a band present at 386 bp were confirmed as positive for clinically significant *Legionella* sp. by DNA sequencing as described previously (2).

Statistics. Bayesian calculations were performed to determine sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV). Fisher's exact test (one tail) was used to determine statistical significance. In each comparative analysis of DFA testing, PCR or culture was considered the

TABLE 1. Comparison of results from DFA testing, culture, and PCR testing for *Bordetella* sp. and *Legionella* sp. specimens (2002 to 2005)

Species	Test method	No. of positive samples (%)	Total no. tested	Sensitivity[a]	Specificity[a]	PPV[a]	NPV[a]
Bordetella sp.	DFA	184 (3.0)	6,195	73 (18)	98 (99)	26 (88)	99.7 (81)
	Culture	150 (2.4)	6,251				
	PCR	2,557 (9.5)	26,929				
Legionella sp.	DFA	31 (0.2)	12,597	11	100	67	99.5
	Culture	85 (0.6)	13,574				
	PCR	27 (3.8)	716				

[a] Values are percentages. Numbers in parentheses are those calculated using PCR as the gold standard; others were calculated using culture as the gold standard.

"gold standard." The study was approved by the institutional review board of the University of Utah, Salt Lake City.

RESULTS

***Bordetella* sp.** Of 360 samples tested for *Bordetella* sp. by both DFA and PCR methods, 14 were positive by both methods, 65 were PCR positive only, and 2 were positive by the DFA test only. Compared with PCR, DFA testing had a sensitivity of 18% (95% confidence interval [95% CI], 10.9 to 27.6%), a specificity of 99% (95% CI, 97.4 to 99.8%), a PPV of 88%, and an NPV of 81%. Of 1,384 samples tested for *Bordetella* by both DFA and culture methods, 11 were positive by both methods, 4 were culture positive only, and 32 were positive by the DFA test only. Compared with culture, DFA testing had a sensitivity of 73% (95% CI, 48.1 to 89.1%), a specificity of 98% (95% CI, 96.7 to 98.3%), a PPV of 25%, and an NPV of 99.7%. Overall, the results of DFA testing were positive significantly less often than those of PCR ($P < 0.0001$), and the results of culture were positive significantly less often than those of DFA testing ($P < 0.028$) (Table 1).

***Legionella* sp.** The results of DFA and culture methods were positive for *Legionella* sp. significantly less often than those of PCR ($P < 0.0001$) (Table 1). Of 62 samples tested for *Legionella* sp. by both DFA and PCR methods, none were positive by DFA testing and 3 were positive by PCR. Of 3,914 samples tested for *Legionella* sp. by both DFA and culture methods, 2 were positive by both methods, 17 were culture positive only, and 1 was positive by the DFA test only. Compared with culture, DFA testing had a sensitivity of 11% (95% CI, 2.9 to 31.4%), a specificity of 100% (95% CI, 99.9 to 100%), a PPV of 67%, and an NPV of 99.5%.

DISCUSSION

With waning vaccine-induced immunity, in the past two decades *B. pertussis* infection has reemerged among adolescent and adult populations. The incidence of infection in young infants, for whom pertussis carries increased risks of morbidity and mortality, is also increasing (13). Likewise, infections with non-*pneumophila Legionella* species in immunocompromised patients have become increasingly recognized as potentially fatal (1). Prior to the advent of PCR testing, DFA testing served as a rapid diagnostic method for *Bordetella* and *Legionella* infections despite poor diagnostic accuracy (7, 14, 15). While laboratorians are aware that DFA testing is an insensitive and cumbersome assay, the number of physician requests for DFA testing for *Bordetella* and *Legionella* sp. has actually increased by year in our laboratory.

Historically, *B. pertussis* DFA testing was recommended as a screening tool (15), and clinicians relied on this test because of its quick turnaround time, sensitivity comparable to that of culture (11, 16), and reported specificity (5, 6, 12). However, significant cross-reactivity of *Bordetella* antibodies with normal nasopharyngeal flora has been described previously (4), and in our study, compared with PCR, the DFA test's PPV and NPV were less than 90%. Since the study was retrospective and no specimen was available for repeat testing, we were unable to resolve the results for 32 specimens that were positive for *Bordetella* by DFA testing and negative by culture. Without clinical history, we hypothesize that in this subgroup, the microorganisms may have been nonviable or that, more likely, the antibody cross-reacted with other bacteria. Although other studies found poor specificity with *Bordetella* DFA testing (4, 7), we could not confirm this observation, perhaps owing in part to the smaller numbers of samples in our study and the lack of clinical history to correlate our findings. Nonetheless, the overall suboptimal performance of *Bordetella* DFA testing makes its routine applicability in the clinical setting limited, particularly when PCR is available.

The utility of *Legionella* DFA testing is also questionable as a rapid first-line diagnostic method when other tests with higher sensitivity, such as PCR and urine antigen testing, are available. In this study, less than 0.5% of more than 12,000 specimens tested positive by the DFA method, and DFA testing detected only 2 of the 19 specimens found to be culture positive for *L. pneumophila*. Previous studies corroborate our finding of a low sensitivity of DFA testing versus culture (8, 9). Our numbers were too small to adequately assess the DFA method compared to PCR, precluding statistically significant conclusions in this case. However, the low numbers of positive specimens (three PCR-positive and no DFA-positive specimens) are not unexpected given the low overall frequency of *Legionella* infections.

Fluorescent-antibody testing remains useful for confirming organism identification from suspected colonies upon culture. However, for the detection of *Bordetella* sp. and *Legionella* sp. from direct specimens, the PPV and NPV of DFA are not optimal for patient care. Resources for clinical laboratories are becoming increasingly limited, and although DFA testing for both *B. pertussis* and *Legionella* sp. may be popular, with rapid results, it requires time and technical expertise to perform and interpret. Additionally, DFA test results have the potential to

mislead clinicians and adversely impact patient care. Therefore, we recommend that clinical microbiologists not offer DFA testing to diagnose infections caused by *Bordetella* and *Legionella* species.

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