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The Journal of Molecular Diagnostics, Vol. 20, No. 3, May 2018

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the Journal of Molecular Diagnostics
jmd.amjpathol.org

Evaluation of Two Commercial Real-Time PCR Kits for *Aspergillus* DNA Detection in Bronchoalveolar Lavage Fluid in Patients with Invasive Pulmonary Aspergillosis

Julie Denis,* Faezeh Forouzanfar,*† Raoul Herbrecht,‡ Elise Toussaint,‡ Romain Kessler,§ Marcela Sabou,* Ermanno Candolfi,* and Valérie Letsher-Bru*

From the Laboratoire de Parasitologie et de Mycologie Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg; the Institut de Parasitologie et de Pathologie Tropicale,† Fédération de Médecine Translationnelle, Université de Strasbourg, Strasbourg; the Service d'Oncologie et d'Hématologie,‡ Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg & Université de Strasbourg, Strasbourg; and the Pôle Pathologie thoracique,§ CHU de Strasbourg, Strasbourg, France*

Accepted for publication December 22, 2018.

Address correspondence to Julie Denis, M.D., Laboratoire de Parasitologie et de Mycologie Médicale, Hôpitaux Universitaires de Strasbourg, 1 Place de l'Hôpital, 67000 Strasbourg, France. E-mail: denisj@unistra.fr.

Invasive pulmonary aspergillosis (IPA) is a common complication of immunosuppression. Rapid diagnosis using molecular techniques is essential to improve patient survival. PCR techniques are promising in enhancing *Aspergillus* detection in blood and respiratory samples. We evaluate for the first time the performances of two commercial real-time PCR kits, the *A. fumigatus* Bio-Evolution and the MycoGENIE *A. fumigatus* for the detection of *A. fumigatus* DNA in bronchoalveolar lavage (BAL) from patients with and without IPA. Seventy-three BAL samples were included. Thirty-one of them corresponded to patients with probable IPA, 11 to patients with possible IPA, and 31 to patients without aspergillosis, according to the 2008 European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria. In the probable IPA group, *A. fumigatus* Bio-Evolution and the MycoGENIE *A. fumigatus* real-time PCR kits showed a specificity of 100% and a sensitivity of 81% and 71%, respectively. The *A. fumigatus* Bio-Evolution detected *Aspergillus* DNA in the 14 BAL samples with a positive *Aspergillus* culture result, whereas the MycoGENIE *A. fumigatus* PCR result was positive only for 12. In the possible IPA group, there were no positive real-time PCR or positive *Aspergillus* culture results. For the patients without aspergillosis, no positive result was observed for real-time PCR kit, despite the presence of various other non-*Aspergillus* pathogens in this group. Our study demonstrates an excellent specificity and a good sensitivity of *A. fumigatus* DNA detection in BAL samples with both kits. *(J Mol Diagn 2018, 20: 298–306; https://doi.org/10.1016/j.jmoldx.2017.12.005)*

Invasive pulmonary aspergillosis (IPA) is a common complication of immunosuppression, particularly during deep and prolonged neutropenia. Hematologic malignant tumors, hematopoietic stem cell transplantation (HSCT), and solid organ transplantation are major risk factors for IPA, followed by solid tumor and multiple other immunosuppressive conditions. *Aspergillus fumigatus* is the most frequent species isolated during aspergillosis (80%), but other species, such as *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*, can also be found.[1] IPA mortality is substantial (>30%) and is the highest in hematologic patients (up to 90% for HSCT).[2–6] Some studies have found survival improvement in patients receiving an early and appropriate antifungal therapy.[7,8] A rapid and accurate diagnosis of aspergillosis is essential to initiate early treatment. IPA is diagnosed according to clinical, radiologic, histologic, and microbiological criteria.[9] Microbiological methods consist of direct examination and culture of respiratory or other relevant samples and of fungal antigen detection. Because of low culture sensitivity, microbiological evidence of *Aspergillus*

Disclosures: None declared.

https://doi.org/10.1016/j.jmoldx.2017.12.005

presence is difficult to establish in respiratory samples.[10] Antigen detection techniques have improved IPA diagnosis. Galactomannan is an accurate marker recommended for IPA diagnosis in serum[11] and commonly used in bronchoalveolar lavage (BAL) fluids with an index threshold of 0.5 recommended by the manufacturer. Some laboratories use higher thresholds on respiratory samples, from 0.8 to 1,[12] to improve the test specificity, with variable results, which explains the lack of standardization among laboratories for galactomannan detection in respiratory samples. Serum (1→3)-β-D-glucan can also be used for this diagnosis, but it is not specific for *Aspergillus*.[11,13–16] PCR techniques seem to be promising to enhance *Aspergillus* detection in blood and respiratory samples.[11] Many in-house PCR tests have been developed in blood and respiratory samples but are still lacking standardization.[17–19] Thus, there is a need for standardized PCR techniques for better IPA diagnosis. Commercial real-time PCR kits are now available to detect *Aspergillus* in blood and in respiratory specimens, but only a few of them have been evaluated.[20–23] Two of them, the *A. fumigatus* Bio-Evolution (Bio-Evolution, Bry-sur-Marne, France) and the MycoGENIE *A. fumigatus* (Ademtech, Pessac, France) kits, have never been compared in clinical respiratory samples. In this study, we evaluated and compared in our routine conditions the performances of these two commercially available PCR kits for the detection of *A. fumigatus* DNA in BAL samples from patients with and without IPA.

Materials and Methods

Specimens

The study was conducted retrospectively on 73 BAL samples addressed to the microbiology laboratory of the Strasbourg University Hospital between February 2014 and November 2015 for routine mycologic, bacteriologic, and virologic examination. Routine mycologic analysis on BAL samples consisted of direct examination (Musto staining), semi-quantitative culture, determination of galactomannan index (Platelia *Aspergillus* antigen, Bio-Rad, Schiltigheim, France), and *Pneumocystis jirovecii* detection by PCR. The semi-quantitative cultures were realized as follows: 2 to 6 mL of BAL, depending on initial BAL volume received, was centrifuged (15 minutes, 2500 × *g*), and 100 μL of the pellet was cultivated onto ChromID *Candida* medium (bioMérieux, Paris, France) and incubated for 9 days at 35°C followed by 7 more days at 27°C. Morphologic *A. fumigatus* identification was confirmed by β-tubulin sequencing and comparison to the Genbank, Centraalbureau voor Schimmelcultures (CBS), and European Molecular Biology Laboratory databases. The mean inhibitory concentrations (MICs) to amphotericin B, caspofungin, fluconazole, voriconazole, and posaconazole were determined by Etest (bioMérieux). Determination of galactomannan index (Platelia *Aspergillus* antigen, Bio-Rad) was performed on the BAL supernatant and concomitant serum samples. DNA for *P. jirovecii* detection was obtained using a manual extraction kit QIAamp DNA mini kit (Qiagen, Courtaboeuf, France).

Real-Time PCR

For the retrospective study, the DNA previously extracted from the BAL for the routine *P. jirovecii* PCR was used. DNA was extracted using an extraction kit (QIAamp DNA mini kit, Qiagen) available for DNA/RNA extraction. A variable BAL sample volume (2 to 6 mL), depending on initial sample volume received, was centrifuged and 200 μL of the pellet was extracted according to the manufacturer's recommendations with 200 μL of elution volume. Positive and negative extraction controls were used for each extraction run and checked during the *P. jirovecii* PCR. DNA was stored at −20°C with one freezing/thawing cycle. *Aspergillus* DNA was detected by using two commercialized real-time PCR kits, the *A. fumigatus* Bio-Evolution kit and the MycoGENIE *A. fumigatus* kit.

The *A. fumigatus* Bio-Evolution PCR kit targets the *ITS1* region and allows the specific detection of *A. fumigatus* DNA with a limit of detection (LOD) of 5 copies/μL, validated on serum and sinus biopsy specimens. This real-time PCR was performed according to the manufacturer's instructions with 5 μL of DNA extract and the kit inhibition control amplified on LightCycler 480 (Roche, Meylan, France). A quantitation cycle <38 was considered to be positive. The positive control, which can be used to quantify DNA, is provided in the kit.

The MycoGENIE *A. fumigatus* multiplex kit detects the *28S* rRNA of *A. fumigatus* with a LOD of 1 copy/μL and the major azole resistance mutation TR34/L98H with a LOD of 6 copies/μL. It is validated on respiratory tract, biopsy, and serum samples. Real-time PCR was performed according to the manufacturer's instructions using 10 μL of DNA extract and the kit inhibition control amplified on LightCycler 480 (Roche, Meylan, France). A positive control from an *A. fumigatus* sensu stricto culture was used. A quantitation cycle <40 was considered to be positive for *A. fumigatus* detection and mutation detection.

Statistical Analysis

For each BAL sample, the following data were recorded: age and sex of the patient, underlying condition, presence and duration of antifungal therapy before the BAL test, mycologic results (direct examination and BAL culture, isolation of *Aspergillus* from other respiratory specimens during the episode), BAL galactomannan index (threshold of 0.5 according to manufacturer's recommendations), sera galactomannan index, and all other pathogens recovered in BAL during the episode. The episodes were classified as probable IPA, possible IPA, and non-IPA episodes according to the 2008 and 2016 European Organization for Research and Treatment of Cancer/Mycoses Study Group

(EORTC/MSG) criteria (excluding PCR) for patients with cancer, severe neutropenia, HSCT or solid organ transplantation, or T-cell immunosuppressive or steroid treatment.[9] For patients who lacked these underlying conditions, the specific algorithm developed for intensive care unit patients was applied.[24]

For both kits, the clinical sensitivity and specificity were calculated according to this final diagnosis (proven/probable IPA for the clinical sensitivity). Nonparametric binomial and McNemar tests were used for the statistical analysis.

Results

Seventy-three BAL samples were included in the study. Thirty-one of them corresponded to probable IPA, 11 to possible IPA, and 31 to nonaspergillosis pulmonary episodes according to the 2008 EORTC/MSG criteria without considering *Aspergillus* PCR results. No proven IPAs were diagnosed during the study.

Probable IPA

The demographic and underlying condition data are presented in Table 1 and the detailed microbiological and galactomannan results in Table 2. The underlying conditions were hematologic malignant tumors [$n = 15$ (49%)], solid organ transplant [$n = 5$ (16%)], allogenic bone marrow transplant [$n = 4$ (13%)], solid tumor [$n = 1$ (3%)], and other conditions [$n = 6$ (19%)]. Neutropenia was reported for 14 patients (45%). All patients presented clinical criteria for IPA with dense, well-circumscribed nodules with or without halo sign or pulmonary condensation. Fifteen patients (48%) received anti-*Aspergillus* antifungal treatment at the time of the BAL sampling, with a mean duration of 13 days (range, 1 to 37 days) before the BAL. Fourteen BALs (45%) tested positive for *A. fumigatus* sensu stricto in culture with a semiquantitative count varying from 1 to >40 colonies, and 27 BALs (87%) tested positive for *Aspergillus* galactomannan.

With the *A. fumigatus* Bio-Evolution kit, clinical sensitivity and specificity were 81% (25 of 31 positive BAL results) and 100%, respectively (Table 3). PCR results were positive for all 14 BAL samples with positive *A. fumigatus* culture and for 21 of 27 (78%) with positive galactomannan index. All six BAL samples with a negative PCR result had a negative culture result and a positive galactomannan index. Median galactomannan index was >5 (range, 0 to > 5) in the positive PCR group and 0.79 (range, 0.59 to > 5) in the negative PCR group. For patients with a negative PCR, no *Aspergillus* was found in other concomitant respiratory samples, such as sputum or tracheal aspiration. No inhibitory effects were detected.

For the MycoGENIE *A. fumigatus*, the PCR result was positive for 22 of 31 BAL specimens in the IPA probable group, giving a sensitivity of 71% (Table 3). All 22 BAL specimens were also PCR positive with the *A. fumigatus* Bio-Evolution kit. Specificity was 100%. The PCR result was positive for 12 of 14 (86%) of the BAL samples, with positive *A. fumigatus* culture results, with the two samples with negative PCR results corresponding to BALs with low fungal load (1 and 6 colonies). The PCR result was positive for 18 of 27 (67%) of the BALs with a positive galactomannan index. Six samples with negative PCR results had a negative culture result and a positive galactomannan index. Median galactomannan index was >5 (range, 0 to > 5) in the positive PCR group and 1.12 (range, 0.59 to > 5) in the negative PCR group. No inhibitory effects were detected. No azole resistances were identified consistently with the results of the MICs previously determined by Etest. These two reagents showed comparable performance ($P = 0.24$, McNemar test) for *A. fumigatus* DNA detection.

Previous antifungal treatment did not appear to be a cause of false-negative result because the percentage of positive PCRs was comparable between patients with and without antifungal treatment (*A. fumigatus* Bio-Evolution 80% and 81% in treated and nontreated patients, respectively; $P > 0.99$; MycoGENIE *A. fumigatus* 80% and 63% in treated and nontreated patients, respectively; $P = 0.44$, nonparametric binomial test).

Possible IPA

Underlying conditions were hematologic malignant tumors [8/11 (73%)], allogenic bone marrow transplant [1/11 (9%)], and other conditions [2/11 (18%)]. Neutropenia was noted for eight patients (73%). Six patients (55%) were undergoing anti-*Aspergillus* antifungal treatment before BAL sampling, with a mean duration of 8 days (range, 1 to 16 days) before the BAL. According to the EORTC/MSG 2008 criteria for possible IPA, every patient of this group presented one of the following clinical criteria: a dense, well-circumscribed nodule with or without halo sign or an air-crescent sign, a negative galactomannan index, and negative respiratory samples cultures. No positive PCR result was observed on BAL samples from this group.

Non-*Aspergillus* Pulmonary Episodes

Underlying conditions were solid organ transplant [2/31 (71%)], hematologic malignant tumors [6/31 (19%)], allogenic bone marrow transplant [2/31 (6%)], and solid tumor [1/31 (3%)]. Neutropenia was noted only for 3 patients (10%). Eight patients (26%) were receiving antifungal treatment at the moment of the BAL, with a mean duration of 17 days (range, 2 to 38 days) before BAL, mainly for posttransplant prophylaxis or curative treatment of another fungal infection.

The final diagnosis was a documented pulmonary infection attributable to another pathogen for 14 patients (three bacterial infections, two viral infections, five *P. jirovecii* pneumonias, one mucormycosis, one cryptococcosis, one pulmonary and disseminated toxoplasmosis, and one

Table 1 Epidemiologic, Clinical Characteristics, and Treatment of Patients with Probable Invasive Pulmonary Aspergillosis

BAL specimen no.	Age, years/sex	Underlying conditions	Pulmonary nodule(s)	Neutropenia	Antifungal treatment before BAL (duration)
1	56/M	Kidney and pancreas transplant (2001)	Yes (halo)	No	Voriconazole (1 day)
2	65/F	NHL, hypogammaglobulinemia, CMV disease, ICU	Yes	Yes	Voriconazole (26 days)
3	55/M	Lung transplant (30 days before)	Yes	No	Micafungin and liposomal amphotericin B nebulization (30 days)
4	71/F	NHL, end-stage postviral liver disease, ICU	Yes	Yes	Liposomal amphotericin B (37 days)
5	66/M	AML	Yes (halo)	Yes	Liposomal amphotericin B (1 day)
6	56/F	AML, AlloHSCT (10 days before)	Yes	Yes	None
7	64/M	COPD, Influenza A (H1N1) pneumonia, steroids, ICU	Yes	No	None
8	34/M	AML	Yes (halo)	Yes	Voriconazole (1 day)
9	84/M	NHL, steroids	Yes (halo)	Yes	None
10	86/M	AML	Yes (halo)	Yes	None
11	54/M	Myasthenia, steroids, azathioprine	Yes	No	Caspofungin (2 days)
12	60/F	Ovarian cancer, bronchial stenosis	Yes	No	None
13	57/F	AML	Yes (halo)	Yes	None
14	50/F	Kidney transplant (2013)	Yes	No	Voriconazole (2 days)
15	75/F	AML	Yes	No	None
16	50/M	ALL, steroids	Yes halo	Yes	None
17	86/M	NHL, cryoglobulinemia, steroids	Yes	No	None
18[1]	86/M	NHL, cryoglobulinemia, steroids	Yes	No	Voriconazole (9 days)
19	62/M	Allogenic HSCT (2012), influenza A (H1N1) pneumonia, ICU	Yes	No	Voriconazole (10 days)
20	32/M	Kidney transplant (2013)	Yes	Yes	None
21	47/M	End-stage postviral liver disease, ICU	Yes	No	None
22[2]	47/M	End-stage postviral liver disease, ICU	Yes	No	Liposomal amphotericin B (14 days)
23	62/F	AML, allogenic HSCT (2013)	Yes	Yes	Voriconazole (12 days)
24	58/M	Lung transplant (2012)	Yes	No	Voriconazole (4 days)
25	67/M	AML	Yes (halo)	Yes	None
26	60/M	AML	Yes	Yes	Voriconazole (1 day)
27	69/M	MDS, COPD, steroids, ICU	Yes	No	None
28	72/M	COPD, inhaled steroids, ICU	Yes	No	None
29	55/M	HIV positive, NHL, allogenic HSCT (2014), GVHD	Yes	Yes	Voriconazole (32 days) then caspofungin (2 days)
30	67/M	AML	Yes	Yes	None
31	76/M	Congestive heart failure, ECMO, ICU	Yes	No	None

F, female; M, male; AML, acute myeloid leukemia; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; GVHD, graft-*versus*-host disease; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma.

P. jirovecii and cytomegalovirus coinfection), noninfectious pulmonary diseases for two patients, and routine control BALs in 15 lung transplant patients.

No positive result was observed with either of the two PCR kits, despite the presence of various other pathogens in this group. The specificity was 100% for both kits.

Discussion

Our study evaluated two commercially available real-time PCR kits for *A. fumigatus* DNA detection in retrospective BAL samples from patients with probable IPA, patients with possible IPA, and patients without aspergillosis. Our study population displayed the risk factors usually found in individuals with probable or possible IPA, such as hematologic malignant tumors, neutropenia, solid organ transplant or allogenic bone marrow transplant, immunosuppressive therapy, or terminal organ failure.[25–27]

The two evaluated kits showed excellent specificities (100% for both kits), with no cross-reactivity with the other pathogens tested, and a good clinical sensitivity for probable IPA, with no significant differences between the two kits

Table 2 *Aspergillus fumigatus* Bioevolution and MycoGENIE *A. fumigatus* Ademtech (CT) RT-PCR Assay, Culture, and Serologic Characteristics for 31 BAL Samples of Patients with Probable Invasive Pulmonary Aspergillosis

BAL								
Sample no.	Real-time *A. fumigatus* PCR kit (Ct)	MycoGENIE (Ct)	Culture	Direct examination.	Galactomannan index	Serum galactomannan result	Other *Aspergillus*-positive culture	Other pathogens in the BAL sample
1	Positive (28)	Positive (26)	*A. fumigatus* >40 colonies	Hyphae	>5	Positive	Tracheal aspiration	None
2	Positive (35)	Positive (33)	Negative	Negative	0.99	Negative	Tracheal aspiration	CMV, *Coronavirus*
3	Positive (35)	Positive (34)	*A. fumigatus* 10–20 colonies	Negative	3.47	Positive	Bronchial lavage	None
4	Positive (22)	Positive (21)	*A. fumigatus* >40 colonies	Hyphae	>5	Positive	Tracheal aspiration	None
5	Negative	Negative	*Candida albicans* 1 colony	Negative	>5	Negative	None	None
6	Negative	Negative	Negative	Negative	1.59	Negative	None	Enterovirus
7	Positive (35)	Positive (34)	*A. fumigatus* 5 colonies	Hyphae	0.07	Negative	None	Influenza A (H1N1)
8	Positive (34)	Positive (31)	Negative	Negative	>5	Positive	None	None
9	Negative	Negative	Negative	Negative	0.79	Negative	None	*Pseudomonas aeruginosa*
10	Positive (35)	Negative	*C. albicans* >40 colonies	Negative	1.12	Negative	None	*Staphylococcus epidermidis*, *Pneumocystis*
11	Positive (35)	Positive (31)	*C. albicans* 10–20 colonies	Negative	0.24	Negative	Tracheal aspiration	CMV, *Pneumocystis*
12	Positive (35)	Positive (39)	*C. albicans* >40 colonies	Negative	0.74	Negative	None	*Pneumocystis*
13	Negative	Negative	*Penicillium* species 1 colony	Negative	0.61	Negative	None	*Stenotrophomonas maltophilia*
14	Negative	Negative	Negative	Negative	1.40	Positive	None	None
15	Positive (35)	Negative	*A. fumigatus* 1 colony	Negative	0.76	Negative	None	*Enterococcus faecium*, *Pneumocystis*
16	Positive (35)	Positive (34)	Negative	Negative	>5	Positive	None	CMV
17	Positive (33)	Positive (31)	*A. fumigatus* 1 col., *A. niger* 3 col.	Negative	>5	Positive	Subsequent BAL	*Pneumocystis*
18	Positive (28)	Positive (26)	*A. fumigatus* 6 colonies, *A. niger* 1 colony, *Trichosporon asahii* >40 colonies	Hyphae	>5	Positive	None	*Pneumocystis*, *Influenza*
19	Positive (30)	Positive (28)	*A. fumigatus* 20–40 colonies	Hyphae	>5	Negative	None	Influenza A (H1N1), *Pseudomonas aeruginosa*
20	Positive (35)	Negative	*A. fumigatus* 6 colonies	Negative	>5	Negative	Tracheal aspiration	*Staphylococcus aureus*
21	Positive (25)	Positive (23)	*A. fumigatus* 20–40 colonies	Hyphae	>5	Negative	Tracheal aspiration	None
22	Positive (35)	Positive (33)	Negative	Hyphae	0.18	Negative	None	*Enterococcus faecium*
23	Negative	Negative	Negative	Hyphae	0.59	Negative	None	*Enterococcus faecium*, *Coronavirus*, *Pneumocystis*
24	Positive (34)	Positive (31)	*A. fumigatus* 1 colony	Hyphae	0.48	Negative	Bronchial aspiration	None
25	Positive (35)	Positive (33)	Negative	Negative	>5	Positive	None	*Enterococcus faecium*
26	Positive (35)	Positive (32)	Negative	Negative	>5	Positive	Stool	None
27	Positive (32)	Positive (30)	*A. fumigatus* 1 colony	Negative	2.75	Negative	Prior BAL	*Parainfluenza* virus
28	Positive (28)	Positive (26)	*A. fumigatus* >40 colonies	Negative	>5	Positive	None	*Pseudomonas aeruginosa*
29	Positive (35)	Positive (33)	Negative	Negative	2.11	Positive	Sputum	*Stenotrophomonas maltophilia*, *Parainfluenza* virus
30	Positive (35)	Positive (32)	Negative	Negative	>5	Positive	None	None
31	Positive (31)	Positive (28)	*A. fumigatus* 5 colonies	Negative	0.61	Negative	Tracheal aspiration	None

BAL, bronchoalveolar lavage; CMV, cytomegalovirus.

(81% for *A. fumigatus* Bio-Evolution versus 71% for MycoGENIE, $P = 0.24$). Three BAL results were discordant between the two PCR kits. All three BAL samples had a positive galactomannan index (1.12, 0.76, and >5); two of them had positive *A. fumigatus* culture results (one and six colonies). None of the patients with discordant BAL results had received previous antifungal therapy. The two samples with a positive culture were not detected by the MycoGENIE kit despite using a larger extraction volume and displaying a lower LOD. This LOD was determined by the manufacturer with the MycoGENIE *A. fumigatus* DNA extraction reagent. Use of a manual Qiagen extraction could explain the decreased analytical sensitivity and require a reevaluation of the LOD.

Table 3 RT-PCRs, Culture, and Galactomannan Assay Results in Patients Diagnosed with Probable Invasive Pulmonary Aspergillosis

	A. fumigatus real-time PCR kit, *n* (%)		MycoGENIE, *n* (%)	
Test result	Positive	Negative	Positive	Negative
Culture (*A. fumigatus*)				
Positive ($n = 14$)	14 (100)	0	12 (86)	2 (14)
Negative ($n = 17$)	11 (65)	6 (35)	10 (59)	7 (41)
Galactomannan				
Positive ($n = 27$)	21 (78)	6 (22)	18 (67)	9 (33)
Negative ($n = 4$)	4 (100)	0	4 (100)	0
Total ($n = 31$)	25 (81)	6 (19)	22 (71)	9 (29)

Several studies have tested in-house PCR and other commercial PCR kits on respiratory samples. The clinical sensitivity of in-house PCRs varies among studies (67% to 100%) according to the patient population.[17–19,28,29] Many PCR kits are commercialized for the detection of *Aspergillus* (AsperGenius, MycAssay, *A. fumigatus* Genesig, Fungiplex Renishaw, etc.), but only a few of them have been tested on respiratory samples (BAL, bronchial lavage, or sputum) of patients at risk for aspergillosis. The studies using the AsperGenius kit showed 65.5% to 84.2% sensitivity and 80% to 91.4% specificity with cross-reactivity with *Rhizopus oryzae* and *Penicillium chrysogenum*.[23,30,31] The MycAssay *Aspergillus* kit had various sensitivities (80% to 100%) and specificities (92.9% to 98.6%, cross-reactivity with *A. flavus*).[21,32,33] The MycoGENIE *A. fumigatus* study showed 92.9% sensitivity (versus culture) and 90.1% specificity.[34] Variation in sensitivity may be explained by various PCR targets, including 28S rRNA (AsperGenius, MycoGENIE), 18S rRNA (MycAssay), and ITS1 (*A. fumigatus* Bio-Evolution), as well as by different methods of DNA extraction. Although Wheat et al[2] described equivalent performances for manual and automated DNA extraction processes, a better sensitivity was observed by others with automated extraction.[31–33,35,36] In a multicenter analysis, White et al[37] demonstrated a better PCR sensitivity using larger sample volumes and small elution volumes ($\leq$100 μL). In our study, DNA was previously manually extracted for *P. jirovecii* routine PCR, and the kits were evaluated in our routine conditions. BAL-extracted volume depended on the volume of the initial sample received and varied from 2 to 6 mL. A study showed an equivalent efficiency for pellet and supernatant extraction for *A. fumigatus* detection,[38] whereas others revealed better efficiency extraction using pellet.[31] Here, BALs were concentrated by centrifugation and 200 μL of the pellet were extracted, as recommended by the extraction kit's manufacturer. Elution volume used during the extraction process was 200 μL, according to our routine extraction protocol. An optimized elution volume (50 or 100 μL) may improve detection capacities of the tested PCR.

Previous antifungal therapy may affect PCR sensitivity results, but study results are controversial. Some authors showed no effect of a prior antifungal treatment,[28] whereas others observed a decreased PCR sensitivity.[29] Here, previous antifungal treatment did not significantly alter PCR results and could not explain the negative PCRs in the probable IPA group.

The *A. fumigatus* Bio-Evolution and MycoGENIE *A. fumigatus* kits increase *A. fumigatus* detection in BAL and showed a higher clinical sensitivity than culture, with 13 (42%) and 8 (26%) BALs having a positive PCR and a negative culture result, respectively. A higher sensitivity detection of *Aspergillus* in respiratory samples allowed upgrading from possible IPA to probable IPA in some studies.[18,39] Here, no PCR was positive in the possible IPA group. A very low fungal load could explain the poor detectability of *A. fumigatus* in possible IPA, despite the low limit of detection. A way to enhance detection sensitivity in this group would be to analyze multiple respiratory (other than BAL) or serum samples in these patients.

These two kits only allow identification of *A. fumigatus* sensu lato, unlike other commercial kits that detect *Aspergillus* species or several species from the genus *Aspergillus* (AsperGenius, MycAssay). *A. fumigatus* sensu lato represents 80% of IPA, and its detection is essential for IPA diagnosis; thus, the lack of detection of other *Aspergillus* species could be problematic.[1] No *A. fumigatus* cryptic species were isolated in this study; thus, the detection of *A. fumigatus* cryptic species could not be studied. However, an extensive study to evaluate the detection of cryptic *A. fumigatus* species would be valuable. Nevertheless, identification of *Aspergillus* species at species level in the case of a negative BAL culture results has improved the diagnosis accuracy and could allow the quick adjustment of antifungal therapy, especially when a non-*Aspergillus* infection was suspected.

The MycoGENIE *A. fumigatus* kit also allows detection of the major azole resistance mutation TR34/L98H. An increase of azole *A. fumigatus* resistance in Europe has been recently reported.[40,41] Detection of this azole resistance mutation by PCR is interesting for appropriate treatment, particularly when no strain can be isolated. In our study, no mutations were detected, confirming the MICs previously determined by Etest and consistent with the first MycoGENIE *A. fumigatus* study.[34] Until now, only rare cases of azole-resistant strains have been isolated in Eastern France, allowing us to use the MIC determination to test sensitivity to many azoles or other drugs, such as amphotericin B.

Aspergillus DNA detection in BAL fluids is a promising tool in IPA diagnosis. In our study, the two PCR kits confirmed *A. fumigatus* DNA presence in nine BAL samples with negative culture results and positive galactomannan indexes. Among them, two had BAL galactomannan indexes between 1 and 5, and two had an index between 0.5 and 1. The galactomannan positivity threshold is still controversial in BAL. In our study, the positive galactomannan index threshold used was 0.5, according to the manufacturer's recommendations, but some authors use 0.8 or 1.0.[12,42–44] For galactomannan indexex between 0.5 and

1.0, PCR would be particularly helpful to confirm *Aspergillus* presence. Moreover, PCR would be interesting in patients with both negative culture results and galactomannan indexes in BAL but with a serum galactomannan index $\geq$0.5 to confirm the presence of pulmonary *Aspergillus*.

For *Aspergillus* DNA detection in IPA diagnosis, several types of samples can be used. A meta-analysis about in-house PCR and commercial kits using blood samples (plasma or sera) showed a mean sensitivity of 80.5% and a specificity of 78.5% for IPA diagnosis.[45] Another meta-analysis compared *Aspergillus* PCR on BAL and serum samples and obtained a lower sensitivity for BAL compared with serum (76.9% to 79.9% versus 84% to 88%, respectively) but a higher specificity in BAL (93.7% to 94.5% versus 75% to 76% in serum) in patients with IPA.[44] These two types of samples seem to be complementary for IPA diagnosis. Several studies propose serum DNA detection as a noninvasive screening technique for the population at risk of IPA because of its feasibility and specificity. BAL fluids are more difficult to obtain and thus could be used to confirm *Aspergillus* presence and to enhance the sensitivity of *Aspergillus* detection in respiratory samples.[44] However, *Aspergillus* DNA detection in BAL does not differentiate colonization from an invasive pulmonary infection or from an allergic aspergillosis and, as culture or galactomannan, remains just a detection method of *Aspergillus* presence.

Conclusions

To improve IPA diagnosis, standardized and validated molecular techniques are required. Our study demonstrates excellent specificity and good sensitivity in *A. fumigatus* DNA detection on BAL samples with the *A. fumigatus* Bio-Evolution and MycoGENIE *A. fumigatus* kits. Further study with an increased number of IPA and a greater range of species would be valuable.

Acknowledgment

We thank Dr. Francois Severac for help with statistical questions.

Supplemental Data

Supplementary data related to this article can be found at *https://doi.org/10.1016/j.jmoldx.2017.12.005*.

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