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PCR Detection of *Pneumocystis carinii* in Bronchoalveolar Lavage Specimens: Analysis of Sensitivity and Specificity

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Although PCR detection of *Pneumocystis carinii* DNA has been described, little is known about the sensitivity or specificity of the assay in routine laboratory practice. We had the unique opportunity to use a bronchoalveolar lavage (BAL) specimen bank with samples for which the direct examination results for *P. carinii* were known. DNA purified from 129 selected specimens was amplified by using the primers described previously (A. E. Wakefield, F. J. Pixley, S. Banerji, K. Sinclair, R. F. Miller, E. R. Moton, and J. M. Hopkin, Mol. Biochem. Parasitol. 43:69–76, 1990). Of the 129 specimens, 37 were positive for *P. carinii* by direct examination. All 37 specimens were positive for *P. carinii* by PCR, yielding a 100% sensitivity and 100% negative predictive value for the assay. An additional 23 specimens were repeatedly positive for *P. carinii* by PCR but were not positive by direct examination. Review of the patient charts for these specimens with discordant results demonstrated that five of the patients were actually positive for *P. carinii*, as determined by either biopsy or examination of repeat or prior BAL specimens. A response to empiric therapy for *P. carinii* pneumonia was seen in an additional two patients. Of the remaining specimens, 8 produced no significant isolates other than *P. carinii*, while 12 contained culture-confirmed significant respiratory pathogens in addition to *P. carinii* (two fungal, nine bacterial, and one viral pathogen). Cytomegalovirus, which was of unknown significance, was isolated from 16 additional specimens. Overall, the specificity of the PCR assay was 79.3% compared to the results of direct examination. We hypothesized that the apparently poor specificity of the PCR assay was due to the increased sensitivity of the assay compared to that of direct examination. The sensitivity of the PCR assay was therefore assessed with BAL specimens containing *P. carinii* cysts. Serial dilutions of this preparation were evaluated by direct examination and PCR. PCR was found to be 100-fold more sensitive than direct examination, which detected one to two cysts per amplification. No false-positive results were detected in controls containing no DNA or by using target DNA from various fungal, viral, or bacterial respiratory pathogens. We conclude that PCR detection of *P. carinii* in BAL specimens is very sensitive and should be considered for patients whose specimens do not yield a diagnosis. The increased sensitivity of the PCR assay may help to identify those patients with low-titer infections who might benefit from directed antibiotic therapy for *P. carinii* and would otherwise be missed by direct examination alone.

Pneumocystis carinii was first identified as a human respiratory pathogen in the early 1900s. The patient populations at risk for developing *P. carinii* pneumonia (PCP) have evolved since the organism's discovery. *P. carinii* was first recognized as causing pneumonitis in rats. In the original reports of Chagas and Carini, *P. carinii* was initially misidentified as a protozoan (1). Recent work has documented the fungal nature of *P. carinii* (11). Infection with this organism emerged as a significant cause of pneumonia in neonates and malnourished and immunosuppressed individuals in Europe after World War II (10, 15). Additional groups at risk for PCP were recognized in the 1960s and 1970s, including individuals with hematologic or other malignancies receiving cytotoxic drugs, individuals receiving long-term prednisone therapy, children with primary immunodeficiencies, and patients receiving immunosuppressive drug therapy for solid-organ transplantation (13, 34, 38, 46). Isolated cases and miniepidemics have been described in the literature (8, 38, 40). It was not until the advent of human immunodeficiency virus (HIV) infection and AIDS, however, that PCP became an illness that was commonly considered in the differential diagnosis of community-acquired pneumonia. Approximately 25,000 cases are reported in the United States each year. Prior to the use of prophylactic therapy for PCP in HIV-positive patients, PCP was seen in 80% of all patients with AIDS and represented the AIDS-defining illness for 60% of people with AIDS (6). Despite the extensive use of prophylaxis, approximately 20% of patients with HIV infection still develop PCP (36).

The diagnosis of PCP has likewise undergone an evolution. When it was first described, PCP was a diagnosis based on clinical and histologic criteria. Histologic identification was made on the basis of a frothy intra-alveolar infiltrate in hematoxylin-eosin-stained sections of open lung biopsy specimens or autopsy tissues. One or more of a variety of stains including Gomori's methenamine silver (GMS), Gram-Weigert, Papanicolaou, Giemsa, and toluidine blue stains were also used to identify the cysts or trophozoites present in the tissue sections. More recently, transbronchial biopsy specimens have been replaced by examination of induced sputa (30) or bronchoalveolar lavage (BAL) fluid (17). BAL fluid has assumed a major role in the laboratory diagnosis of *P. carinii* in patients with and without AIDS (5, 18). New, more sensitive stains have become available to detect cysts in lavage fluids and induced sputum specimens. The nonspecific fluorochrome calcofluor white (3,

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23) and indirect immunofluorescence stains that use antipneumocystis monoclonal antibodies (16, 24) enhanced the sensitivity and specificity of organism detection.

Because of the lack of culture techniques and the reliance on direct examination, the laboratory diagnosis of PCP lacks sensitivity, especially for patients in whom the organism burden is low. The widespread use of prophylactic therapies for PCP in HIV-positive patients has led to difficulties in detecting PCP due to atypical patterns of clinical presentation of patients with the disease and the small numbers of organisms detected after antimicrobial treatment (22). The underlying disease process may also affect the number of organisms present in a laboratory specimen, such that HIV-infected patients have a much higher organism burden compared to that in patients without this viral infection (27). Wakefield et al. (43) have recently described the use of PCR to enhance the detection of *P. carinii* in clinical specimens. We describe our experience in using PCR with these primers to detect *P. carinii* DNA in 129 BAL specimens from 122 patients seen at the Mayo Clinic.

MATERIALS AND METHODS

Specimen description. BAL specimens were obtained in sterile saline by standard techniques (28). Excess specimen remaining after the completion of all diagnostic testing was saved for experimental examination. Permission for specimen use was obtained as part of the informed consent prior to the lavage procedure. BAL specimens were collected either prospectively as fresh specimens (stored at 4°C) or from the BAL specimen storage bank (stored at −70°C). One hundred twenty-nine specimens obtained over the period from 1989 to 1995 were selected. These represented known *P. carinii*-positive specimens, direct examination-negative specimens, and repeat or prior specimens from patients with a laboratory diagnosis of *P. carinii* infection at some time in their evaluation at the Mayo Clinic.

DNA purification. DNA was purified from specimens and controls by the IsoQuick extraction method (Orca Research, Inc., Bothell, Wash.). One milliliter of well-mixed BAL fluid was centrifuged at 14,000 × *g* in a microcentrifuge for 5 min. The cell pellet was lysed with guanidine thiocyanate, and the nucleic acids were extracted on the nuclease binding matrix. The DNA was precipitated with 1/10 volume of 6 M sodium acetate, 1 volume of isopropyl alcohol, and 2 μl of 10% glycogen. The pellet was collected after 10 min of centrifugation at 14,000 × *g* in the microcentrifuge. After washing in 70% ethanol, the pellet was dried and then resuspended in 200 μl of RNase-free water for amplification. DNAs for viral and bacterial controls were extracted by the same techniques from cell cultures infected with 10^6 to 10^8 viral infectious units or from 1 ml of a suspension of bacteria with a turbidity equivalent to that of a McFarland no. 1 standard (approximately 3×10^8 CFU/ml).

Amplification primers. PCR primer sequences for the mitochondrial 5S rRNA gene of *P. carinii* were previously described by Wakefield et al. (43). Primers for the beta-globin gene, also described previously (37), served as positive controls for all patient samples being amplified. Primers were synthesized by the Mayo Molecular Core Facility. The DNA was purified with NAP-25 columns (Pharmacia, Piscataway, N.J.) and was concentrated by lyophilization.

Amplification protocol. The standard reaction mixture was described previously (12). Five microliters of target DNA was added to each 45-μl aliquot of reaction mixture. Specimens were cycled 60 times for 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C, with a final elongation time of an additional 1 min. All specimens were irradiated with UV light after completion of cycling to cross-link the incorporated isopsoralen, reducing the possibility of cross contamination of amplified products to other samples.

Agarose gel electrophoresis. Three percent agarose gels in Tris-acetate-EDTA buffer with ethidium bromide were chilled at 4°C for at least 1 h prior to electrophoresis. Ten microliters of specimen or control PCR products was added to the lanes of the gel. At least one molecular weight standard was included on each gel to confirm amplicon sizes. Gels were run at 190 V for 30 to 60 min or until good separation of molecular weight standard bands was observed. The bands were visualized with UV light and were documented on Polaroid black and white film.

Southern blot procedure. Sodium hydroxide-denatured DNA was transferred from the gel to a nylon membrane by capillary action in 10× SSC (1× SSC buffer is 0.15 M NaCl plus 0.015 M sodium citrate buffer. After overnight transfer, the DNA was cross-linked to the nylon membrane by exposure to UV irradiation.

Probe synthesis. The nucleotide sequences of the 346-bp amplicon specific for *P. carinii* was published previously (43). Nucleotide sequences internal to the original amplicon were selected for primers to produce a nearly full-length probe (sense primer, TAT TTA TTG TAA GAA ATA GTT; antisense primer, CTG GTT TTC TGC GAA AAT TGT). All primer sets were synthesized by the Mayo Molecular Core Facility. The *P. carinii* genomic DNA target was amplified without isopsoralen to generate a PCR product. A chemiluminescent label was attached to the probe with the ECL direct nucleic acid labeling kit and detection system (Amersham, Arlington Heights, Ill.). The labeled probes were added and were allowed to hybridize to the membrane overnight. The membranes were washed twice with 50 ml of wash buffer (6 μM urea, 0.4% sodium dodecyl sulfate, and 0.5× SSC) at 40°C for 20 min and twice with 50 ml of 2× SSC at room temperature for 5 min. The membranes were then placed in 30 ml of detection reagent provided in the ECL kit for 1 min at room temperature and were exposed to X-ray film for 1 min and 60 min.

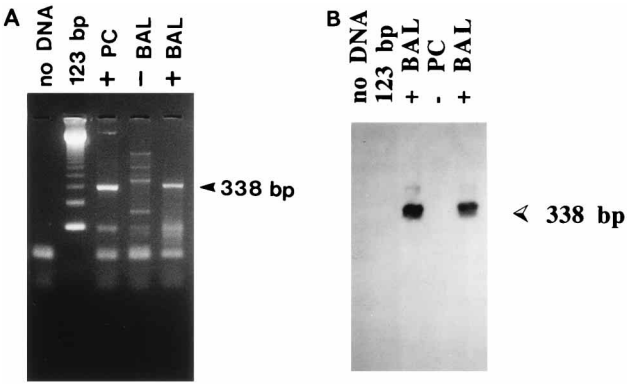

FIG. 1. Three percent agarose gel electrophoresis (A) and corresponding Southern blot (B) demonstrating typical PCR amplification findings. Following PCR amplification, 5 μl of amplicon from control and BAL specimens was applied to a 3% agarose gel for electrophoretic separation. The 338-bp band produced when the *P. carinii* template was present was readily observed in both the positive control specimens and BAL specimens containing *P. carinii*. Neither the no-DNA blank nor the BAL specimen negative for *P. carinii* contained bands migrating at 338 bp. Although a band often migrated at a slightly higher molecular mass than the anticipated 338-bp *P. carinii* band, with a 40-min electrophoretic separation, these bands were clearly differentiated on the gel and did not contain the *P. carinii* sequence, as determined by blot analysis.

Analysis of PCR data. All patient specimens were analyzed independently to obtain knowledge of direct examination and culture results. Samples with discrepant gel, blotting, or direct test results were amplified two additional times (two separate amplification runs). A positive result was accepted as agreement of two of three or three of three of the test determinations.

Direct examination of BAL specimens for *P. carinii* detection. BAL specimens were diluted to produce a single monolayer of cells after cytocentrifugation. Patient specimens were analyzed in duplicate, with positive and negative controls stained concurrently to ensure adequate staining characteristics. The slides were fixed for 2 min in high-pressure liquid chromatography-grade methanol and air dried. The slides were then stained with either GMS or calcofluor white (Fungifluor Solution A; Polysciences, Warrington, Pa.). The slides were examined directly (with GMS staining) or were examined with a Zeiss fluorescence microscope by using a BP400-440 nm excitation filter and an LT470 nm barrier filter for excitation at 400 to 440 nm and emission at a light frequency of >470 nm. Slides were identified as positive for *P. carinii* only if characteristic cyst walls with comma-shaped intracystic bodies were seen. Results for all patients were confirmed by a second observer.

Retrospective chart review. All patient charts were reviewed if a laboratory diagnosis of *P. carinii* was made by direct examination or PCR. The following data were collected: primary clinical diagnosis, HIV status, results of prior or subsequent BAL examinations, prior or subsequent diagnosis of PCP, history of prophylaxis for *P. carinii*, response to empiric treatment for PCP, clinical impression at the time of BAL examination, culture results for the BAL specimen, and the results of any other significant laboratory diagnostic testing performed.

Statistical analysis. The direct examination for *P. carinii* by using either GMS or calcofluor white stain was the "gold standard" for the diagnosis of *P. carinii* infection.

RESULTS

Specimens containing *P. carinii*-specific DNA produced a well-defined band that migrated at the anticipated size of 338 bp (Fig. 1). These bands were probe positive by subsequent Southern blotting. For some amplification events, negative specimens also produced a band that migrated at a slightly higher molecular size. This band could be differentiated by size and did not bind the *P. carinii*-specific probe.

TABLE 1. Correlation of patient HIV status with PCR and direct examination results for patients with one or more *P. carinii* positive test results

HIV infection	No. of patients with the following test result[a]:		
	Direct examination +, PCR gel +, PCR probe + (n = 37)	Direct examination −, PCR gel +, PCR probe + (n = 18)	Direct examination −, PCR gel −, PCR probe + (n = 5)
Yes	7	1	0
No	21	5	3
Unknown	9	12	2

[a] +, positive test result; −, negative test result.

A total of 129 specimens from 122 patients were amplified, and the investigators were blinded to the direct examination results. Of the 129 specimens from 122 patients, 37 were positive by direct examination. All 37 specimens were positive for *P. carinii* by PCR gel and Southern blot analyses, yielding a 100% sensitivity and 100% negative predictive value for the PCR assay. Twenty-three of the remaining specimens (negative for *P. carinii* by direct examination) were positive by PCR after gel electrophoresis (n = 18) or Southern blot (n = 23) analysis (specificity, 75%; positive predictive value, 62%).

To address this apparently low specificity and low positive predictive value for the PCR assay, we conducted a retrospective chart review to identify indicators that might have contributed to the discrepant results. Data collected from chart reviews consisted of HIV status, other underlying disease processes, subsequent or prior diagnoses of *P. carinii*, prophylaxis for *P. carinii*, culture results for the BAL specimen tested, and the clinical diagnosis and response to treatment. The patients with positive results for *P. carinii* were predominantly negative for HIV infection (n = 29) or were untested for HIV infection (n = 23) (Table 1). Eight of the patients who tested positive for *P. carinii* by all methods were HIV positive. Seven of the eight specimens from this group were detected by direct examination. In contrast, of the 52 positive specimens in the HIV-negative or HIV-untested groups, 30 were found to be positive by direct examination, while 22 yielded positive results by PCR alone. Ninety-six percent of the discrepant results (22 of 23 specimens) were for samples from patients who were HIV negative or whose HIV status was unknown. The population untested for HIV represented patients with other underlying illnesses that explained their immunosuppression. They were largely without significant risk factors to warrant testing for HIV, and no evidence of infection with this virus was detected in their subsequent clinical course (Table 2).

TABLE 2. Analysis of underlying illness associated with all *P. carinii*-PCR positive samples

Associated disease process	No. of patients with the following test result[a]:		
	Direct examination +, PCR gel +, PCR probe + (n = 37)	Direct examination −, PCR gel +, PCR probe + (n = 18)	Direct examination −, PCR gel −, PCR probe + (n = 5)
HIV infection or AIDS	7	1	0
Transplantation	8	4	0
Malignancy			
Hematologic	5	5	3
Other	9	0	1
Autoimmune	2	4	0
Asthma or respiratory	3	3	1
Chronic liver disease	1	0	0
Unknown	2	1	0

[a] +, positive test result; −, negative test result.

TABLE 3. Analysis of patient and specimen characteristics with discrepancies in diagnostic tests for *P. carinii*

Clinical diagnosis for respiratory symptoms	No. of patients with the following test result[a]:	
	Direct examination −, PCR gel and blot + (n = 18)	Direct examination −, PCR blot + (n = 5)
PCP diagnosis confirmed		
Biopsy specimen positive for *P. carinii*	1	0
Prior or subsequent BAL specimen positive for *P. carinii*	3	1
PCP diagnosis suggested, response to empiric therapy	2	0
No definitive diagnosis made	7	1
Other potential pathogens identified in BAL specimen		
Viral pathogens[b]	13	4
Bacterial pathogens[c]	6	1
Fungal pathogens[d]	1	1

[a] +, positive test result; −, negative test result.

[b] All viral isolates were CMV. Only one of the gel-positive specimens was treated as CMV pneumonitis. All other isolates were considered clinically insignificant.

[c] Bacterial isolations included one patient each infected with *Legionella pneumophila*, *Proteus mirabilis*, *Pseudomonas maltophilia*, *Haemophilus parainfluenzae*, *S. aureus*, *Branhamella catarrhalis*, and *M. avium-M. intracellulare*, and two patients infected with *H. influenzae.*

[d] Fungal isolations consisted of one patient each infected with *C. immitis* and *A. fumigatus.*

Seventeen specimens contained other respiratory pathogens, in addition to *P. carinii* (Table 3). All 17 specimens contained cytomegalovirus (CMV); one BAL specimen was obtained from a patient with clinical features consistent with CMV pneumonitis. Seven of the specimens also contained bacterial pathogens, and two additional specimens contained significant fungal isolates. Therefore, 28.3% of all *P. carinii*-positive specimens in this series contained additional isolates.

The specificity of the PCR amplification for *P. carinii* was experimentally determined by testing DNAs from several other respiratory tract pathogens with primers to generate the 338-bp amplicon. No cross-reactive amplification products were detected by gel or blot analysis when DNA from *Escherichia coli*, *Streptococcus pneumoniae*, viridans group streptococci, *Klebsiella pneumoniae*, *Haemophilus influenzae*, CMV, herpes simplex virus, Epstein-Barr virus, varicella-zoster virus, *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Mycobacterium tuberculosis*, *Mycobacterium avium-M. intracellulare*, or *Histoplasma capsulatum* was used.

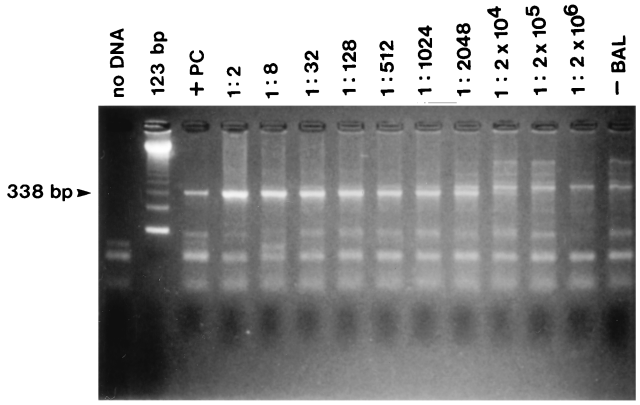

FIG. 2. Three percent agarose gel electrophoresis demonstrating PCR amplification of the *P. carinii* sequence in serially diluted *P. carinii*-containing BAL fluid. *P. carinii* organisms were expressed from an infected autopsy lung specimen. The suspension was concentrated by centrifugation and was then serially diluted in a BAL specimen that was negative for *P. carinii*. A portion of the specimen was used for detection of *P. carinii* cysts by direct examination, while the second aliquot was extracted and amplified as described in the text. The amplicon is detected to a dilution of $1{:}2 \times 10^4$ on both the gel (shown) and the corresponding blot (data not shown). Calculations from the corresponding direct examinations demonstrated a sensitivity of 1,000 cysts/ml of BAL fluid for the direct examination compared to about 10 cysts/ml for the PCR assay (approximately 1 to 2 cysts per amplification reaction).

Chart review for the specimen characteristics and further diagnostic testing indicated that for one patient, the direct examination of the BAL specimen was negative for *P. carinii* but that the PCR assay was positive. The corresponding transbronchial biopsy specimen was found to contain *P. carinii* organisms, however. BAL specimens from four additional patients were positive by direct examination for *P. carinii* with other samples prior or subsequent to the positive PCR result. These five patients were therefore considered true positives for *P. carinii* infection. Recalculation of the specificity of the assay indicates that the PCR assay was at least 79.3% specific and 80% efficient. Two additional patients probably had PCP, as judged by their responses to empiric therapy. Fourteen of the 23 patients with a positive *P. carinii* PCR test result had recently taken or were currently receiving steroid therapy. Five of the patients not receiving therapy for *P. carinii* died during the hospitalization. Four of these patients did not respond to treatment for other organisms cultured from the BAL specimen (one each *C. immitis*, *M. avium-M. intracellulare*, *H. influenzae*, and *Staphylococcus aureus*). The fifth patient died because of respiratory failure of unknown etiology.

The sensitivity of detection by PCR was compared to that by direct examination by using serial dilutions of a *P. carinii*-containing BAL specimen; three samples were tested in parallel by direct examination and amplification. A lower limit of 1,000 cysts per ml of original BAL specimen could be detected by direct examination. The amplification method demonstrated a 100-fold increase in sensitivity, detecting approximately 10 cysts per ml (or 1 to 2 cysts in each amplification reaction mixture) (Fig. 2).

DISCUSSION

Detection of *P. carinii* in lung tissue, BAL specimens, or sputum specimens has classically required direct visualization of cysts or trophozoites in tissue or cytologic specimens by special staining methods. The efficiency of these techniques is limited by sampling error, the inability to concentrate the BAL or sputum specimen beyond the level at which good organism morphology can be obtained for microscopic preparations, the inability of most stains to be used effectively for both cysts and trophozoites, and observer skill for detecting these morphologic forms of the organism. PCR detection of *P. carinii* from BAL specimens addresses all of these issues. Sampling is improved by increasing the volume of BAL specimen examined. The DNA extraction and purification process essentially concentrates the specimen, allowing for a 100-fold increase in the volume of specimen assayed compared to that used for direct examination. This concentration step allows for the detection of one to two cysts per amplification reaction, and the results are in good agreement with those in previous reports (31). Furthermore, DNA from both the cyst and trophozoite forms is detected by PCR, whereas the fluorescent stains for *P. carinii* can reliably detect only the cyst form in these specimens. Finally, PCR detection of *P. carinii* provides a definitive laboratory diagnosis with either the presence or the absence of the 338-bp amplicon band. Direct examination, in contrast to PCR, is a much more subjective diagnosis with the GMS stain; *P. carinii* is approximately the same size as the yeast forms of *C. albicans*. To the unskilled observer, these two organisms might be mistaken. Moreover, even the more sensitive fluorescent stains require a skilled observer for accurate interpretation of the results. Calcofluor white does not stain trophozoites present in a specimen (23), while the monoclonal antibody (fluorescent) stains trophozoites in a way that does not allow for a definitive identification (16).

How sensitive is too sensitive for *P. carinii* detection? This question assumes that small numbers of *P. carinii* organisms may be insignificant in causing disease, representing either normal flora or an asymptomatic carrier state. The results of studies addressing this issue are conflicting. Autopsy studies demonstrated a lack of carrier state (33), and case reports have demonstrated rare cases of asymptomatic carriage (9, 39, 41). In contrast, a high rate of seropositivity for *P. carinii* autoantibodies is seen in both the adult and pediatric populations (32). This generally reflects seroconversion secondary to an asymptomatic infection. The occurrence of *P. carinii* in small epidemics suggests that infectious spread does occur in humans, probably by the same respiratory route seen in rats (20, 21). We also know from studies with rat models that an asymptomatic carrier state can eventually evolve into PCP in patients with prolonged immunosuppression with corticosteroids (14, 19). In patients without AIDS, corticosteroid therapy is an extremely common factor predisposing patients to PCP (47). In our current study, 60.9% of the patients who had any discrepant test results for *P. carinii* were receiving corticosteroid therapy. In the case of steroid-induced immunosuppression, even a carrier state for *P. carinii* might precede clinical PCP and therefore prove to be significant.

The experience with the laboratory diagnosis of PCP by PCR has been limited to several studies providing conflicting results. Several studies found patients with false-positive results by PCR; these patients were followed for several months, but their conditions did not progress to PCP (25, 29, 42). Others found progression to PCP or diagnosis of PCP by alternative methods in their group with false-positive PCR results (25, 26, 35, 42, 44, 45). The increased sensitivity of the PCR assay compared to that of direct examination has therefore become a double-edged sword. In our current study, the increased sensitivity of PCR produced an apparently poor specificity for the assay (68.2%), if we use direct examination as the gold standard. Analysis of the patient population, however, suggests that the diagnosis of PCP may have been missed by direct examination in at least five of our patients. These five patients

were confirmed to have PCP by prior or subsequent diagnostic testing. This suggests that direct examination was actually 88.1% sensitive, whereas PCR was 100% sensitive. The specificity of the direct examination remained 100%, whereas that of PCR was 78.3%. If we considered the two patients who responded to empiric therapy for PCP as true positives, then the direct examination would be only 84.1% sensitive, while the PCR assay's apparent specificity would have increased to 81.2%. Eleven additional patients had culture-negative pneumonitis or were infected with microbial isolates that were interpreted as clinically insignificant. The increased sensitivity of the PCR assay would have provided a potential diagnosis, and therefore therapy, for a total of 18 of the 23 patients with discrepant results for *P. carinii* infection (78.3% of the false-positive group). Overall, 17 of the 23 patients originally identified as having false-positive results (56.5%) were found to have identifiable possible pathogens in their lavage fluid specimens. CMV was detected in cultures of specimens from all 17 of these patients. All but one of these cultures were thought to be clinically insignificant. Nine of these same specimens also contained bacterial or fungal isolates considered to be significant. Prior studies have demonstrated that PCP is frequently associated with another infection. Baughman (4) isolated an additional pathogen in cultures of specimens from more than 15% of the patients studied, while Limper et al. (27) isolated CMV in cultures of BAL specimens from 47.4% of their HIV-positive and 26.8% of their HIV-negative patients. Coexisting aerobic bacteria were cultured from 11 of 75 (14.7%) of the patients examined.

Other applications of PCR in diagnosing PCP include its use in the detection of *P. carinii* from blood (2) or induced sputum (7). While detection of *P. carinii* with either of these specimens represents a significantly less invasive procedure for the patient, the diagnostic yield compared to that of direct examination of BAL specimens is quite poor. Detection of *P. carinii* from blood was limited to those patients with extrapulmonary involvement in two separate studies (29, 35), while Atzori et al. (2) found 90% agreement between the results of PCR and direct detection when they used a more complicated nested PCR protocol to test their series of HIV-positive patients. Detection of *P. carinii* in induced sputum by PCR is best seen in those patients with a very large organism burden, such as those patients who are HIV positive and who have not received prophylaxis. Chouaid et al. (7) demonstrated only a 46.5% sensitivity for their HIV-positive patients and concluded that PCR with induced sputa is not cost-effective, with all patients with negative results still requiring a BAL procedure in addition to the PCR assay (7). Wakefield et al. (44) reported 90% sensitivity of PCR detection of *P. carinii* in induced sputa using silver staining as the reference method. The discrepancy between these two reports may reflect the use of different staining methods as the gold standard or may reflect a lack of consistency in specimen quality for induced sputa which tend to be very much dependent on patient coaching. The use of PCR of sputum has not been adequately evaluated with HIV-negative patients, a population of individuals who generally do not have tremendous organism burdens.

We conclude that PCR detection of *P. carinii* is a very sensitive test, identifying five more positive patients than direct examination in our current series. The apparent low specificity of the assay reflects the 100-fold increase in sensitivity of PCR compared to that of direct examination. This increased sensitivity will make us address the issue of the significance of a positive PCR result with a negative direct examination result. A positive PCR result would provide an earlier diagnosis by PCR rather than by staining, a possible diagnosis, or a concomitant diagnosis for patients who might benefit from directed therapy for PCP.

REFERENCES

1. **Armengol, C. E.** 1995. A historical review of *Pneumocystis carinii*. JAMA **273:**747–751.
2. **Atzori, C., J. J. Lu, B. Jiang, M. S. Barlett, G. Orlando, S. F. Queener, J. W. Smith, A. Cargnel, and C. H. Lee.** 1995. Diagnosis of *Pneumocystis carinii* pneumonia in AIDS patients by using polymerase chain reaction on serum specimens. J. Infect. Dis. **172:**1623–1626.
3. **Baselski, V. S., M. K. Robison, L. W. Pifer, and D. R. Woods.** 1990. Rapid detection of *Pneumocystis carinii* in bronchoalveolar lavage samples by using Cellufluor staining. J. Clin. Microbiol. **28:**393–394.
4. **Baughman, R. P.** 1994. Use of broncoscopy in the diagnosis of infection in the immunocompromised host. Thorax **49:**3–7.
5. **Broaddus, C., M. D. Dake, M. S. Stulbarg, W. Blumenfeld, W. K. Hadley, J. A. Golden, and P. C. Hopewell.** 1985. Bronchoalveolar lavage and transbronchial biopsy for the diagnosis of pulmonary infections in the acquired immunodeficiency syndrome. Ann. Intern. Med. **102:**747–752.
6. **Centers for Disease Control.** 1986. CDC update. Acquired immunodeficiency syndrome—United States. Morbid. Mortal. Weekly Rep. **35:**17–21.
7. **Chouaid, C., P. Roux, I. Lavard, J. L. Poirot, and B. Housset.** 1995. Use of the polymerase chain reaction technique on induced-sputum samples for the diagnosis of *Pneumocystis carinii* pneumonia in HIV-infected patients: a clinical and cost-analysis study. Am. J. Clin. Pathol. **104:**72–75.
8. **Chusid, M. J., and K. A. Heyrman.** 1978. An outbreak of *Pneumocystis carinii* pneumonia at a pediatric hospital. Pediatrics **62:**1031–1035.
9. **Contini, C., R. Romani, V. Vullo, S. Delta, and F. Sorice.** 1992. Does laboratory recovery of *Pneumocystis carinii* always mean clinically significant disease? AIDS **6:**1558–1559.
10. **Deamer, W. C., and H. U. Zolinger.** 1953. Interstitial "plasma cell" pneumonia of premature and young infants. Pediatrics **12:**11–22.
11. **Edman, J. C., J. A. Kovacs, H. Masur, D. V. Santi, H. J. Elwood, and M. L. Sogin.** 1988. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature **334:**519–522.
12. **Espy, M. J., R. Patel, C. V. Paya, and T. F. Smith.** 1995. Comparison of three methods for extraction of viral nucleic acids from blood cultures. J. Clin. Microbiol. **33:**41–44.
13. **Esterly, J. A., and N. E. Warner.** 1965. *Pneumocystis carinii* pneumonia: twelve cases in patients with neoplastic lymphoreticular disease. Arch. Pathol. **80:**433–441.
14. **Frenkel, J. K., J. T. Good, and J. A. Schultz.** 1966. Latent Pneumocystis infection of rats, relapse, and chemotherapy. Lab. Invest. **15:**1559–1577.
15. **Gajdusek, D. C.** 1957. *Pneumocystis carinii*—etiologic agent of interstitial plasma cell pneumonia of premature and young infants. Pediatrics **19:**543–565.
16. **Gill, V. J., G. Evans, F. Stock, J. E. Parrillo, H. Masur, and J. A. Kovacs.** 1987. Detection of *Pneumocystis carinii* by fluorescent-antibody stain using a combination of three monoclonal antibodies. J. Clin. Microbiol. **25:**1837–1840.
17. **Golden, J. A., H. Hollander, M. S. Stulbarg, and G. Gamsu.** 1986. Bronchoalveolar lavage as the exclusive diagnostic modality for *Pneumocystis carinii* pneumonia: a prospective study among patients with acquired immunodeficiency syndrome. Chest **90:**18–22.
18. **Griffiths, M. H., G. Kocjan, R. F. Miller, and P. Godfrey-Faussett.** 1989. Diagnosis of pulmonary disease in human immunodeficiency virus infection: role of transbronchial biopsy and bronchoalveolar lavage. Thorax **44:**554–558.
19. **Hughes, W. T., P. C. McNabb, T. D. Makres, and S. Feldman.** 1974. Efficacy of trimethoprim and sulfamethoxazole in the prevention and treatment of *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **5:**289–293.
20. **Hughes, W. T.** 1982. Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. J. Infect. Dis. **145:**842–848.
21. **Jacobs, J. L., D. M. Libby, R. A. Winters, D. M. Gelmont, E. D. Fried, B. J. Hartman, and J. Laurence.** 1991. A cluster of *Pneumocystis carinii* pneumonia in adults without predisposing illnesses. N. Engl. J. Med. **324:**246–250.
22. **Jules-Elysee, K. M., D. E. Stover, M. B. Zaman, E. M. Bernard, and D. A. White.** 1990. Aerosolized pentamidine: effect on diagnosis and presentation of *Pneumocystis carinii* pneumonia. Ann. Intern. Med. **112:**750–757.
23. **Kim, Y. K., S. Parulekar, P. K. W. Yu, R. J. Pisani, T. F. Smith, and J. P. Anhalt.** 1990. Evaluation of calcofluor white stain for the detection of *Pneumocystis carinii*. Parasitology **13:**307–310.
24. **Kovacs, J. A., V. L. Ng, H. Masur, G. Leoung, W. K. Haley, G. Evans, H. C. Lane, F. P. Ognibene, J. Shelhamer, J. E. Parrillo, and V. J. Gill.** 1988. Diagnosis of *Pneumocystis carinii* pneumonia: improved detection in sputum with use of monoclonal antibodies. N. Engl. J. Med. **318:**589–593.
25. **Leigh, T. R., A. E. Wakefield, S. E. Peters, J. M. Hopkins, and J. V. Collins.** 1992. Comparison of DNA amplification and immunofluorescence for detecting *Pneumocystis carinii* in patients receiving immunosuppressive therapy. Transplantation **54:**468–470.
26. **Leigh, T. R., B. G. Gazzard, A. Rowbottom, and J. V. Collins.** 1993. Quan-

titative and qualitative comparison of DNA amplification by PCR with immunofluorescence staining for diagnosis of *Pneumocystis carinii* pneumonia. J. Clin. Pathol. **46:**140–144.
27. **Limper, A. H., K. P. Offord, T. F. Smith, and W. J. Martin.** 1989. *Pneumocystis carinii* pneumonia: differences in lung parasite number and inflammation in patients with and without AIDS. Am. Rev. Respir. Dis. **140:**1204–1208.
28. **Limper, A. H., U. Specks, W. M. Brutinel, W. J. Martin, and M. S. Rohrbach.** 1993. Interlobar variation in the recovery of bronchoalveolar lavage fluid, cell populations, and angiotensin-converting enzyme in normal volunteers. J. Lab. Clin. Med. **121:**785–791.
29. **Lipschick, G. Y., V. J. Gill, J. D. Lundgren, V. A. Andrawis, N. A. Nelson, J. O. Nielsen, F. P. Ognibene, and J. A. Kovacs.** 1992. Improved diagnosis of *Pneumocystis carinii* infection by polymerase chain reaction on induced sputum and blood. Lancet **340:**203–206.
30. **Masur, H., V. J. Gill, F. P. Ogenbene, J. Shelhamer, C. Godwin, and J. A. Kovacs.** 1988. Diagnosis of Pneumocystis pneumonia by induced sputum technique in patients without acquired immunodeficiency syndrome. Ann. Intern. Med. **109:**755–756.
31. **Olsson, M., K. Elvin, S. Lofdahl, and E. Linder.** 1993. Detection of *Pneumocystis carinii* DNA in sputum and bronchoalveolar lavage samples by polymerase chain reaction. J. Clin. Microbiol. **31:**221–226.
32. **Peglow, S. L., A. G. Smulian, M. J. Linke, C. L. Pogue, S. Nurre, J. Crisler, J. Phair, J. W. M. Gold, D. Armstrong, and P. D. Walzer.** 1990. Serologic responses to *Pneumocystis carinii* antigens in health and disease. J. Infect. Dis. **161:**296–306.
33. **Peters, S. E., A. E. Wakefield, K. Sinclair, P. M. Millard, and J. M. Hopkin.** 1992. A search for *Pneumocystis carinii* in post-mortem lungs by DNA amplification. J. Pathol. **166:**195–198.
34. **Pifer, L. L., W. T. Hughes, S. Stagno, and D. Woods.** 1978. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. Pediatrics **61:**35–41.
35. **Roux, P., I. Lavard, J. L. Poirot, C. Chouaid, M. Denis, J. L. Oliver, M. Nigou, and M. Miltgen.** 1994. Usefulness of PCR for detection of *Pneumocystis carinii* DNA. J. Clin. Microbiol. **32:**2324–2326.
36. **Saah, A. J., D. R. Hoover, Y. Peng, J. P. Phair, B. Visscher, L. A. Kingsley, and L. K. Schrager.** 1995. Predictors for failure of *Pneumocystis carinii* pneumonia prophylaxis. JAMA **273:**1197–1202.
37. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
38. **Santiago-Delpin, E. A., E. Mora, Z. A. Gonzalez, L. A. Morales-Otero, and R. Bermudez.** 1988. Factors in an outbreak of *Pneumocystis carinii* in a transplant unit. Transplant. Proc. **20:**462–465.
39. **Sheldon, W. H.** 1959. Subclinical Pneumocystis pneumonitis. Am. J. Dis. Child. **97:**287–297.
40. **Singer, C., D. Armstrong, P. P. Rosen, and D. Schottenfeld.** 1975. *Pneumocystis carinii* pneumonia: a cluster of eleven cases. Ann. Intern. Med. **82:**772–777.
41. **Stiller, R. A., I. L. Paradis, and J. H. Dauber.** 1992. Subclinical pneumonitis due to *Pneumocystis carinii* in a young adult with elevated antibody titers to Epstein-Barr virus. J. Infect. Dis. **166:**926–930.
42. **Tamburrini, E., P. Mencarini, A. DeLuca, G. Maiuro, G. Ventura, A. Antinori, A. Ammassari, E. Visconti, L. Ortona, A. Siracusano, E. Ortona, and G. Vicari.** 1993. Diagnosis of *Pneumocystis carinii* pneumonia: specificity and sensitivity of polymerase chain reaction in comparison with immunofluorescence in bronchoalveolar lavage specimens. J. Med. Microbiol. **38:**449–453.
43. **Wakefield, A. E., F. J. Pixley, S. Banerji, K. Sinclair, R. F. Miller, E. R. Moton, and J. M. Hopkin.** 1990. Amplification of mitochondrial ribosomal RNA sequences from *Pneumocystis carinii* DNA of rat and human origin. Mol. Biochem. Parasitol. **43:**69–76.
44. **Wakefield, A. E., L. Guiver, R. F. Miller, and J. M. Hopkin.** 1991. DNA amplification on induced sputum samples for diagnosis of *Pneumocystis carinii* pneumonia. Lancet **337:**1378–1380.
45. **Wakefield, A. E., R. F. Miller, L. A. Guiver, and J. M. Hopkin.** 1994. Granulomatous *Pneumocystis carinii* pneumonia: DNA amplification studies on bronchoscopic alveolar lavage samples. J. Clin. Pathol. **47:**664–666.
46. **Walzer, P. D., D. P. Perl, D. J. Krogstad, P. G. Rawson, and M. G. Schultz.** 1974. *Pneumocystis carinii* pneumonia in the United States: epidemiologic, diagnostic, and clinical features. Ann. Intern. Med. **80:**83–93.
47. **Yale, S. H., and A. H. Limper.** 1996. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illnesses and prior corticosteroid therapy. Mayo Clin. Proc. **71:**5–13.