

# Comparison of an *Aspergillus* Real-time Polymerase Chain Reaction Assay With Galactomannan Testing of Bronchoalveolar Lavage Fluid for the Diagnosis of Invasive Pulmonary Aspergillosis in Lung Transplant Recipients

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**Background.** Early diagnosis and treatment of invasive pulmonary aspergillosis (IPA) improves outcome.

**Methods.** We compared the performance of publicly available pan-*Aspergillus*–*Aspergillus fumigatus*–, and *Aspergillus terreus*–specific real-time polymerase chain reaction (PCR) assays with the Platelia galactomannan (GM) assay in 150 bronchoalveolar lavage (BAL) samples from lung transplant recipients (16 proven/probable IPA, 26 *Aspergillus* colonization, 11 non-*Aspergillus* mold colonization, and 97 negative controls).

**Results.** The sensitivity and specificity of pan-*Aspergillus* PCR (optimal quantification cycle [Cq],  $\leq 35.0$  by receiver operating characteristic analysis) and GM ( $\geq 5$ ) for diagnosing IPA were 100% (95% confidence interval, 79%–100%) and 88% (79%–92%), and 93% (68%–100%) and 89% (82%–93%), respectively. The sensitivity and specificity of *A. fumigatus*–specific PCR were 85% (55%–89%) and 96% (91%–98%), respectively. *A. terreus*–specific PCR was positive for the 1 patient with IPA due to this species; specificity was 99% (148 of 149 samples). *Aspergillus* PCR identified 1 patient with IPA not diagnosed by GM. For BAL samples associated with *Aspergillus* colonization, the specificity of GM (92%) was higher than that of pan-*Aspergillus* PCR (50%;  $P = .003$ ). Among negative control samples, the specificity of pan-*Aspergillus* PCR (97%) was higher than that of BAL GM (88%;  $P = .03$ ). Positive results for both BAL PCR and GM testing improved the specificity to 97% with minimal detriment to sensitivity (93%).

**Conclusions.** A recently developed pan-*Aspergillus* PCR assay and GM testing of BAL fluid may facilitate the diagnosis of IPA after lung transplantation. *A. fumigatus*– and *A. terreus*–specific real-time PCR assays may be useful in rapidly identifying the most common cause of IPA and a species that is intrinsically resistant to amphotericin B, respectively.

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Invasive pulmonary aspergillosis (IPA) is a significant cause of morbidity and mortality in lung transplant recipients [1]. Indeed, the respiratory tracts of ~40% of lung transplant recipients become colonized with *Aspergillus* spp. [2], and 6%–16% of patients develop IPA [3]. Despite better antifungal agents and more aggressive surveillance and prophylactic strategies, mortality associated with IPA remains high [4]. In part, high mortality stems from delayed diagnosis [5]. As

such, there has been much effort in developing rapid and more sensitive diagnostic tools such as galactomannan (GM) detection and *Aspergillus* polymerase chain reaction (PCR) assays. Although the serum GM enzyme immunoassay (EIA) (Bio-Rad Platelia) is a well-accepted diagnostic tool for aspergillosis [6], its sensitivity in lung transplant recipients is poor [7]. Recently, GM testing has been applied to bronchoalveolar lavage (BAL) samples. However, data on the performance of BAL testing in lung transplant recipients remain limited [7–9]. *Aspergillus* PCR of BAL fluid has also been investigated for the diagnosis of IPA [10–14], but lack of standardization and heterogeneity of methods between studies have contributed to variable performance. To date, sensitivities have ranged from 36% to 100% [15, 16]. For this reason, *Aspergillus* PCR is promising as a diagnostic tool but faces barriers to its implementation in clinical practice [17].

The present study compares the performance of an *Aspergillus* real-time PCR assay, which was validated elsewhere according to relevant guidelines [18] and in a rabbit model of IPA [19], with that of GM testing on BAL fluid samples for diagnosing IPA in lung transplant recipients. In addition to a pan-*Aspergillus* PCR that targets 18S ribosomal DNA common to all *Aspergillus* species, we assessed *Aspergillus fumigatus*- and *Aspergillus terreus*-specific assays that target ITS-1 regions of ribosomal DNA.

## METHODS

We conducted a retrospective study among lung transplant recipients at the University of Pittsburgh Medical Center between 2000 and 2010 who underwent bronchoscopy for surveillance or diagnostic evaluation. The study was approved by our institutional review board. Residual BAL fluid from the clinical microbiology laboratory was collected and stored without preservation at  $-80^{\circ}\text{C}$ . In determining the performance characteristics of tests, only the first BAL sample associated with an episode of IPA was included in the evaluation.

### Definitions

Two investigators reviewed medical records and imaging studies to classify patients as having fungal colonization, or proven, probable, or no fungal infection [6]. “Proven IPA” required histopathologic examination of lung or tracheobronchial biopsy showing hyphae with morphology suggestive of *Aspergillus* and respiratory culture growing *Aspergillus* spp. “Probable IPA” required presence of either a dense, well-circumscribed lesion, air crescent sign or cavity on chest computed tomographic (CT) scan and positive respiratory culture for *Aspergillus* spp. Serum GM and  $(1\rightarrow3)\text{-}\beta\text{-D}$ -glucan testing were not routinely performed among transplant patients at our institution. Detection of GM in BAL fluid was not included in the definition of IPA in this study. Patients with a BAL culture positive for *Aspergillus* or

non-*Aspergillus* molds but with a normal bronchoscopic and chest CT scan findings were classified as having *Aspergillus* and non-*Aspergillus* mold colonization, respectively. To ensure accuracy of this classification, colonized patients must not have received a mold-active antifungal for  $\geq 7$  days within 4 weeks of the BAL sample and must have undergone a follow-up evaluation at  $\geq 6$  months after positive fungal culture to ensure that they did not develop IPA.

### Test Performance

Frozen BAL samples were shipped overnight, on dry ice, in batch to Viracor-IBT Laboratories for PCR and GM testing. The samples were thawed at room temperature and vortexed before analyses. The pan-*Aspergillus*, *A. fumigatus*, and *A. terreus* PCR assays were performed using primers, probes, and methods developed at Viracor-IBT Laboratories in collaboration with the Immunocompromised Host Section of the National Cancer Institute [20] [Table 1]. The AllPrep DNA/RNA Mini Kit (QIAGEN) was used for extractions with modifications. Five hundred microliters of BAL fluid was pelleted at 15,000 g for 5 min, and the pellet was resuspended in the kit lysis buffer. After bead beating, the lysate was extracted using the QIAGEN QIA-Cube instrument and eluted in 100  $\mu\text{L}$ . Negative extraction control and germling positive controls were included in each extraction run. For 9 samples, only 400  $\mu\text{L}$  was available, and all was used for DNA extraction. A universal internal control was added to each sample. Each sample was tested in duplicate. The final quantification cycle (Cq), the point at which sample fluorescence rose above the established threshold level, was obtained. GM was measured on 300  $\mu\text{L}$  of BAL fluid using the Platelia *Aspergillus* EIA (Bio-Rad Laboratories). Both PCR and GM assays were performed in a blinded fashion, and results were sent to the investigators at the University of Pittsburgh for data analysis.

### Statistical Analysis

Sensitivity and specificity were calculated for PCR and GM EIA based on the assumption that the reference standard for diagnosis was the clinical definition of IPA given above. A receiver operating characteristic (ROC) analysis was conducted to determine the optimal cutoffs for PCR and GM. To compare sensitivity and specificity between BAL GM and PCR, we used the McNemar  $\chi^2$  test for patients with IPA and no IPA [21].

## RESULTS

### Testing of BAL Samples

A total of 150 BAL samples from 137 unique patients were included in this study [Table 2]. Sixteen samples were associated with IPA (5 proven, 11 probable), 26 with colonization by *Aspergillus* spp., and 11 with colonization by molds other than

**Table 1. Primers and Probes Used in the *Aspergillus* Real-time Polymerase Chain Reaction Assays**

Assay	Primer or probe	Primer sequence (5' → 3') <sup>a</sup>	Accession no.	Position
pan- <i>Aspergillus</i>	Forward primer	GTGGAGTGATTTGTCTGCTTAATTG	AF548061 <sup>d</sup>	1215–1239
	Probe <sup>b</sup>	CGGCCCTTAAATAGCCCGGTCCG	AF548061 <sup>d</sup>	1258–1280
	Reverse primer	TCTAAGGGCATCACAGACCTGTT	AF548061 <sup>d</sup>	1345–1367
<i>A. fumigatus</i>	Forward primer	GCCCGCCGTTTCGAC	GU319985	86–100
	Probe <sup>b</sup>	CCCGCCGAAGACCCCAACATG	GU319985	136–156
	Reverse primer	CCGTTGTTGAAAGTTTAACTGATTAC	GU319985	195–221
<i>A. terreus</i>	Forward primer	CATTACCGAGTGCGGGTCTTTA	GU256759	12–33
	Probe <sup>b</sup>	CCCAACTCCCACCCGTGACTATTG	GU256759	37–61
	Reverse primer	CCCGCCGAAGCAACAAG	GU256759	65–81
UIC	Forward primer	CAGCAGAACACCCCATC	U57606	1162–1179
	Probe <sup>c</sup>	AACCACTACCTGAGCACCCAGTCC	U57606	1207–1230
	Reverse primer	GTGATCGCGCTTCTCGTT	U57606	1249–1266

<sup>a</sup> Primer concentrations were empirically optimized. *Aspergillus* target and universal internal control (UIC) primers had final reaction concentrations of 800 and 200 nmol/L, respectively.

<sup>b</sup> All *Aspergillus* probes possessed a 5' FAM reporter and 3' dark quencher.

<sup>c</sup> The UIC probe possessed a 5' VIC reporter dye and a 3' TAMRA quencher.

<sup>d</sup> The pan-*Aspergillus* oligonucleotides are designed to hybridize to the 18S ribosomal DNA region of all *Aspergillus* spp. The sequence accession number for the pan-*Aspergillus* assay is based on *A. fumigatus* AF548061.

*Aspergillus*, and 97 were not associated with *Aspergillus* or other molds (negative controls). PCR and GM testing were performed on 150 and 148 BAL samples, respectively; the BAL volume from 1 patient with IPA and 1 negative control was insufficient to perform GM. The distributions of Cq values and GM indexes are presented in Figure 1.

### Performance of BAL PCR and GM

The median Cq value among patients with IPA (29.67 [interquartile range (IQR), 23.18–32.81]) was lower than that among patients with *Aspergillus* colonization (35.31 [31.7–40.06];  $P < .0001$ ), patients with non-*Aspergillus* mold colonization (45 [40.51–45];  $P < .0001$ ); or negative controls (45 [40.12–45];  $P < .0001$ ). The Cq value was lower for patients with *Aspergillus* colonization than for patients colonized with non-*Aspergillus* molds ( $P = .003$ ), but there was no difference in Cq values between those colonized with non-*Aspergillus* molds and controls ( $P = .76$ ).

ROC analysis identified Cq values of  $\leq 35$  as the optimal cutoff [Figure 2A]; Cq was  $\leq 35$  for 100% of BAL samples from patients with IPA (16 of 16), compared with 50% of those with *Aspergillus* colonization (13 of 26), 18% of those with non-*Aspergillus* mold colonization (2 of 11), and 3% of controls (3 of 97) [Table 3]. The sensitivity and specificity of this cutoff in the diagnosis of IPA are presented in Table 4.

The median BAL GM index among patients with IPA (2.71 [IQR, .77–6.73]) was significantly higher than that among patients with *Aspergillus* colonization (.32 [.22–.38];  $P < .0001$ ), patients with non-*Aspergillus* mold colonization (.23 [.18–.40];  $P = .002$ ), or controls (.23 [.17–.37];  $P < .0001$ ). There was no

difference in BAL GM indexes between patients colonized with *Aspergillus* or other molds and controls ( $P = .43$ ). In addition, 93% of BAL samples associated with IPA (14 of 15), 7% (2 of 26) from patients with *Aspergillus* colonization, 9% (1 of 11) from patients with non-*Aspergillus* mold colonization, and 12% (12 of 97) from controls had BAL GM indexes  $\geq .5$  [Table 3]. The corresponding values for BAL GM indexes  $\geq 1.0$  were 67% (10 of 15 samples), 4% (1 of 26), 0% (0 of 11) and 3% (3 of 97), respectively. ROC analysis identified  $\geq .5$  as the optimal cutoff for BAL GM indexes [Figure 2B]. The performance of this index for diagnosing IPA is presented in Table 4.

Fifty percent of patients with IPA (8 of 16) received a mold-active antifungal before BAL [Figures 3A and 3B]. None of the corresponding BAL samples yielded negative PCR results. One negative BAL GM was noted for a patient who had received a mold-active antifungal agent for  $> 1$  month.

### Correlation Between BAL PCR and GM

Both PCR and GM testing were performed in 148 BAL samples [Table 3; Figure 4]. In the 15 BAL samples from patients with IPA tested by both methods, the Pearson correlation coefficient  $r$  for correlation between Cq and GM indexes was  $-.72$  (95% confidence interval [CI],  $-.90$  to  $-.32$ ;  $P = .003$ ). In addition, the agreement between Cq and GM index at cutoffs of  $\leq 35$  and  $\geq .5$ , respectively, was 93% (14 of 15 samples). Overall, there was no significant difference in the sensitivity of BAL PCR and GM ( $P = 1.00$ ; McNemar's test).

Among the samples from patients with *Aspergillus* colonization, the agreement between PCR and GM was 58% (15 of

**Table 2. Characteristics of Patients Enrolled in This Study**

Characteristic <sup>a</sup>	Patients with IPA (n = 16) <sup>b</sup>	Patients with <i>Aspergillus</i> colonization (n = 26)	Patients with non- <i>Aspergillus</i> mold colonization (n = 11)	Negative controls (n = 97)
Age, median + standard deviation, years	54.7 ± 10.8	62.7 ± 10.76	53.0 ± 13.1	58.5 ± 11.8
Sex, % (no.) of samples from women	62 (10/16)	38 (10/26)	64 (7/11)	47 (46/97)
Common underlying lung diseases				
COPD/emphysema	1	7	4	44
Idiopathic pulmonary fibrosis	1	4	0	6
Cystic fibrosis	6	11	0	27
Type of lung transplant				
Double lung	11	17	9	65
Single lung	5	9	1	29
Heart-lung	0	0	1	3
Microbiology	<i>Aspergillus fumigatus</i> (13) <sup>c</sup> ; <i>Aspergillus niger</i> (2) <sup>d</sup> ; <i>Aspergillus terreus</i> (1)	<i>Aspergillus fumigatus</i> (12); <i>A. fumigatus</i> (7); <i>A. niger</i> (5); <i>Aspergillus ustus</i> (1); <i>Aspergillus clavatus</i> (1)	<i>Penicillium</i> (7); <i>Cladosporium</i> (1); <i>Fusarium</i> (1); <i>Scopulariopsis</i> (1); <i>Syncephalastrum</i> (1)	...
Interval from transplantation to BAL, median (IQR), months	23.0 (11.0–36.5)	13.1 (7.5–21.3)	13.2 (5.6–27.0)	11.6 (3.9–21.0)

<sup>a</sup> BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; IQR, interquartile range.

<sup>b</sup> Five patients had proven invasive pulmonary aspergillosis (IPA), and 11 had probable IPA. Proven IPA included tracheobronchitis alone (n = 1), lung parenchymal IPA alone (n = 2), both proven tracheobronchitis and proven parenchymal IPA (n = 1), and both proven tracheobronchitis and probable parenchymal IPA (n = 1). All cases of probable IPA were parenchymal disease; 1 of these patients also had proven aspergillosis of the vocal cords.

<sup>c</sup> *A. niger* was also cultured from 1 sample; this sample is included as *A. fumigatus*.

<sup>d</sup> *A. niger* as sole pathogen.

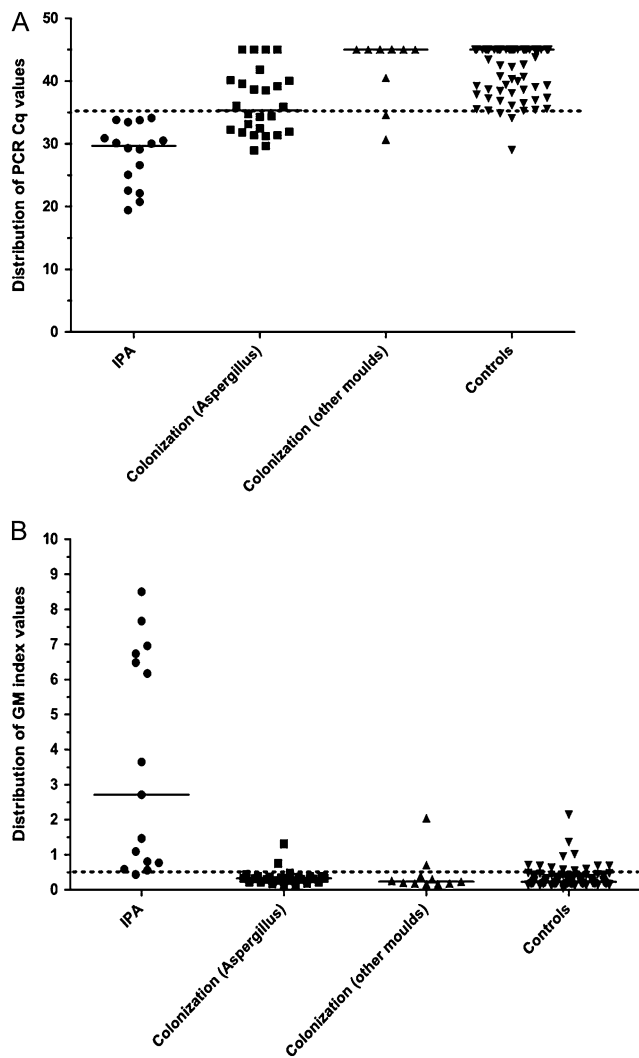
26 samples). For samples with *Aspergillus* colonization, the specificity of BAL PCR was significantly less than that of GM (50% [13 of 26] vs 92% [24 of 26];  $P = .003$ ) [Figure 4]. Among the samples from patients with non-*Aspergillus* mold colonization, the agreement between PCR and GM was 100% (11 of 11 samples). Overall, 29% of samples (2 of 7) colonized with *Penicillium* yielded false-positive PCR and GM results. Among the control samples, the agreement between PCR and GM was 88% (85/96). The specificity of PCR was 97% (93 of 96 samples), higher than that of GM (88% [84 of 96 samples];  $P = .03$ ).

#### Performance of *Aspergillus* Species-specific PCR

For *A. fumigatus*- and *A. terreus*-specific PCR, the cutoff for test positivity was  $\leq 40$  (data not shown). The sensitivity and specificity of *A. fumigatus*-specific PCR for diagnosing IPA due to *A. fumigatus* were 85% (CI, 55%–98%) (11 of 13 samples) and 96% (CI, 92%–99%) (132 of 137 samples), respectively. Results of *A. terreus*-specific real-time PCR were positive for the 1 patient with IPA due to this species; the specificity was 99% (148 of 149 samples).

## DISCUSSION

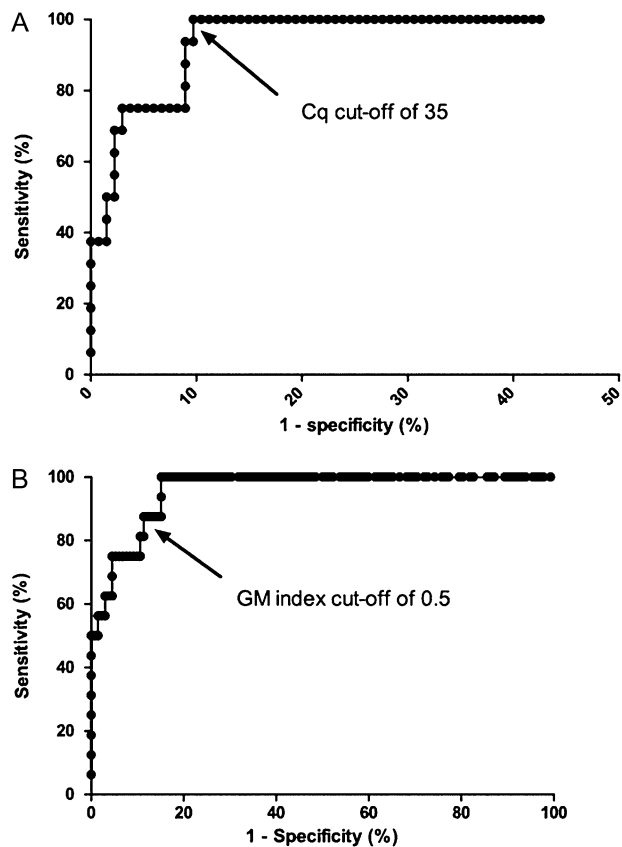
In this study, we demonstrated that a real-time PCR assay of BAL fluid has value in diagnosing IPA among lung transplant recipients. Using a Cq cutoff of  $\leq 35$ , the sensitivity and specificity of PCR in diagnosing proven or probable IPA were 100% (16 of 16 samples) and 88% (118 of 134 samples), respectively. In other studies of BAL fluid from patients with hematologic malignancies and hematopoietic stem cell transplant (HSCT) recipients, the sensitivity and specificity of PCR assays for diagnosing IPA ranged from 36% to 100% [15, 16]. It is difficult to compare our study with earlier reports because of the lack of standardization of PCR techniques [15, 16], including significant heterogeneity in the volumes of BAL fluid tested, the fraction of BAL samples used for extraction (eg, supernatant, cell pellet, whole sample), BAL extraction methods, the target genes and PCR format used, and the threshold for detection of fungal DNA [15, 16]. Nevertheless, this is the first reported study that validates a PCR assay as a diagnostic test for IPA. If further validated in other patient populations and by multicenter studies, this assay should be a valuable adjunctive tool for



**Figure 1.** Distribution of BAL PCR Cq (A) and galactomannan index values by categories of disease. Median quantification cycle (Cq) values and galactomannan (GM) indexes were significantly lower and higher, respectively, in patients with invasive pulmonary aspergillosis (IPA) than in patients with *Aspergillus* or non-*Aspergillus* mold colonization or controls. Dashed lines represent Cq cutoff of 35 (A) and GM cutoff of .5 (B); horizontal bars, median values; PCR, polymerase chain reaction.

diagnosing IPA. Furthermore, the assay may be a useful addition to the European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria for classification of invasive aspergillosis [6], which provide standardized definitions for clinical trials and epidemiologic studies.

False positivity is well recognized as a potential limitation of PCR assays for diagnosis of aspergillosis [15, 22]. In fact, our false-positive rate of 12% (16 of 134 samples) is in line with the 8% rate reported in the literature [15, 16, 22]. False-positive PCR results can be procedure related (eg, due to *Aspergillus* contamination from the environment, cross-reactivity of PCR primers and probes with sequences from other organisms) or related to clinical factors (eg, *Aspergillus* colonization) [15].



**Figure 2.** Receiver operating curves for BAL PCR (A) and GM (B). Receiver operating characteristic analyses identified quantification cycle (Cq) values  $\leq 35$  and galactomannan (GM) indexes  $\geq .5$  as optimal cutoff values for diagnosing invasive pulmonary aspergillosis.

In our study, 81% of false-positive PCR results (13 of 16 samples) were due to *Aspergillus* colonization of the airways. This clinical false positivity is the trade-off for molecular techniques aimed at detecting *Aspergillus* at the lowest concentration possible, regardless of the role in IPA or colonization. For this reason, *Aspergillus* PCR assays are more accurately considered detection tests rather than diagnostic tests [15, 23]. As such, the ultimate performance in diagnosing IPA in the clinic will depend on judicious ordering and interpretation of the test in settings where the disease is reasonably suspected.

Along these lines, it is notable that the PCR assay was positive in only 50% (13/26) of BAL fluid colonized with *Aspergillus*. The disparate results between the 100% sensitivity of the PCR assay in detecting IPA and the 50% sensitivity in detecting *Aspergillus* colonization may be explained by the pathogenesis of IPA. The life cycle of *Aspergillus* in the airway is a dynamic process, and disease develops as a result of the interaction between fungal burden and virulence on the one hand, and the status of host immunity on the other [24]. Conidia are inhaled from the environment and deposited in the alveoli. In a suitable environment, the conidia swell, germinate, and invade surrounding

**Table 3. Bronchoalveolar Lavage (BAL) Polymerase Chain Reaction (PCR) and Galactomannan (GM) Assay Results Classified by *Aspergillus* Infection**

Infection	BAL GM index			Assay not done
	≥1.0	.5–.99	<.5	
<b>Invasive pulmonary aspergillosis (n = 16)</b>				
PCR positive <sup>a</sup>	10	4	1 <sup>b</sup>	1
PCR negative	0	0	0	0
<b><i>Aspergillus</i> colonization (n = 26)</b>				
PCR positive	1 <sup>c</sup>	1 <sup>c</sup>	11	0
PCR negative	0	0	13	0
<b>Colonization by molds other than <i>Aspergillus</i> (n = 11)</b>				
PCR positive	1 <sup>d</sup>	1 <sup>d</sup>	0	0
PCR negative	0	0	9	0
<b>No infection (n = 97)</b>				
PCR positive	0	2 <sup>e</sup>	1	0
PCR negative	3 <sup>f</sup>	7 <sup>f</sup>	83	1

<sup>a</sup> Positive PCR results were defined as quantification cycle (Cq) values of ≤35 (cutoff identified by receiver operating characteristic analysis).

<sup>b</sup> This patient had probable invasive pulmonary aspergillosis. The PCR Cq value was 34.13, and the GM index .44.

<sup>c</sup> These 2 BAL samples had Cq values of 31.4 and 29, respectively, and GM indexes of 1.31 and .75.

<sup>d</sup> These 2 BAL samples were from patients colonized with *Penicillium* (Cq values, 30.7 and 24.6, respectively; GM indexes, 2.0 and .7).

<sup>e</sup> The first patient had a recurrent history of colonization with *Aspergillus fumigatus*, but the fungal culture at the time of BAL was negative. The BAL Cq value for this patient was 29.0, and the GM index was .68. The equivalent values for the second patient were 34.1 and .57, respectively.

<sup>f</sup> None of these 10 patients with false-positive GM indexes had previous *Aspergillus* colonization or had received piperacillin-tazobactam or amoxicillin-clavulanate within 1 month before BAL.

lung tissues [24]. In IPA, various fungal morphologies may be present, including conidia, germlings, and hyphae, whereas conidia predominate during colonization. The DNA extraction method used in this study is much more efficient for germlings than conidia (Viracor-IBT internal data, communicated by Mark Wissel), which may account for the greater sensitivity in detecting *Aspergillus* DNA during IPA than in detecting colonization. Alternatively, the results may reflect higher fungal burdens in BAL fluid from patients with IPA.

The remaining 19% of false-positive PCR tests (3 of 16) were due to procedural factors. The first 2 were associated

with *Penicillium* colonization. *Penicillium* has a close phylogenetic relationship with *Aspergillus*. Most species of *Penicillium* share 100% identity in the pan-*Aspergillus* primer and probe binding sites. Molds not phylogenically related to *Aspergillus*, such as *Cladosporium*, *Syncephalostrom*, *Scopulariopsis*, and *Fusarium*, did not cause false-positive results. The third false-positive result was noted in BAL fluid from a patient with intermittent airway colonization with *A. fumigatus*. Although the BAL culture was negative at the time of the PCR sample, *A. fumigatus* was cultured 3 months later. The culture at the time of PCR, therefore, might have been false negative, and the PCR assay may have been more sensitive than culture in detecting *Aspergillus*. Taken together, our results show that the PCR assay is highly specific for detecting *Aspergillus* spp., yielding very few false-positive results in patients without *Aspergillus* colonization or disease.

BAL GM also performed well in diagnosing IPA; with a cutoff of ≥.5, the sensitivity and specificity were 93% and 89%, respectively. Increasing the cutoff to ≥1.0 improved the specificity to 97% at the expense of decreasing sensitivity to 67%. Although the sensitivity of BAL GM in our study was similar to that reported elsewhere in lung transplant patients [7, 9], our false-positive rate of 11% (16 of 148 results) was significantly lower. In contrast to *Aspergillus* PCR, only 19% of the false-positive BAL GM results (3 of 16) were due to *Aspergillus* (n = 2) or *Penicillium* (n = 1) colonization. The cause of false-positive BAL GM results in the remaining 81% (13 of 16) could not be determined. None of these patients, however, received piperacillin-tazobactam or amoxicillin-clavulanate, well-described causes of false-positive GM results [25].

For IPA, the concordance between BAL PCR and GM was 93% (14 of 15 results). One case of IPA was detected with PCR but missed with GM, and there were no cases of IPA with positive GM but negative PCR results. For *Aspergillus* colonization, the concordance between the tests was only 58%; the specificity of BAL GM (92%) in this setting was significantly higher than that of PCR (50%; *P* = .003). On the other hand, the concordance for negative controls was 88%, and the specificity of BAL GM (88%) was significantly lower than that of PCR (97%; *P* = .03). In other words, the major cause of false-positive results for the PCR assay was *Aspergillus* colonization, whereas the causes of false-positive results for GM were unknown. Positive results by both BAL PCR and GM testing improved the specificity to 97% with minimal detriment to sensitivity (93%).

This study included only lung transplant recipients, and the results cannot be extrapolated to other populations at risk for IPA, such as patients with hematologic malignancy or HSCT recipients. In fact, it is well recognized that different host factors can lead to different pathogenesis of IPA [26], which in turn might affect host inflammatory responses. Moreover, a unique problem in lung transplantation is the frequency of fungal

**Table 4. Performance of Bronchoalveolar Lavage (BAL) Polymerase Chain Reaction (PCR) and Galactomannan (GM) Assays in the Diagnosis of Invasive Pulmonary Aspergillosis**

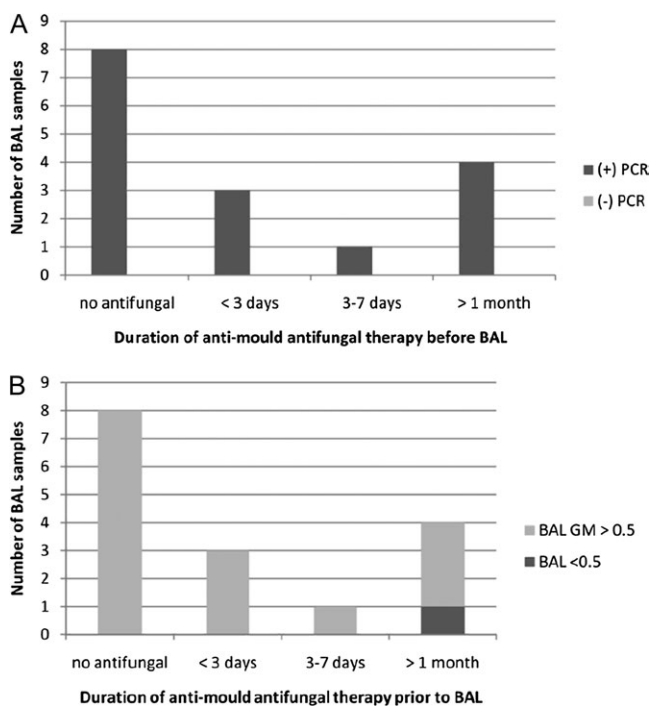
Assay result	Performance, % (95% CI) [no. of samples] <sup>a</sup>				
	Sensitivity	Specificity	PPV	NPV	Likelihood ratio
BAL PCR <sup>b</sup>	100 (79–100) [16/16]	88 (79–92) [118/134]	50 (30–65) [16/32]	100 (97–100) [118/118]	8.4
BAL GM index $\geq 1.0$ <sup>c</sup>	67 (38–88) [10/15]	97 (92–99) [129/133]	71 (42–92) [10/14]	96 (92–99) [129/134]	22.2
BAL GM index $\geq .5$ <sup>c</sup>	93 (68–100) [14/15]	89 (82–93) [118/133]	48 (29–67) [14/29]	99 (95–100) [118/119]	8.3

<sup>a</sup> Positive predictive value (PPV) and negative predictive value (NPV) were calculated for the distribution of disease categories in this study (ie, valid for a population with invasive pulmonary aspergillosis in 11%, *Aspergillus* colonization in 17%, non-*Aspergillus* mold colonization in 7%, and nonfungal infection in 79%). CI, confidence interval.

<sup>b</sup> Positive PCR results were defined as quantification cycle (Cq) values of  $\leq 35$  (cutoff determined by receiver operating characteristic analysis).

<sup>c</sup> A total of 148 BAL samples were available for GM testing.

colonization of the airway. Up to 46% of lung transplant recipients become colonized with *Aspergillus* after transplantation [2], but only a small proportion develop invasive disease [1]. On

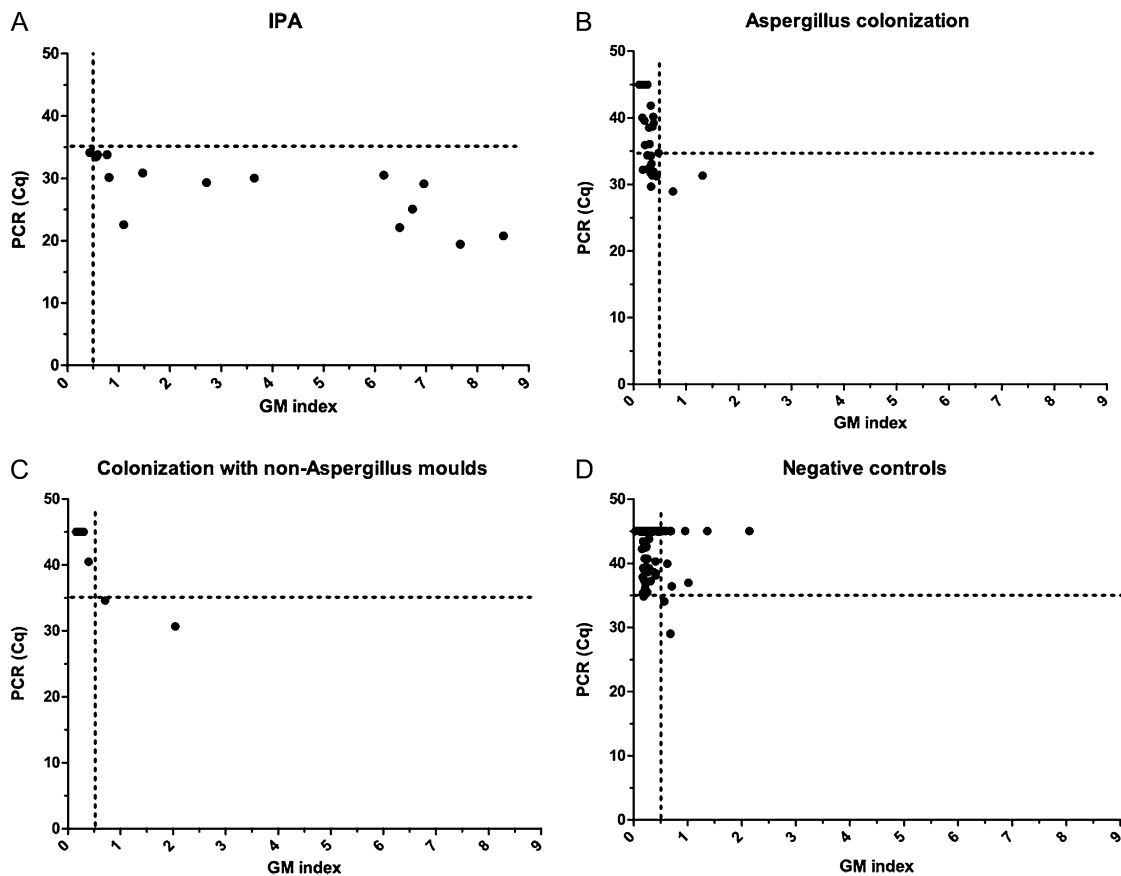


**Figure 3.** Effects of mould-active antifungal therapy on BAL PCR (A) and GM results (B). Mold-active antifungal therapy did not affect bronchoalveolar lavage (BAL) polymerase chain reaction (PCR) results and had a minimal impact on galactomannan (GM) results. Among patients with invasive pulmonary aspergillosis (IPA), 50% (8 of 16) received a mold-active antifungal before BAL. Three patients received < 3 days of antifungal therapy (2 patients received a combination of voriconazole and caspofungin, and 1 received aerosolized amphotericin B alone). One patient received 6 days of voriconazole and 1 day of caspofungin and aerosolized amphotericin B. Four patients received  $\geq 1$  month of voriconazole (2 patients received  $\sim 4$  weeks, 1 received  $\sim 5$  weeks) or itraconazole (1 patient,  $\sim 3$  months). One patient with a false-negative BAL GM result (GM index, .44) received >1 month of voriconazole. Among the patients receiving >7 days of voriconazole for antifungal prophylaxis, only 1 had a voriconazole trough level measured at the time of breakthrough IPA (.71  $\mu\text{g/mL}$ ).

the other hand, the predictive value of a positive culture for IPA in HSCT recipients or patients with hematologic malignancy is significantly higher [27, 28]. As such, it would be expected that the specificity of BAL PCR in these populations will also be higher than among the lung transplant recipients.

Our study has several limitations that should be acknowledged. First, the reference standard for defining proven and probable IPA is imperfect and may not accurately identify all cases. In this pilot study, for example, a positive culture was required to define a case of IPA. Because the sensitivity of culture in patients with IPA ranges from 50% to 80%, our definition probably excluded some cases. In our transplant program, we do not use indirect tests for diagnosing IPA, such as serum GM or  $\beta$ -D-glucan detection, owing to their low sensitivity and specificity [7, 29]. For this reason, we had to rely on chest CT findings to define probable IPA cases. Lung transplant recipients may not have “typical” radiologic findings suggestive of invasive fungal infection as observed in patients with hematologic malignancy or HSCT recipients, such as dense well-circumscribed lesions with or without halo signs [30, 31]. Indeed, 2 of our 3 patients with IPA proven to involve the lung parenchyma did not have “typical” CT patterns. Second, the retrospective nature of our study precluded the evaluation of PCR results for disease screening and monitoring response to therapy. Future prospective studies are needed to address these issues.

In conclusion, our data demonstrate that *Aspergillus* PCR and GM testing of BAL samples may facilitate the diagnosis of IPA in lung transplant recipients. Both tests offer the potential advantage over culture-based diagnostic methods of short turnaround time, which may facilitate more timely initiation of antifungal therapy. In distinguishing between clinically important species, PCR may afford a further advantage over indirect markers, such as GM. *A. terreus*, for example, is a less common cause of IPA than *A. fumigatus*, but it is important to recognize because of its resistance to amphotericin B in vitro and in vivo [32–35]. The PCR assay described in this study demonstrates very high sensitivity and negative predictive value. Employed



**Figure 4.** Correlation between PCR Cq and GM index, by categories of disease classification. There were no significant differences in performance between PCR and GM assays in samples from patients with invasive pulmonary aspergillosis (IPA) and non-*Aspergillus* mold colonization. GM was more specific than PCR in samples from patients with *Aspergillus* colonization, and PCR was more specific than GM in negative controls. Dotted lines represent optimal cutoffs for PCR (horizontal lines) and GM (vertical lines); Cq, quantification cycle value.

judiciously in settings in which IPA is suspected clinically, therefore, the assay is likely to be an important adjunctive diagnostic test.

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