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Evaluation of Commercially Available Real-Time Polymerase Chain Reaction Assays for the Diagnosis of Invasive Aspergillosis in Patients with Haematological Malignancies

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Abstract Early diagnosis of invasive aspergillosis (IA) is a challenge. Non-specific clinical and radiologic findings, as well as difficulties in conventional diagnostic method application, may delay correct diagnosis. Nowadays, nucleic acid-based assays have reduced the need for conventional antigen detection and culture-based methods and provided new opportunities for patient care. Aspergillus PCR is now included in the latest European Cancer Research and Treatment Organization/Mycosis Study Group definition updates. We evaluated the performance of commercial real-time polymerase chain reaction (PCR) MycAssay *Aspergillus* PCR and Artus *Aspergillus* RG PCR assays and compared the results with galactomannan enzyme immunoassay. During 41 febrile neutropenic episodes, 168 serum samples were collected from 32 patients with haematological malignancies. IA diagnosis was established according to the revised guidelines of the European Organization for Research and Treatment of Cancer/Mycoses Study Group. Twenty-one probable episodes were identified. There were no proven IA cases in the study. In 20 episodes, patients did not fulfil the established criteria for the IA diagnosis. Artus *Aspergillus* RG PCR assay had a sensitivity of 47.6% and specificity of 100%, while those of MycAssay *Aspergillus* PCR were 61.9% and 100%, respectively. Two different PCR assays were used in this study. Although there are many studies that evaluated MycAssay Aspergillus PCR, data regarding Artus Aspergillus RG PCR assay are scarce. We found moderate sensitivity and high specificity in the diagnosis of IA in patients with haematological malignancy in both PCR methods. Our results demonstrated that commercial PCR assays can be applied for the early diagnosis and pre-emptive treatment of IA.

Keywords Invasive aspergillosis · Galactomannan · Polymerase chain reaction · Diagnosis · Commercial · Enzyme immunoassay

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Introduction

Invasive aspergillosis (IA) is a rapidly progressing infection with high mortality and morbidity rates, and

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therefore, an early and accurate diagnosis is crucial for the treatment of the immunocompromised patients [1, 2]. According to the European Cancer Research and Treatment Organization/Mycosis Study Group (EORTC/MSG) guidelines, potential IA cases can be classified as proven, probable, or possible, based on the respective degree of diagnostic certainty [3].

Aspergillus spp. culture and histopathologic or direct microscopic examinations of tissue samples obtained using sterile procedures are required in order to diagnose the proven IA case. However, potential complications, e.g. bleeding in patients with thrombocytopenia, make tissue sampling difficult, while microscopic examinations and culture have low sensitivity. The time required to obtain the results delays accurate diagnosis as well [3].

The presence of galactomannan (GM) and β-D-glucan (BDG) antigens represents the mycological criterion necessary for the establishment of probable IA diagnosis. However, BDG is not specific for *Aspergillus* and the clinical experience with this assay is limited [1]. Because of the false positive results in GM enzyme immunoassay (EIA), experts recommend alternative non-culture-based and non-invasive diagnostic tests such as polymerase chain reaction (PCR) [4]. With respect to Aspergillus PCR, the Fungal PCR Initiative (FPCRI) has made significant progress towards setting a standard. The data for Aspergillus PCR testing in adults were strong enough for serum, plasma, whole blood, and BAL fluid. There are not many commercial PCR assays. PCR performance was comparable to GM and BDG detection despite technological variability [5]. Aspergillus PCR can be used to detect invasive aspergillosis before the onset of clinical symptoms. White et al. [6] investigated the performance of the Aspergillus PCR and found that serum PCR was positive 10.8 days before the diagnosis of IA. Moreover, a combination of PCR/GM EIA has been shown to be an effective diagnostic strategy which reduced unnecessary antifungal treatment [7].

In this study, we aimed to evaluate commercial MycAssay *Aspergillus* real-time PCR (Myconostica, Ltd, Manchester, UK) and Artus *Aspergillus* RG PCR (Qiagen, Hilden, Germany) assays for the diagnosis of IA in patients with haematological malignancies and compare the results with those obtained using a GM EIA.

Materials and Methods

Study Design and GM EIA Testing

Adult febrile neutropenic patients with haematological malignancies who were admitted to the Adult Haematology and Haematopoietic Stem Cell Transplantation (HSCT) units of Akdeniz University Hospital, Antalya, Turkey, between January 2015 and January 2016 were included in the study. Febrile neutropenia was defined as one instance of neutrophil count of $< 500/mm^3$ and a fever above 38.5 °C. Serum samples were obtained weekly twice from patients during their febrile neutropenic episodes for the GM antigen analyses performed using EIA. Residual serum samples were stored at − 80 °C for the PCR analyses using the commercial *Aspergillus* PCR assays.

The demographical, clinical, and laboratory data such as the underlying type of haematological malignancy, chemotherapy regime, HSCT, prophylactic regime, empirical therapy, high-resolution computed tomography (HRCT) findings, cultures, and histopathologic examination were recorded. Cases were defined as proven, probable, or possible IA, according to the revised EORTC/MSG criteria [3], and the patients were followed until discharge or death. Other febrile neutropenic patients who received systemic antifungal prophylaxis or empirical therapy but who did not fulfil the established IA criteria were considered to be without IA infection. These patients have been used as controls.

Serum samples were tested using EIA for the detection of the GM antigen and commercial *Aspergillus* PCR assays. GM was detected using the Platelia *Aspergillus* EIA (Bio-Rad, Marnes-la-Coquette, France), according to the manufacturer's instructions. Optical density index value of ≥ 0.5 was used to determine the positivity of the samples.

The study and all experimental procedures were approved by the Ethics Committee of the Akdeniz University Medical Faculty (approval number: 70904504/130; September 18, 2013). Informed consents were obtained from all patients before enrolling them in this study.

DNA Extraction and PCR Assays

DNA was isolated from the serum samples with QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was extracted from a minimum 500 µL of serum sample in a final elution volume of 60 µL. One negative control and a known positive control specimens were used for the monitoring of each extraction process. DNA sample aliquots were stored at − 20 °C until PCR analysis.

Artus *Aspergillus* RG PCR (Qiagen, Hilden, Germany) is a commercial multiplex real-time assay, used for the specific amplification of 110-bp regions of *Aspergillus fumigatus, Aspergillus flavus,* and *Aspergillus terreus* genomes. PCR assay was performed using the RotorGene Q (Qiagen, Hilden, Germany) with 10 µL of purified DNA template in a final reaction volume of 25 µL, using the following cycling conditions: 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s, 65 °C for 30 s, and 72 °C for 20 s. Amplification data were obtained with RotorGeneQ software 2.0 (threshold of 0.05) in green channel for *A. fumigatus*, orange channel for *A. terreus*, crimson channel for *A. flavus*, and in the yellow channel for the internal amplification control. Analytical sensitivity for all targets was ≤ 10 DNA copies/µL.

MycAssay *Aspergillus* PCR (Myconostica, Ltd, Manchester, UK) is a commercially designed assay for the detection of genomic DNA from 15 different *Aspergillus* species (including *A. fumigatus*, *A. flavus*, *A. terreus*, *Aspergillus nidulans,* and *Aspergillus niger*). In this assay, 18S ribosomal RNA gene is targeted. PCR assay was performed using the RotorGene Q (Qiagen, Hilden, Germany) instrument with 10 µL of purified DNA template in a final reaction volume of 25 µL, using the following cycling conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 55 °C for 55 s, and 72 °C for 20 s. Amplification data were obtained with RotorGeneQ software 2.0 (threshold of 0.05) in green channel for *Aspergillus* DNA detection and yellow channel for the internal amplification control. Specimens with the crossing threshold (Ct) values ≤ 39, corresponding to a target sensitivity of ≤ 50 DNA copies/µL, which was approximately equivalent to one *Aspergillus* genome, were considered positive.

Statistical Analysis

The data were analysed using SPSS for Windows, Version 22.0 (SPSS Inc., Chicago, Illinois, USA), and the results were presented as the average values ± standard deviations (SD), number, and percentage. Fisher's exact test and χ^2 test were used for frequency comparison between groups and the determination of two-sided p values. Kappa value was calculated to determine the concordance between tests. p values < 0.05 were considered statistically significant. The performance of the investigated tests for the establishment of IA diagnosis was determined by measuring sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and likelihood ratios of the tests. EORTC revised guideline was used as the reference gold standard [3].

Results

Patient characteristics and the diagnosis of IA

Thirty-two adult patients (18 male and 14 female) with haematological malignancies who were at risk of IA were included in the study. The mean age of patients was 54.47 ± 16.48 (range, 25–81 years). Twenty-five patients had one, five patients had two, and two patients had three febrile neutropenic episodes. Demographical characteristics of patients are given in Table 1.

A total of 41 febrile neutropenic episodes were observed. Twenty-one (51.2%) probable IA episodes were identified according to the revised EORTC/MSG criteria [3]. All probable IA episodes were defined using the HRCT findings and GM analyses. No episodes were defined as proven IA, because no histopathologic samples were found to be positive, nor the fungal cultures demonstrated the presence of *Aspergillus* spp. or other filamentous fungi. Twenty episodes (48.8%) did not fulfil EORTC/MSG criteria for the IA diagnosis. In total, 168 serum samples were obtained from all patients during all febrile neutropenic episodes, while 100 serum samples were collected during probable IA episodes. The characteristics of patients with probable IA episodes and the results obtained by the GM EIA and PCR assays are summarized in Table 2.

Table 1 Demographical characteristics of patients

Demographical characteristics	n = 32 (%)
Age (mean ± SD)	54.47 ± 16.48
Gender	
Male	18 (56.25%)
Female	14 (43.75%)
Hospital unit	
Haematology unit	20 (62.5%)
Intensive Care unit	9 (28.1%)
Adult Haematopoietic Stem Cell Transplantation (HSCT) unit	3 (9.4%)
Total hospitalization (day)	49.75 ± 31.35
Haematological malignancy	
Acute myeloblastic leukaemia (AML)	15(46.9%)
Myelodysplastic syndrome-AML transformed (MDS-AML)	6 (18.8%)
Acute lymphoid leukaemia (ALL)	4 (12.5%)
Graft-versus-host disease (GVHD)	2 (6.25%)
Non-Hodgkin's lymphoma (NHL)	2 (6.25%)
Myelodysplastic syndrome (MDS)	1 (3.1%)
Multiple myeloma (MM)	1 (3.1%)
Chronic lymphoid leukaemia (CLL)	1 (3.1%)
Chemotherapy (CT) regimen	
Induction	17 (53.1%)
Relapse resistance	10 (31.3%)
Allogeneic HSCT regimen	3 (9.4%)
Consolidation	1 (3.1%)
CT has not started (new diagnosis)	1 (3.1%)

Performance of Artus Aspergillus RG PCR and MycAssay Aspergillus PCR

A total of 168 serum samples obtained from 41 febrile neutropenic episodes of 32 patients were screened for the GM antigen, and positive results were obtained in 58 (34.5%) of 168 serum samples and in 30 (73.2%) of 41 episodes, with the optical density indices ranging between 0.5 and 8.2 for GM antigen. The GM antigen was found as negative for 26.9% of the episodes, while one sample was positive in 34.1% of the episodes. The percentage of two or more consecutive positive samples was 31.7%. Positivity in any two sera was detected in 7.3% of the episodes.

Positive results using Artus *Aspergillus* RG PCR were obtained in 18 (10.8%) of 168 serum samples and 10 (24.4%) of 41 episodes (10 probable IA cases). Of these 10 episodes, *A. flavus* was detected in five, *A. fumigatus* in three, and *A. terreus* in two episodes. Twenty-one (12.5%) of 168 serum samples and 13 (31.7%) of 41 episodes (13 probable IA cases) were found to be positive using MycAssay *Aspergillus* PCR assay. Artus *Aspergillus* RG PCR and MycAssay *Aspergillus* PCR tests were found as negative for 75.6% and 68.3% of the episodes; one sample was positive in 12.2% and 19.5% of the episodes and two or more consecutive positives were detected in 12.2% and 12.2%, respectively. Per-episode (classified as proven/probable IA) performances of both PCR tests are presented in Table 3.

Comparison of the Results Obtained in the PCR and GM Assays

Ten probable IA episode samples were shown to be positive for the tested fungi, according to the results obtained with both Artus *Aspergillus* RG PCR and MycAssay *Aspergillus* PCR. The results from these molecular tests did not agree for three probable IA episodes. A very good degree of agreement between

Table 2 Detailed clinical data obtained by analysing patients with probable IA episodes

Episode N	Sex/ age	Haematologic malignancy	HRCT findings	Serum number	GM positive serum number	Consecutive GM positivity	GM index (lowest–highest)	Artus *Aspergillus* RG PCR (positive serum number)	Consecutive Artus *Aspergillus* RG PCR positivity	MycAssay *Aspergillus* PCR (positive serum number)	Consecutive MycAssay *Aspergillus* PCR positivity
1	M/54	MDS	Nodule-halo	7	7	6	1.28–5.64	*A. fumigatus* (4)	3	Positive (4)	3
2	F/67	MDS-AML	Nodule-halo-cavity	14	4	2	0.52–1.74	*A. fumigatus* (2)	1	Positive (2)	1
3	F/76	ALL	Nodule-halo-cavity	2	2	1	0.5–0.61	Negative	0	Negative	0
4	M/70	MM	Nodule-halo	4	2	1	0.69–0.71	Negative	0	Negative	0
5	F/53	AML	Nodule	2	2	1	2.71–3.22	*A. flavus* (2)	1	Positive (2)	1
6	M/53	AML	Nodule-halo	8	2	0	0.69–0.94	Negative	0	Negative	0
7	M/53	AML	Nodule-halo	2	1	0	2.02	*A. flavus* (1)	0	Positive (1)	0
8	M/27	MDS-AML	Nodule-halo	7	3	2	0.55–2.14	Negative	0	Positive (1)	0
9	F/81	AML	Nodule-halo	8	2	1	0.5–0.55	Negative	0	Negative	0
10	F/81	AML	Nodule	1	1	0	7.0	*A.flavus* (1)	0	Positive (1)	0
11	M/63	ALL	Nodule	1	1	0	3.71	*A.fumigatus* (1)	0	Positive (1)	0
12	M/62	MDS-AML	Nodule-halo-cavity	6	4	3	0.51–1.14	Negative	0	Negative	0
13	M/62	MDS-AML	Nodule-halo-cavity	10	2	1	0.5–0.57	Negative	0	Negative	0
14	M/62	MDS-AML	Nodule-halo-cavity	5	4	3	1.04–7.0	*A.terreus* (3)	2	Positive (3)	2
15	F/59	AML	Nodule-halo	8	2	1	0.5–0.61	Negative	0	Negative	0
16	M/27	ALL	Nodule-halo	1	1	0	8.2	*A.terreus* (1)	0	Positive (1)	0
17	M/40	AML	Nodule	4	2	1	1.38–4.91	*A.flavus* (2)	1	Positive (2)	1
18	M/62	NHL	Nodule-halo	2	2	1	0.66–0.67	Negative	0	Negative	0
19	F/33	GVHD	Nodule	5	2	0	0.6–0.8	Negative	0	Positive (1)	0
20	M/66	ALL	Nodule	2	1	0	5.25	Negative	0	Positive (1)	0
21	M/47	AML	Nodule-halo	1	1	0	6.73	*A.flavus* (1)	0	Positive (1)	0

ALL acute lymphoid leukaemia, *AML* acute myeloblastic leukaemia, *CLL* chronic lymphoid leukaemia, *GM* galactomannan, *GVHD* graft-versus-host disease, *HRCT* high-resolution computed tomography, *MDS* myelodysplastic syndrome, *MDS-AML* myelodysplastic syndrome-AML transformed, *MM* multiple myeloma, *NHL* non-Hodgkin's lymphoma

Table 3 Per-episode (probable invasive aspergillosis) performances of the investigated tests

Parameter	Per-episode	
	Artus *Aspergillus* PCR	MycAssay *Aspergillus* PCR
Sensitivity (%) (95% CI)	47.6 (25.7–70.2)	61.9 (38.4–81.8)
Specificity (%) (95% CI)	100 (83.1–100)	100 (83.1–100)
PPV (%) (95% CI)	100	100
NPV (%) (95% CI)	64.5 (54.7–73.2)	71.4 (59.1–81.1)
Accuracy (%) (95% CI)	74 (58–86)	81 (66–92)
Positive likelihood ratio	0.48	0.62
Negative likelihood ratio	0.52	0.38

PPV positive predictive value, *NPV* negative predictive value

Artus *Aspergillus* RG PCR and MycAssay *Aspergillus* PCR ($\kappa = 0.913$; $p < 0.001$) was achieved, with a moderate degree of agreement between the GM EIA and Artus *Aspergillus* RG PCR, GM EIA and MycAssay *Aspergillus* PCR ($\kappa = 0.404$ and 0.463, respectively; $p < 0.001$). GM EIA, Artus *Aspergillus* RG PCR, and MycAssay *Aspergillus* PCR results in different episode groups are shown in Table 4. No samples, shown to be negative according to the GM EIA, were shown to be positive in the PCR assays. The mean Ct value of serum samples that were shown to be positive by Artus *Aspergillus* RG PCR was 33.91 ± 3.58, while this value was 36.07 ± 2.71 when using MycAssay *Aspergillus* PCR.

Factors Affecting the Performance of PCR Assays

All patients were administered with prophylactic antifungal therapy. Posaconazole (68.3%) was the most commonly used antifungal agent, followed by fluconazole (26.8%) and voriconazole (4.9%). Empirical antifungal therapy was administered in 27 (65.9%) episodes. Antifungal agents commonly used as empirical treatment were liposomal amphotericin B and voriconazole. A statistically significant difference was found between the prophylactic ($p = 0.017$) or empirical ($p < 0.001$) use of antifungal agents and PCR test results.

Positive results were obtained in one case (1.9%) using Artus *Aspergillus* RG PCR analyses with the volume of 500 µL and 17 (14.8%) samples with volumes higher than 500 µL, and the difference was shown to be statistically significant ($p = 0.014$). When using MycAssay *Aspergillus* PCR, positive results were obtained from two serum samples (3.8%) with a volume of 500 µL and 19 (16.5%) samples with more than 500 µL, which was also demonstrated to be significantly different ($p = 0.020$).

For the serum GM EIA, 139 of 168 serum samples were shown to have GM index < 1. Among these, using Artus *Aspergillus* RG PCR, we did not detect any positive samples. Of 29 samples with GM index > 1, 18 (62.1%) samples were shown to be positive when using the same assay. High GM index level (> 1) was more often identified as positive when using PCR, and the difference was statistically significant ($p < 0.001$). One out of the 139 samples with GM index < 1 was shown to be positive when using MycAssay *Aspergillus* PCR, while 20 (69%) out of 29 samples with GM index > 1 were positive, as shown with the same assay. The difference was shown to be statistically significant as well ($p < 0.001$). Samples

Table 4 Galactomannan EIA, Artus *Aspergillus* RG PCR, and MycAssay *Aspergillus* PCR results in different episode groups

Test results all episodes ($n = 41$)	Probable IA		No IA	
	Positive	Negative	Positive	Negative
Galactomannan EIA	21	0	9	11
Artus RG PCR	10	11	0	20
MycAssay PCR	13	8	0	20

IA invasive aspergillosis

with high GM index level (> 1) were more often identified as positive when using PCR, compared with the samples with low GM indices.

Discussion

The establishment of IA diagnosis remains a challenge, and therefore, different PCR techniques are emerging as new diagnostic tools. Clinicians should be aware of the methods and performance characteristics of the tests used. According to both American and European Clinical Guidelines, the results of PCR assays should be evaluated with other diagnostic tests [8, 9]. On the other hand, Aspergillus PCR which has been evaluated for more than 25 years is now included in the latest EORTC/MSG definition updates [10]. The European Society for Clinical Microbiology and Infectious Diseases, the European Confederation of Medical Mycology, and the European Respiratory Society (ESCMID-ECMM-ERS) recommended a prospective screening of haematological patients carrying a high risk of the development of IA by combining GM EIA and PCR assays, to improve the diagnostic accuracy and accelerate the diagnosis.

In this study, we aimed to evaluate two commercial PCR assays and compare the results with those obtained with the GM EIA for the diagnosis of IA in patients with haematological malignancies. We determined the sensitivity and specificity of MycAssay *Aspergillus* PCR as 61.9% and 100% and Artus *Aspergillus* PCR as 47.6% and 100%, respectively. The performance of MycAssay *Aspergillus* PCR in our study was similar to the previous reports, in which sensitivity was shown to range from 43.8 to 70% and specificity from 57.8 to 97.6% [11–14].

Furthermore, we observed a very good agreement between the results obtained with MycAssay *Aspergillus* PCR and Artus *Aspergillus* RG PCR. Positive results using Artus *Aspergillus* RG PCR were obtained in 10 (24.4%) of 41 episodes (10 probable IA cases). Of these 10 episodes, *A. flavus* was detected in five, *A. fumigatus* in three, and *A. terreus* in two episodes. Thirteen (31.7%) of 41 episodes (13 probable IA cases) were found to be positive using MycAssay *Aspergillus* PCR assay. While Artus *Aspergillus* RG PCR assay detects only three *Aspergillus* species, MycAssay *Aspergillus* PCR can detect 15 species, which may explain the higher number of positive samples detected by MycAssay *Aspergillus* PCR.

In the present study, nine episodes were positive and eleven episodes were negative for the GM in the group without IA. None were positive by the PCR assays. The GM indices for these nine episodes were between 0.5 and 0.92, and empirical piperacillin–tazobactam treatment was provided to eight episodes, while one episode was shown to be during graft-versus-host disease (GVHD). The positive results obtained by GM EIA in this group may be false positives due to a variety of factors, including the cross-reactivity with other kinds of moulds, antibacterial treatment with piperacillin–tazobactam or amoxicillin–clavulanate for 100 days after HSCT, and gastrointestinal mucositis due to GVHD [15, 16].

We determined that the performance of PCR can be affected by antifungal therapy, sample volume, and the GM index level, while the underlying disease, chemotherapeutic regimen, duration of neutropenia, and the administration of T cell suppressive therapy did not affect the performance of these assays [15]. Simultaneous use of antifungal agents may reduce PCR sensitivity and affect the results [16, 17]. In our study, prophylactic antifungal therapy was used in all episodes, and empirical antifungal therapy was administered in 27 (65.9%) episodes. Although antifungal therapy does not affect DNA extraction, it can reduce the amount of available target in the samples. The European Aspergillus PCR Initiative (EAPCRI) group analysed 29 different PCR protocols for the detection of fungal infection in serum samples. They showed that high sample volumes and DNA elution volume under 100 μL increased sensitivity [18]. In our study, we confirmed that a high volume of sample increased the performance of the PCR methods. Previously, no significant difference in the proportion of positive PCR results was found between different GM index groups [12]. However, we showed that samples with high GM index level (> 1) were more often identified as positive when using PCR, compared to the samples with low GM indices.

Aspergillus DNA is not constantly present in blood during an IA episode, and therefore, sequential sampling is crucial for establishing the diagnosis. Cuenca-Estrella et al. [19] showed the importance of serial detection of *Aspergillus* DNA with a large sample number from each patient for early diagnosis. White et al. [20] reported that the sensitivity and

specificity of a PCR assay for IA diagnosis were 80% and 77.8%, respectively. The use of consecutive positive samples increased the specificity to 100%, while reducing the sensitivity to 50%. Mengoli et al. [17] demonstrated that single PCR analysis showed a sensitivity and specificity of 88% and 75%, respectively, while the use of consecutive samples led to 75% sensitivity and 87% specificity in the detection of proven/probable IA. One negative PCR result was shown to be sufficient for the exclusion of possible IA diagnosis, while obtaining consecutive positive PCR results was required for the confirmation of the diagnosis.

In the present study, the percentages of GM EIA and PCR positivity for two or more consecutive serum samples were 31.7% and 12.2%, respectively, during the IA episodes. Sonmez et al. [21] reported 5.5% and 1% positivity with GM EIA and *Aspergillus fumigatus* PCR test for two or more consecutive serum samples, respectively. We think that differences between patient populations may be the main cause of this discrepancy.

As with the GM EIA, the PCR method can be studied in non-serum samples. In the study of Zhang et al. [22], the performance of PCR and GM assays was similar in serum samples, but in BAL samples, the specificity of PCR was found to be higher than GM. Furthermore, Zarrinfar et al. [23] showed that there may be performance differences between PCR methods in clinical samples.

There is no proven case in our study, and the patients were not regularly sampled. Large number of samples was not collected from every patient due to their exitus or patient referral to another centre. Another limitation of our study is the lack of PCR analyses performed in duplicate, as recommended by EAPCRI.

In conclusion, both investigated PCR assays have moderate sensitivity and high specificity in the establishment of IA diagnosis in patients with haematological malignancies. Yet, it looks fairly good on per-episode basis particularly, when the borderline GM EIA results are excluded and concordance between the assays seems high. These assays can be used as adjunct diagnostic tools for the early diagnosis and pre-emptive treatments of adult patients with haematological malignancies.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest.

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