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Laboratory and Clinical Evaluation of a Commercial DNA Probe for the Detection of *Legionella* spp.

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We prospectively compared a commercially available *Legionella* DNA probe with culture and direct immunofluorescence. The analytical sensitivities of the DNA probe and direct immunofluorescence were equal. Both tests detected 4×10^3 CFU of *Legionella pneumophila* or *Legionella micdadei* per ml in the pulmonary secretions of experimentally infected guinea pigs. The diagnostic sensitivity of the reagent was evaluated by using 809 samples of respiratory secretions. Of 51 DNA probe-positive specimens, 31 came from patients with culture-confirmed legionellosis. Two culture-positive specimens had negative DNA probe tests. The sensitivity and specificity of the DNA probe were 93.9 and 97.4%, respectively. The sensitivity and specificity of direct immunofluorescence were 68.9 and 99.6%, respectively. The low specificity of the DNA probe resulted in an unacceptable positive predictive value (60.8%). False-positive DNA probe tests were not due to nonspecific binding of the probe or to technical problems but were associated with one lot of probe reagent. Most of the false-positive probe tests had values near the threshold value of ≥4.0 suggested by the manufacturer. Raising the threshold value for a positive test to 7 lowered the sensitivity to 69.2% but raised the specificity to 99.2%. At this level, the performances of the DNA probe and direct fluorescent-antibody testing were equivalent. Respiratory secretions from patients receiving therapy for culture-confirmed *Legionella* infection remained DNA probe positive for up to 8 days, even though cultures and/or direct immunofluorescence tests often became negative. The DNA probe test is a satisfactory replacement for direct immunofluorescence but cannot replace culture for the laboratory diagnosis of *Legionella* infections.

The diagnosis of legionellosis remains a difficult problem for both clinicians and microbiologists. None of the currently available laboratory tests is sufficiently sensitive to detect all cases (9, 17). Because of their respective speed and sensitivity, direct fluorescent-antibody testing (DFA) and culture have been the most widely used methods for the laboratory diagnosis of *Legionella* infections.

Recently, a rapid DNA probe test designed to detect *Legionella* species in clinical specimens has been marketed by Gen-Probe, Inc., San Diego, Calif. In vitro studies have demonstrated that this DNA probe is highly specific for members of the genus *Legionella* (8, 19). In a retrospective study using frozen specimens from patients with culture-confirmed legionellosis, Edelstein et al. (10) found that the DNA probe test had a sensitivity of 76% and a specificity of 100% after exclusion of a number of probe-positive specimens which were either negative on repeat culture or unavailable for repeat culture. Doebbeling et al. (5) have reported on a prospective study in which the sensitivity of the probe was 63% compared with culture, and the specificity was unacceptably low at 95%. By adopting a higher threshold value for a positive DNA probe test, the authors found that the specificity of the test improved markedly without a significant effect on the sensitivity. Both of the above studies concluded that the DNA probe was not as sensitive as culture but was equivalent to DFA testing.

As a prelude to the adoption of this test in our laboratory, we conducted a prospective evaluation of the DNA probe test, comparing it with both culture and DFA. We report here the results of that prospective evaluation, which has resulted in our modification of the interpretive criteria for this test.

(Portions of this work were previously presented at the 88th Annual Meeting of the American Society for Microbiology, 8 to 13 May 1988, Miami, Fla. [abstract C-45].)

MATERIALS AND METHODS

Bacteria. Egg-passaged, virulent strains of *L. pneumophila* and *L. micdadei* were used to infect guinea pigs. *L. pneumophila* subsp. *pneumophila* serogroup 1 Burlington 1 was previously obtained from Washington Winn, Jr. *L. micdadei* EK was originally isolated in eggs from lung tissue obtained from a patient with pneumonia (16) and has been maintained by egg passage since its isolation. Suspensions of both bacteria which had been harvested from infected eggs were stored at −70°C until use.

Infection of guinea pigs. Male Hartley strain guinea pigs were infected with either *L. pneumophila* or *L. micdadei* by intratracheal inoculation as previously described (15). On day 3 after infection, pulmonary secretions were collected by lavaging the lungs of the infected animals with saline.

DNA probe. Commercial lots of the Gen-Probe *Legionella* DNA probe test kit were used in all studies. The DNA probe procedure was carried out as described by the manufacturer. Briefly, 150 μl of specimen was liquefied by adding an equal volume of solubilizer and incubating for 15 min. The solubilized specimen was transferred to tubes containing bacterial lysing reagent and sonicated for 15 min at 60 to 70°C. DNA probe reagent was next added, and the mixture was incubated at 72 ± 1°C for 1 h. Finally, separation reagent was added to the tubes, which were then incubated for an additional 5 min at 72°C and then centrifuged to collect the solid-phase separating reagent. The supernatant fluid was

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discarded, and the pellet containing the separating agent and any hybridized nucleic acid was washed twice by centrifugation. Binding of labeled DNA was measured by counting the tubes for 1 min in a gamma counter. Each test run included a positive and a negative control sample, which were supplied with the kit, and a tube of unhybridized DNA probe as a measure of probe labeling. Results were expressed as a probe ratio, which is the ratio of counts in the specimen to the counts in the negative control sample. The manufacturer's package insert suggested that a ratio of ≥4.0 should be considered positive.

One experiment was conducted with a modified DNA probe procedure to determine whether the apparent false-positive DNA probe tests were the result of DNA hybridization or of nonspecific binding of unhybridized probe to the separation reagent. In this experiment, three aliquots of each specimen were treated with the solubilizing and lysing reagents as described above. After this, the *Legionella* DNA probe reagent was added to two sets of tubes. One set was incubated at 72°C, while in the second set the hybridization reaction was carried out at 85°C. In the third set of samples, the *Legionella* DNA probe was replaced with a *Mycobacterium avium* DNA probe reagent (Gen-Probe) and the hybridization reaction was carried out at the usual temperature.

Specimen processing. A total of 809 clinical specimens, largely sputum samples, which were submitted to our laboratory between 2 April and 31 October 1987 for *Legionella* testing were examined by culture, DFA, and DNA probe testing. DFA testing was performed by using polyclonal reagents prepared against *L. pneumophila* serogroups 1 to 6 and *L. micdadei* (MarDx, Denville, N.J.). The DNA probe and DFA testing were performed by separate technologists, each of whom was unaware of the results of the other test.

Specimens were cultured on buffered charcoal yeast extract (BCYE) agar containing α-ketoglutarate (BCYE-α) and on BCYE-α containing cefamandole (4 μg/ml), polymyxin B (80 U/ml), and anisomycin (80 μg/ml) (BMPA-α) (7), which were prepared in the Clinical Microbiology Laboratory.

After inoculation onto the media, the remainder of each specimen was stored at 5°C. The inoculated plates were incubated at 35°C and examined daily with a dissecting microscope. Those cultures which appeared to be contaminated (i.e., those which contained more than 20 to 25 non-*Legionella* colonies after the initial 48 h of incubation) were recultured on BCYE-α and BMPA-α both before and after treatment with the acid wash procedure of Bopp et al. (2). Additionally, all specimens which were either DFA or DNA probe positive were also recultured after acid washing.

Legionellae were identified by their characteristic morphology on BCYE-α agar and confirmed by DFA testing using monovalent polyclonal antibody reagents prepared against *L. pneumophila* serogroups 1 to 6 and *L. micdadei* (MarDx).

Quality control. Quality control of the BCYE-α and BMPA-α which we prepared was performed by inoculating samples of each batch with 0.1 ml of a dilution of virulent *L. pneumophila* subsp. *pneumophila* serogroup 1 which had been prepared from the lungs of infected guinea pigs. Samples of the bacterial suspension were stored at −70°C. The dilution inoculated onto the plates was one which was expected to produce between 50 and 100 *Legionella* colonies per plate. Medium lots which contained fewer than 50 colonies were discarded. During the latter part of this study, we also tested the diluted quality control suspensions with the DNA probe to determine the usefulness of the probe for medium quality control.

Effect of therapy on DNA probe results. The physicians caring for those patients who had positive DNA probe tests or cultures were requested to submit additional specimens so that test results during therapy could be studied.

Review of patient records. A review of the charts of all patients who had positive DNA probe tests was conducted to compare the characteristics of patients with true- and false-positive DNA probe tests. Noted were the presence of radiographic or clinical evidence of pneumonia, immunosuppressive therapy, and whether additional DNA probe tests were submitted. Empiric treatment with antimicrobial agents active against legionellae within 5 days before the first DNA probe-positive specimen was also noted. In addition, laboratory worksheets of the initial DNA probe-positive specimen from each patient were reviewed, and the presence of other organisms, such as gram-negative rods or yeasts, which might have inhibited the growth of legionellae and thus caused false-positive DNA probe tests was noted. The proportions of patients in the different groups were compared by using Fisher's exact test.

RESULTS

When pulmonary secretions collected by lavage from infected guinea pigs were tested, we found that the analytical sensitivities of the DNA probe and DFA were equal. When the manufacturer's suggested value of ≥4.0 was used as a positive result, the DNA probe could detect about 4×10^3 CFU of either *L. pneumophila* or *L. micdadei* (Fig. 1). Only those specimens which were positive by DNA probe were also positive by DFA. In contrast, culture was over 100-fold more sensitive and detected as few as 12 CFU of either organism per ml.

Legionellosis was diagnosed in 11 patients by culture. Of these patients, nine (81.8%) were also positive by DNA probe and eight (72.7%) were positive by DFA (Table 1). All of the initial diagnostic specimens in these patients were sputum specimens. It is also noteworthy that three (27.3%) of these patients were infected with two species or serogroups of legionellae.

An additional 20 specimens were obtained from 9 of the 11 culture-positive patients for periods of up to 8 days after the institution of antimicrobial therapy. The test results for these specimens are shown in Table 2 along with the initial diagnostic specimen of each patient for comparison. DNA probe ratios remained elevated for all of these specimens (Table 2). Neither the magnitude of the probe ratio nor the results of subsequent DNA probe tests was indicative of the present condition or predictive of the ultimate outcome of a patient. DFA and culture results were more variable; only 9 (45%) of the 20 specimens were DFA positive, and 9 (45%) were culture positive. Eight (40%) were both DFA and culture negative.

Since the 20 DNA probe-positive specimens described above came from patients with culture-confirmed legionellosis, they were assumed to represent true-positive DNA probe tests and were used in our analysis of the sensitivity and specificity of the DNA probe test.

Of 809 clinical specimens, 51 (6.3%) were positive by DNA probe. Of the 51 DNA probe-positive specimens, 31 (60.8%) came from patients with culture-confirmed legionellosis. There were two specimens which were culture positive and DNA probe negative, giving a sensitivity of 93.9% (31 of 33). Twenty (39.2%) of the positive DNA probe tests were from patients with negative cultures, making the specificity of the test unacceptably low at 97.4%. The positive predictive value of the DNA probe was 60.8%, and the negative predictive value was 99.7%.

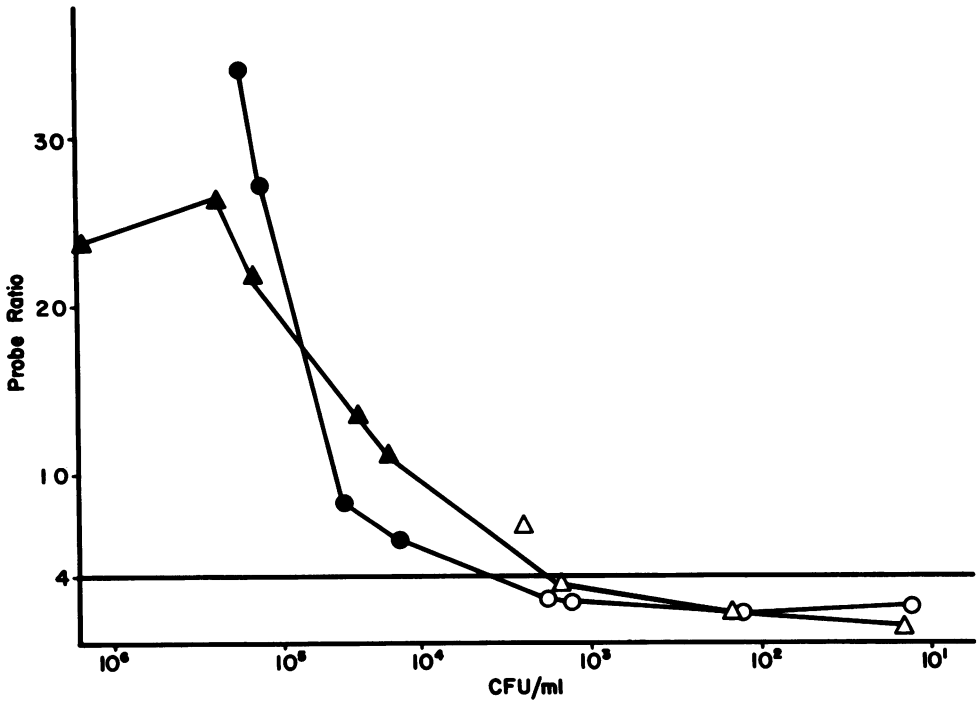

FIG. 1. Detection of *L. pneumophila* (LDB 1) and *L. micdadei* (PPA) in pulmonary secretions from experimentally infected guinea pigs by DNA probe, DFA, and culture. Horizontal line indicates the threshold value suggested by the manufacturer for the DNA probe test. ●, LDB1 DFA positive; ○, LDB1 DFA negative; ▲, PPA DFA positive; △, PPA DFA negative.

Of the 31 true-positive specimens, 29 were also examined by DFA. Twenty (68.9%) of these were also DFA positive. Of the 776 culture-negative specimens, 3 were DFA positive, making the specificity of DFA 99.6%.

When the distribution of the positive DNA probe tests was examined, we found that 15 of the 21 culture-negative specimens and 8 of the 31 true-positive specimens had probe ratios between 4 and 7, making the positive predictive value in this range only 34.8%. The positive predictive value for those tests with probe ratios of ≥7 was 78.2%, with 18 true-positive and 5 culture-negative specimens falling in this range.

TABLE 1. DNA probe, direct immunofluorescence, and culture results of 11 patients with legionellosis

Patient no.	Test result[a]		
	Probe ratio	DFA	Culture
1	1.9	−	LDB 1[b]
2	2.4	−	LDB 1
3	5.3	−	LDB 1 PPA[c]
4	5.6	+	LDB 1
5	5.8	+	LDB 1
6	12.3	+	LDB 1 PPA
7	13.2	+	PPA
8	13.3	+	LDB 1
9	17.0	+	LDB 4[d]
10	19.9	+	LDB 4
11	40.1	+	LDB 1 LDB 6[e]

[a] Results presented are those for the initial diagnostic specimen of each patient.
[b] *L. pneumophila* serogroup 1.
[c] *L. micdadei*.
[d] *L. pneumophila* serogroup 4.
[e] *L. pneumophila* serogroup 6.

Both true- and false-positive specimens produced a hybridization signal only when the hybridization reaction was carried out at 72 and not at 85°C, suggesting that the positive signal in both specimens was originally due to the formation of hybrids, which was inhibited by the higher temperature (Table 3). Also, when the same specimens were reacted with the *M. avium* probe, no hybridization took place, indicating that some component of the specimen was not causing nonspecific binding of unhybridized oligonucleotide probe to the separating reagent and that the hybridization was with a target recognized only by the *Legionella* DNA probe.

Analysis of the test results showed that the test values were not affected by the technologist performing the test or the age of the test kit (data not shown). Comparison of the daily probe ratios with the daily value of the negative control demonstrated that there was little relationship between the magnitude of the negative control on a given day and the value of the probe ratios, suggesting that the false-positive DNA probe tests were not the result of abnormally low negative control tests (Fig. 2A). Further evidence for this suggestion was gained when we scored each test on the basis of percent hybridization of the probe (the ratio of the counts in the specimen to the total counts in the probe) rather than on the probe ratio. There was a strong correlation between probe ratio and percent hybridization (Fig. 2B), suggesting that the DNA probe test results were indeed the result of hybridization rather than artificially low negative control values.

Examination of the distribution of positive DNA probe tests demonstrated that most of the false-positive DNA probe tests occurred during a 6-week period during April and

TABLE 2. Diagnostic test results in specimens from patients receiving therapy for culture-confirmed legionellosis

Patient no.	Test	Test result on illness day[a]:							
		0	1	2	3	4	5	6	8
1	DNA probe	12.3	10.0		7.25	5.8	9.0	4.1	
	DFA	+	−		+	−	−	−	
	Culture	+	−		−	−	+	−	
2	DNA probe	5.6		5.8[b]					23.6
	DFA	+		+					+
	Culture	+		+					−
3	DNA probe	19.9	7.0	8.8					
	DFA	+	−	+					
	Culture	+	+	+					
4	DNA probe	17.0	7.5	7.6	7.7[c]		5.7		
	DFA	+	+	−	−		−		
	Culture	+	+	−	−		−		
5	DNA probe	5.8					9.9		
	DFA	+					+		
	Culture	+					−		
6	DNA probe	13.3				36.7			
	DFA	−				+			
	Culture	+				+			
7	DNA probe	40.1		37.4					
	DFA	+		+					
	Culture	+		+					
8	DNA probe	5.3	6.0						
	DFA	−	−						
	Culture	+	+						
9	DNA probe	13.2		7.2					
	DFA	+		−					
	Culture	+		−					

[a] Day 0 was the day of initial diagnosis.
[b] Another specimen was also probe, culture, and DFA positive on this day.
[c] Another specimen was probe positive but DFA and culture negative on this day.

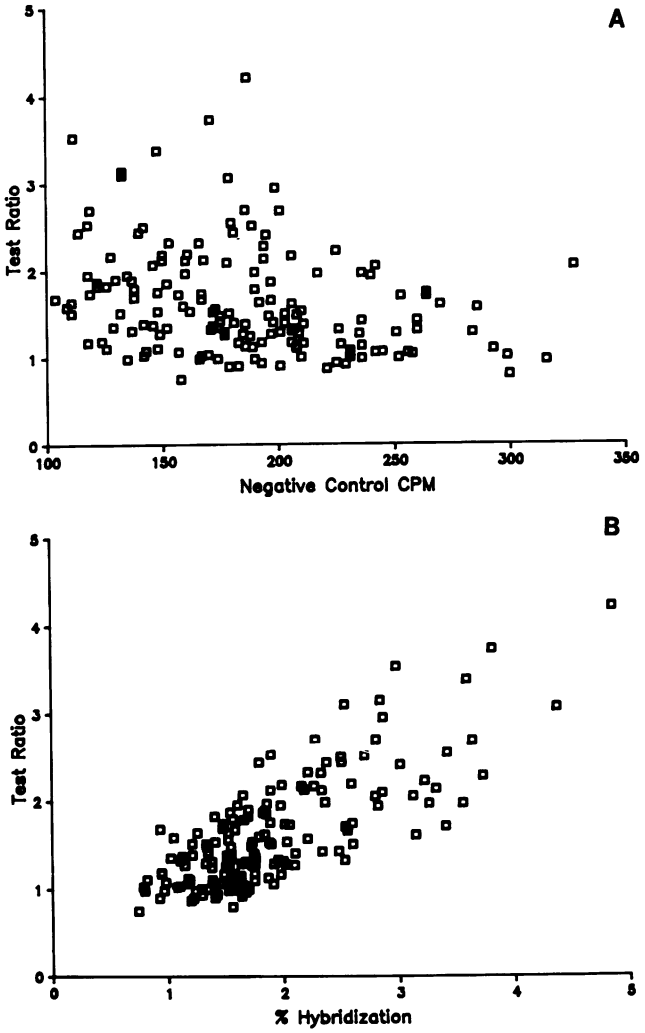

FIG. 2. (A) Distribution of daily DNA probe negative control values and corresponding daily test ratios. (B) Relationship of DNA probe test results scored as percentage of probe hybridized.

May and were associated with a single lot of DNA probe reagent (Fig. 3).

The review of the medical records of the patients demonstrated differences between patients with true-positive and false-positive DNA probe tests. Patients with true-positive tests were significantly more likely to have had clinical and/or radiographic evidence of pneumonia and a positive DFA and were more likely to have positive DNA probe tests on subsequent specimens (Table 4). Both patient groups were equally likely to have had bacteria in their specimens which could have potentially interfered with our ability to grow legionellae. There was also no difference in the proportion of patients in either group which had received empiric antimicrobial therapy with agents which are active against legionellae in the 5 days before the initial DNA probe test was performed. Both patient groups also had equal proportions of immunosuppressed patients and equal mortality rates.

TABLE 3. Effect of temperature and probe composition on DNA probe hybridization results with culture-positive and culture-negative specimens

Specimen no.	Original results		*Legionella* probe hybridization at:				% Hybridization to *M. avium* probe
			72°C		85°C		
	DFA	Culture	Ratio[a]	% Hybridization[b]	Ratio	% Hybridization	
48079	−	−	8.3	13.4	1.4	2.3	1.3
47479	+	+	4.0	6.4	1.2	1.8	1.2

[a] Ratio of counts in specimen to counts in negative control.
[b] Percentage of total probe radioactivity bound to specimen.

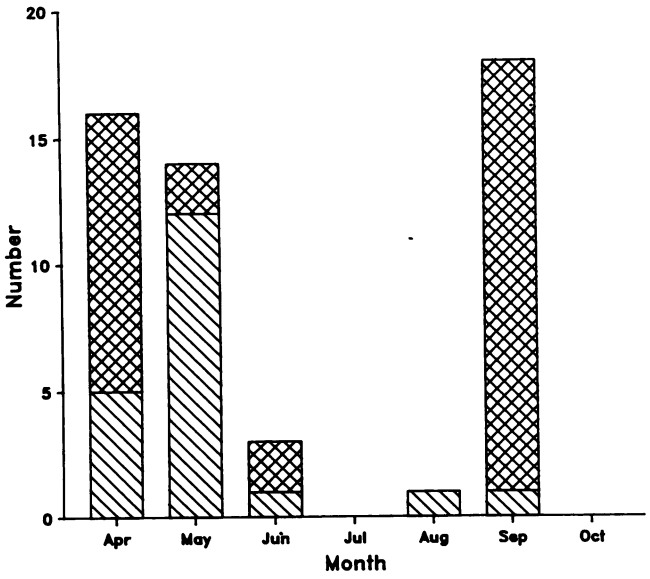

FIG. 3. Distribution of true-positive (▩) and false-positive (▨) DNA probe tests by month.

Since we were unable to demonstrate a biological or technical reason for the false-positive DNA probe tests, we examined the possibility that the predictive values of the DNA probe test could be improved by raising the lower limit for a positive test. We found that test performance could be improved by raising the lower limit for a positive DNA probe test from 4.0 to 7.0. At this point, the sensitivity of the test declined to 69.7%, but the specificity rose to a more acceptable value of 99.2% (Fig. 4). At this level, the performance of the DNA probe was identical to that of DFA.

The DNA probe test was a useful adjunct in our quality control to the BCYE media which we prepared. All lots tested during our study passed the quality control requirements in that each supported the growth of 50 to 100 colonies per 0.1 ml. In addition, testing of the use dilution of our quality control suspension revealed that these suspensions had probe ratios of less than 4.0 on every occasion except one and thus demonstrated that our media were capable of supporting the growth of legionellae from specimens which were DNA probe negative (Fig. 5).

TABLE 4. Comparison of patients with true- or false-positive DNA probe tests

Patient characteristic or observation	No. positive/total of patients with the following DNA probe test result:	
	True positive[a]	False positive[b] (P^c)
Pneumonia	9/9	8/21 (0.0017)
Repeat DNA probe positive (≤5 days)	9/9	0/5 (0.0005)
DFA positive	6/9	3/21 (0.008)
Yeast or gram-negative rod in sputum	8/9	12/21 (NS)
Prior anti-*Legionella* antimicrobial agents (≤5 days)	5/9	10/21 (NS)
Immunosuppression	7/9	12/21 (NS)
Death	2/9	2/21 (NS)

[a] DNA probe positive, culture positive.
[b] DNA probe positive, culture negative.
[c] Determined by Fisher's exact test; NS, not significant.

DISCUSSION

The Gen-Probe DNA probe test is about equal in analytical sensitivity to DFA. We detected about 10^4 CFU of virulent *L. pneumophila* or *L. micdadei* per ml in the pulmonary secretions of infected animals using both the DNA probe and DFA.

Because of its ability to detect all species and serogroups of legionellae, the DNA probe test could offer significant advantages over DFA for the rapid diagnosis of legionellosis. However, since the prevalence of positive specimens is usually very low, a very sensitive and specific test is required so that predictive values will be acceptable. As originally marketed, the DNA probe test was significantly more sensitive than DFA. Unfortunately, the specificity was unacceptably low. After modification of the interpretive criteria, the sensitivity of the DNA probe test (69.7%) was equivalent to that of DFA (68.9%), and the specificities of the two tests (99.2 and 99.6%, respectively) were also equivalent.

The results of our prospective study are very similar to those of the retrospective study of Edelstein et al. (10) in terms of the efficiency of the DNA probe test. In both studies, apparent false-positive DNA probe tests occurred. The false-positive DNA probe tests in the present study appear to result from binding of the DNA probe to a target in a temperature-dependent fashion, since binding was inhibited at 85°C in both true- and false-positive specimens. The observation that no binding occurred in these specimens when a mycobacterial probe was substituted for the *Legionella* reagent suggests that nonspecific binding of unhybridized nucleic acid to hydroxyapatite does not occur either.

Doebbeling et al. (5) have also reported that the specificity of the DNA probe test increased from 95 to 99% when the threshold value for a positive DNA probe was raised to ≥7. The sensitivity of the test also decreased from 63 to 50% when the threshold was changed. It is noteworthy, however, that the rate of false-positive DNA probe tests in that study (4.6%) was higher than in the present study (2.5%) and that their rate of false-positive DFA tests (2.5%) was also significantly higher than ours (0.4%). The most likely explanation for this difference is that these workers used culture as the standard to which the probe and DFA were compared. They also reported only the numbers of specimens which were positive or negative and gave no indication of the numbers of patients involved. In our study, specimens which were culture positive or which came from patients with culture-documented legionellosis were considered to be true-positive tests. It is very likely that our clinical rather than laboratory-based definition of disease is responsible for our higher sensitivity and specificity. Of 31 true-positive specimens, 20 came from patients receiving therapy for culture-documented legionellosis, and we observed a sensitivity of only 45% for culture and 45% for DFA in this group of specimens. It has been demonstrated that patients receiving antimicrobial therapy for legionellosis may shed organisms for variable periods of time, up to 10 days, which may be variably detected by culture and/or DFA (13). In the group of specimens studied by Doebbeling et al., 11 of 21 (52%) of the specimens with false-positive DNA probe tests were also DFA positive (5), suggesting that these false-positive specimens may have come from patients receiving therapy for legionellosis and were culture negative because of therapy.

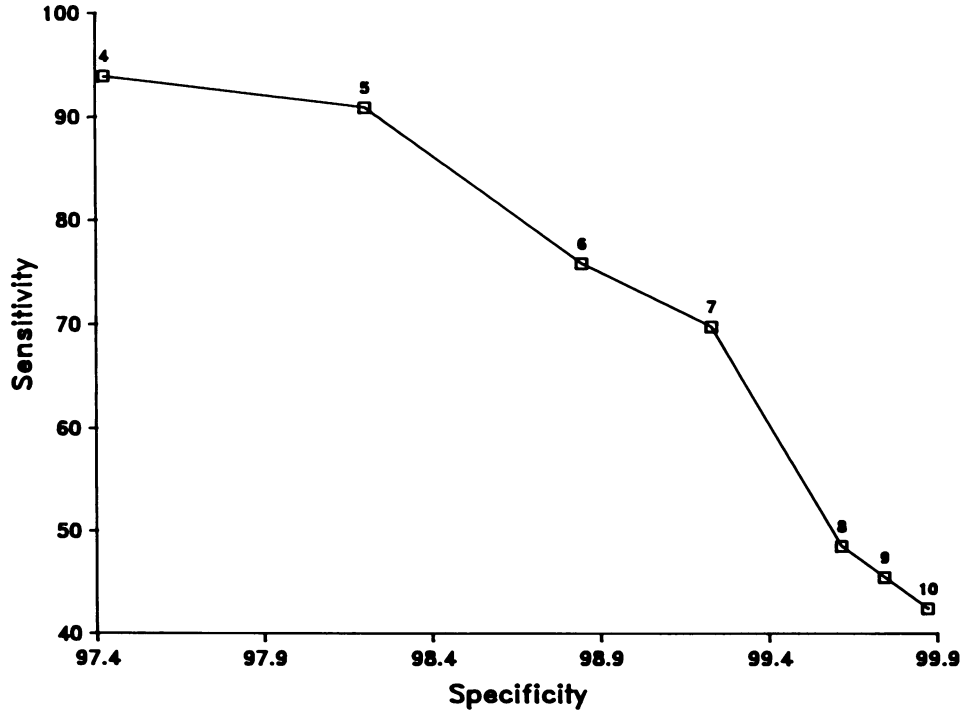

FIG. 4. Effects of various threshold values on the diagnostic performance of the DNA probe test. The threshold values are indicated by the numbers above the datum points.

Indeed, when these workers used culture and/or DFA positivity as an indicator for true positivity, they found that the sensitivity of the DNA probe was 74%, a value very similar to that which we report here (5). This again underscores the fact that laboratories and physicians must take the therapeutic history of the patient into account when interpreting diagnostic tests for legionellae.

Our data suggest that the DNA probe may hybridize with

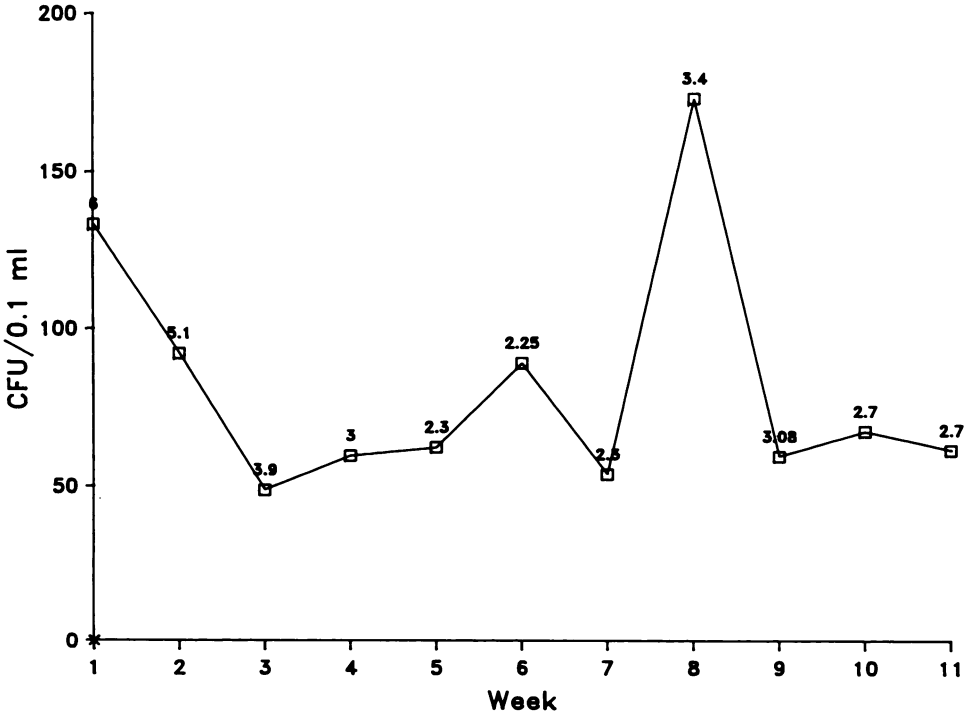

FIG. 5. Colony counts and DNA probe test ratios of quality control specimens used to test each lot of BCYE-α. The DNA probe value for each specimen is indicated by the number above each datum point.

other bacteria present in the patient specimens. However, we were not able to demonstrate hybridization by using bacteria harvested from the blood agar plates used for the routine cultures of several specimens which gave false-positive DNA probe tests (data not shown). It is possible that the organism responsible was not detected in our studies because it is an anaerobe or even a nonbacterial agent. This interpretation is problematic, however, because previous in vitro studies have demonstrated that the DNA probe reagent is highly specific for members of the genus *Legionella* (8, 19).

Another possibility is that the false-positive DNA probe tests resulted from a lack of sensitivity of our culture system. This is suggested by the fact that most of the false-positive DNA probe tests were in the low positive ($\geq$4 to $\leq$7) range and could be interpreted as suggesting that these specimens contained low numbers of legionellae which we could not recover by culture. However, several of our findings do not support this possibility. First, culture in our laboratory was the most sensitive diagnostic test for legionellosis, and the legionellae in 2 of the 11 patients in whom legionellae were detected were detected only by culture. Second, 3 of the 11 patients were found to have dual infection, which is a measure of the assiduousness with which our technologists examine cultures for legionellae. Our experimental design was highly weighted in favor of culture in that all DFA and DNA probe-positive specimens were automatically recultured. Thus, the probe-positive, culture-negative specimens were cultured twice on a total of six plates of media before being accepted as culture negative. In actuality, this practice resulted in our detecting only one additional positive specimen. Also, our media underwent rigid quality control testing which demonstrated that they could support the growth of low numbers of virulent legionellae. Finally, in a chart review we were able to detect differences between the patients who had true- and false-positive DNA probe tests, which supported the accuracy of our laboratory diagnoses. Patients with true-positive DNA probe tests were more likely to have had clinical and radiologic evidence of pneumonia than were those with false-positive tests. Most (8 of 11) patients with a true-positive DNA probe test also had a positive DFA, while only three patients with a false-positive DNA probe test had a positive DFA. Since our studies with guinea pig pulmonary secretions clearly demonstrated that the two tests are of equal analytical sensitivity, it is not surprising that most DNA probe-positive specimens were also DFA positive. Conversely, the lack of DFA positivity among the specimens which gave a false-positive DNA probe test suggests that the positive DNA probe test was not due to the presence of legionellae in the specimen. Finally, patients with both true- and false-positive tests were equally likely to have received antimicrobial agents before the specimen was submitted or to have potentially interfering bacterial flora in their specimens. This suggests that interference from antibiotics or from other organisms in the specimen did not alone account for the negative cultures in the presence of positive probes.

Another related possibility is that the false-positive tests were the result of an as yet unrecognized *Legionella* species in the specimens which could be detected by the DNA probe but not by DFA or culture. This hypothesis cannot, of course, be disproved by our data, but the fact that most of the false-positive probe tests were in the low positive range suggests that an unknown *Legionella* species is not the cause. It is entirely possible that the false-positive specimens contained a microorganism which partially hybridized with the DNA probe and which could not grow on BCYE agar. The phenotypic description of the genus *Legionella*, however, includes their requirement for iron and cysteine as evidenced by growth on BCYE agar (3). Thus, if an organism exists which both hybridizes poorly with the DNA probe and fails to grow on BCYE agar, one might question its inclusion within the genus *Legionella* since it would be genotypically and phenotypically different from the 33 currently recognized species.

Our data also suggest that if the false-positive tests were the result of another organism, the organism must be of low virulence. The low probe ratios in these specimens could have resulted from small numbers of an organism which hybridizes fully with the DNA probe. Most of the patients with false-positive DNA probes did not have clinical evidence of pneumonia. Since colonization of humans by legionellae has not been demonstrated (1, 4), our data then suggest that it would be reasonable to presume that the putative organism is of low virulence and of little clinical significance.

Interpretation of this test and additional clinical studies of the efficiency of the DNA probe may be problematic unless the culture system being used is optimal. Because the DNA probe test is not 100% sensitive, culture must remain the final test for all specimens. However, a laboratory which cannot isolate legionellae from most specimens with DNA/probe ratios in excess of 7 from patients who have not received antibiotics probably has some problem with its culture methods.

Laussucq et al. (14) recently described a cluster of 23 patients at one hospital who had positive *Legionella* DNA probe test results over a 4-month period. Additional cultures and serological testing failed to demonstrate that the patients involved had been infected by legionellae. The authors could not definitely conclude that the cluster of cases resulted from false-positive DNA probe tests because the original probe-positive specimens had not been cultured. There are two striking similarities between their study and the present one. First, 88% of the positive specimens in the reported cluster and 75% of the false-positive specimens in our study had probe ratios between 4 and 7. Second, 67% of the patients reported by Laussucq et al. and 62% of the patients with false-positive probes in our study had no clinical or radiologic evidence of pneumonia. It is thus a definite possibility that this cluster could have resulted from false-positive DNA probes. These findings again emphasize the need for laboratories using the DNA probe (or DFA) to have a good culture system in place beforehand.

It is well recognized that the legionellae become readily adapted to growth on artificial media after a few subcultures. Because of this, laboratory isolates which have been passaged on BCYE agar do not constitute an adequate quality control challenge for the media. We and others have found commercially prepared BCYE agar to be quite variable in quality when subjected to testing procedures which use small numbers of virulent legionellae (12; A. W. Pasculle, personal communication). Studies such as the present one are highly dependent on the sensitivity of the culture techniques employed and cannot be adequately performed without media of documented quality because culture, even with optimal media, is not 100% sensitive. In this regard, we found the DNA probe to be a useful adjunct to our quality control program. The plating efficiency of virulent legionellae even on good BCYE agar is often as low as 10% (18). Thus, if one relies entirely on enumeration of colonies which appear on the agar, the actual size of the inoculum used is

not measured. Using the DNA probe to test the quality control inoculum provided indirect evidence that unusually large inocula were not required to produce colonies on the media, since all quality control specimens were DNA probe negative. Thus, we could demonstrate that our media were of sufficient sensitivity to grow legionellae from DNA probe-negative specimens.

The DNA probe test is not sufficiently sensitive to replace culture for the laboratory diagnosis of legionellosis. Nevertheless, it is an entirely acceptable replacement for DFA testing as a rapid diagnostic test in those laboratories with sufficient test volumes to make the test cost-effective. In our study, after modification of the interpretive criteria, the sensitivity and specificity of the probe were virtually identical to those of DFA. In addition to its theoretical advantage of being able to detect all *Legionella* species and serogroups, there are concrete advantages as well. The DNA probe test requires slightly less hands-on time than the DFA test (20 versus 27 min in our study). Our technologists found the test easier to perform than DFA primarily because the reading of DFA slides is a very tedious and time-consuming task. In addition, reading of DFA tests requires a great degree of operator skill, which may not be present in all laboratories. In this regard, the quantitative nature of the DNA probe test makes it inherently easier to learn and perform reliably. All necessary control reagents were supplied with the kit, and the instructions supplied served their purpose more than adequately.

Another potential advantage of the DNA probe over DFA and culture is its apparent ability to detect patients who may have been partially treated due to prior empiric antimicrobial therapy. Only 45% of the specimens which we obtained after the institution of therapy in patients with culture-documented legionellosis were positive by DFA or culture, and only 30% were positive by both tests. All patients, however, had elevated DNA probe ratios for up to 8 days after the institution of therapy. Our data suggest that a patient who has received prior therapy with agents known to be active against legionellae and who has had sequential DNA probe tests which were positive is very likely to have legionellosis, even if cultures are negative. That the DNA probe might detect noncultivatable organisms is not surprising, since it has been demonstrated that neither erythromycin nor rifampin is bactericidal for extracellular or intracellular legionellae (6, 11).

Laboratories performing this test must have access to information concerning the patient if the test is to be properly interpreted. Since we have clearly shown that patients with culture-documented legionellosis have DNA probe test values which remain elevated for at least a week even though culture and DFA may become negative, the laboratory must know whether a patient has received prior empiric therapy before it can assume that a probe-positive, culture-negative specimen represents a false-positive DNA probe test.

Our data also suggest that one way to interpret DNA probe tests would be to repeat the test on additional specimens. All patients with culture-proven legionellosis had multiple positive DNA probe tests, while the patients with false-postive DNA probe tests were negative on subsequent testing.

The DNA probe test performed most efficiently in our study when a value of ≥ 7 was used as the lower limit for a positive test. Before our submission of this paper for publication, the manufacturer revised the criteria for interpretation of this test. It is now suggested that values between ≥ 4 and <7 be interpreted as equivocal test results. Our data strongly suggest that this interpretive criterion is of little diagnostic value. The positive predictive value for those specimens which fell in this range in the present study (in which the prevalence was 3.8%) was only 34.8%, making such an interpretive category of use only in those situations (large epidemics) in which the prevalence of disease is unusually high.

Practically all of the false-positive test results in this study were associated with a single lot of the DNA probe test. Since about half of our true-positive test results also occurred during this period of time, we were unable to calculate the sensitivity and specificity of the test with the results from this test kit excluded. Nevertheless, the initially high sensitivity of the DNA probe test (93.9%) when values of ≥ 4 were considered positive, compared with 69% of DFA, is encouraging. If the reasons for the false-positive test results can be identified and corrected without changing the sensitivity of the test, the DNA probe test would be superior rather than equivalent to DFA for the rapid diagnosis of legionellosis.

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LITERATURE CITED

1. **Baumgardner, D. J., D. K. Johnson, and D. E. Ross.** 1988. Prevalence of *Legionella* in pharyngeal secretions of patients with pharyngitis. J. Am. Board Fam. Pract. **1**:24–28.
2. **Bopp, C. A., J. W. Sumner, G. K. Morris, and J. G. Wells.** 1981. Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of a selective medium. J. Clin. Microbiol. **13**:714–719.
3. **Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, H. W. Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, J. C. Feeley, W. L. Thacker, P. Skaliy, W. T. Martin, B. J. Brake, B. S. Fields, H. V. McEachern, and L. K. Corcoran.** 1985. Ten new species of *Legionella*. Int. J. Syst. Bacteriol. **35**:50–59.
4. **Bridge, J. A., and P. H. Edelstein.** 1983. Oropharyngeal colonization with *Legionella pneumophila*. J. Clin. Microbiol. **18**: 1108–1112.
5. **Doebbeling, B. N., M. J. Bale, F. P. Koonz, C. M. Helms, R. P. Wenzel, and M. A. Pfaller.** 1988. Prospective evaluation of the Gen-Probe assay for detection of Legionellae in respiratory secretions. Eur. J. Clin. Microbiol. Infect. Dis. **7**:748–752.
6. **Dowling, J. N., R. S. Weyant, and A. W. Pasculle.** 1982. Bactericidal activity of antibiotics against *Legionella micdadei* (Pittsburgh pneumonia agent). Antimicrob. Agents Chemother. **22**:272–276.
7. **Edelstein, P. H.** 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. **14**:298–303.
8. **Edelstein, P. H.** 1986. Evaluation of the Gen-Probe DNA probe for the detection of legionellae in culture. J. Clin. Microbiol. **23**:481–484.
9. **Edelstein, P. H.** 1987. The laboratory diagnosis of Legionnaire's disease. Semin. Respir. Infect. **2**:235–241.
10. **Edelstein, P. H., R. N. Bryan, R. K. Enns, D. E. Kohne, and D. L. Kacian.** 1987. Retrospective study of Gen-Probe rapid diagnostic system for detection of legionellae in frozen clinical respiratory tract samples. J. Clin. Microbiol. **25**:1022–1026.
11. **Horwitz, M. A., and S. C. Silverstein.** 1983. The intracellular multiplication of the Legionnaire's disease bacteria (*Legionella pneumophila*) in human monocytes is reversibly inhibited by erythromycin and rifampin. J. Clin. Invest. **71**:15–26.

12. **Keathly, J. D., and W. C. Winn, Jr.** 1984. Comparison of media for recovery of *Legionella pneumophila* clinical isolates, p. 19–20. *In* C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowsky (ed.), *Legionella*. Proceedings of the 4th Annual Symposium. American Society for Microbiology, Washington, D.C.
13. **Kirby, B. D., K. M. Snyder, R. D. Myer, and S. M. Finegold.** 1980. Legionnaire's disease: report of sixty-five cases and a review of the literature. Medicine (Baltimore) **59**:188–205.
14. **Laussucq, S., D. Schuster, W. J. Alexander, W. L. Thacker, H. W. Wilkinson, and J. S. Spika.** 1988. False-positive DNA probe test for *Legionella* species associated with a cluster of respiratory illnesses. J. Clin. Microbiol. **26**:1442–1444.
15. **Pasculle, A. W., J. N. Dowling, F. N. Frola, D. A. McDevitt, and M. Levi.** 1985. Antimicrobial therapy of experimental *Legionella micdadei* pneumonia in guinea pigs. Antimicrob. Agents Chemother. **28**:730–734.
16. **Pasculle, A. W., R. L. Myerowitz, and C. R. Rinaldo, Jr.** 1979. New agent of pneumonia isolated from renal transplant recipients. Lancet **ii**:58–61.
17. **Reingold, A. L.** 1988. Role of legionellae in acute infections of the lower respiratory tract. Rev. Infect. Dis. **10**:1018–1028.
18. **Weinbaum, D. L., R. R. Benner, J. N. Dowling, A. W. Pasculle, and G. R. Donowitz.** 1984. *Legionella micdadei* interaction with human monocytes. Infect. Immun. **46**:68–73.
19. **Wilkinson, H. W., J. S. Sampson, and B. B. Plikaytis.** 1986. Evaluation of commercial gene probe for identification of *Legionella* cultures. J. Clin. Microbiol. **23**:217–220.