Diagnostic value of DNA and (1→3)- β -D-glucan detection in serum and bronchoalveolar lavage of mice experimentally infected with *Fusarium oxysporum*

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A sensitive and highly specific nested PCR (nPCR) protocol was developed for the specific detection of *Fusarium oxysporum* DNA in clinical specimens. The diagnostic value of *F. oxysporum*-specific DNA and (1→3)- β -D-glucan (BDG) detection was subsequently evaluated in serum and bronchoalveolar lavage (BAL) specimens of mice infected intravenously with *F. oxysporum* conidia. Mice were sacrificed in groups of six daily up to day 8 and then on days 11 and 14. The *F. oxysporum*-specific DNA and BDG in serum and BAL specimens were detected using nPCR and a Fungitell kit, respectively. Cultures of lung homogenate of all of the infected animals yielded *F. oxysporum* and the fungus was also observed in KOH/Calcofluor mounts of 67 % of the tissues. The BDG (cut-off value 80 pg ml⁻¹) and nPCR sensitivity in BAL and serum specimens was 15 and 98 %, and 92 and 75 %, respectively. Combined detection of *F. oxysporum* DNA and BDG in serum enhanced the sensitivity to 98 %. However, the kinetics of the two markers were slightly different. Whilst BDG positivity in serum remained high throughout the infection period, nPCR positivity declined slowly. The data obtained in this study suggest that combined detection of BDG and DNA in serum offers a sensitive and specific diagnostic approach for invasive *Fusarium* infection.

Received 21 March 2007

Accepted 7 September 2007

INTRODUCTION

Infections due to *Fusarium* species are emerging hyalohyphomycoses of immunocompromised patients and are associated with high mortality (Nucci & Anaissie, 2002, 2006; Torres *et al.*, 2003; Dignani & Anaissie, 2004; Walsh *et al.*, 2004). The dismal prognosis of *Fusarium* infection is the result of limited therapeutic options. Fluconazole, itraconazole and caspofungin exhibit little *in vitro* activity against *Fusarium* species (Reuben *et al.*, 1989; Arikan *et al.*, 2001; Dignani *et al.*, 2003). Recent reports suggest that *Fusarium* species not only affect bone marrow transplant recipients and patients with haematological malignancies (Boutati & Anaissie, 1997; Nucci *et al.*, 2004), but also solid-organ transplant patients (Nucci, 2003; Lodato *et al.*, 2006). In some centres, *Fusarium* species are the second most common cause of mould infection after *Aspergillus* species (Vartivarian *et al.*, 1993; Krcmery *et al.*, 1997). The *Fusarium* species most frequently implicated in human infections include *Fusarium solani*, *Fusarium oxysporum* and *Fusarium moniliforme*. As infection with *Fusarium* species may mimic aspergillosis, including similarities in

tissue morphology, patients are usually treated with amphotericin B, an agent that has limited activity against *Fusarium* infection (Anaissie *et al.*, 1992; Boutati & Anaissie, 1997).

Although blood culture sensitivity in disseminated *Fusarium* infections is relatively higher than in aspergillosis, it is still only about 50 % or less (Boutati & Anaissie, 1997; Nucci *et al.*, 2004). Due to lack of diagnostic facilities and clinical awareness, little information is available on the occurrence of disseminated *Fusarium* infections in the Middle East. In a solitary retrospective study from Israel (Nir-Paz *et al.*, 2004), 89 patients with *Fusarium* infection were identified; seven had disseminated disease, 34 had localized invasive disease and 48 had superficial infection. However, the fungus was isolated from the blood of only two (10 %) of the 21 immunocompromised patients. The predominant infecting species identified among the isolates was *F. oxysporum*, followed by *F. solani*.

Early and specific diagnosis of fusariosis is of paramount importance for improved prognosis (Fleming *et al.*, 2002). As culture-based methods have low sensitivity and biopsy specimens from critically ill patients are difficult to obtain, attention has been focused on developing non-culture-based diagnostic approaches. PCR-based molecular

Abbreviations: BAL, bronchoalveolar lavage; BDG, (1→3)- β -D-glucan; nPCR, nested PCR; NPV, negative predictive value; PPV, positive predictive value.

methods have been employed only recently for specific identification of *Fusarium* infection in tissue specimens obtained from ocular, nail and cutaneous infections (Jaeger *et al.*, 2000; Ninet *et al.*, 2005; Lau *et al.*, 2007). In this study, we evaluated the diagnostic usefulness of (1→3)- β -D-glucan (BDG), a non-specific fungal marker, and DNA detection (as a species-specific marker) in serum and bronchoalveolar lavage (BAL) specimens of mice infected intravenously with *F. oxysporum* conidia and followed up for 14 days post-infection (p.i.).

METHODS

Animal experimentation. *F. oxysporum* (CBS 109898) was cultured on Sabouraud dextrose agar plates for 72 h at 30 °C. Conidia were harvested using 0.02 % Tween 80 in 0.9 % NaCl. After washing twice in 0.9 % NaCl by centrifugation, the conidia were suspended in 0.9 % NaCl and counted in a haemocytometer. The haemocytometer count was confirmed by culturing serial tenfold dilutions of the inoculum on Sabouraud dextrose agar and determining the number of c.f.u. Based on experimental studies using *Fusarium* species (Mayayo *et al.*, 1999; Sugiura *et al.*, 2003) and taking into account the lower virulence of *F. oxysporum*, an arbitrary dose of 1×10^7 conidia was used to infect each mouse intravenously. Seventy-two specific-pathogen-free female BALB/c mice (8–12 weeks old, 15–20 g), bred and maintained in the Central Animal House Facility of the Faculty of Medicine, were used. They were housed in groups of six and given food pellets and water *ad libitum*. Twelve healthy mice selected at random were sacrificed, and serum and BAL specimens were collected for use as negative controls for BDG and nested PCR (nPCR) assays. In addition, blood was collected from each animal by cardiac puncture at the time of sacrifice. Each animal was immunosuppressed with four intraperitoneal injections of cyclophosphamide (200 mg kg⁻¹) given 4 days and 1 day before inoculation, on the day of inoculation and 3 days after inoculation. The animals were sacrificed in groups of six every day up to day 8 and then on days 11 and 14 p.i. BAL was collected by exposing the trachea and lavaging the lung four times with 1 ml PBS. The BAL was centrifuged at 10 000 g for 10 min. The supernatant was used for BDG estimations, whilst the sediment was used for DNA extraction (200 μ l).

Culture and microscopic examination. Approximately 5 mm³ portions of lung, liver, spleen, kidney and heart were cultured on Sabouraud dextrose agar (Oxoid) supplemented with chloramphenicol (50 mg l⁻¹). In addition, 100 μ l BAL sample before centrifugation and blood were also cultured. Lung tissue from all of the animals was examined by 10 % potassium hydroxide/0.1 % (w/v) Calcofluor mounts for the presence of fungal elements.

Detection of BDG. BDG was detected with a Fungitell kit (Associates of Cape Cod) following the method recommended by the manufacturer. Briefly, serum or BAL samples (5 μ l) were pretreated for 10 min at 37 °C with 20 μ l 0.6 M KCl/0.125 M KOH and assayed with the Fungitell reagent in a kinetic, chromogenic format for 25–40 min at 37 °C. The A₄₀₅ was read and the concentration of BDG in each sample was calculated from a calibration curve with standard solutions of 6.25–100 pg ml⁻¹.

nPCR. The reference strains of *F. oxysporum* (CBS 109898), *F. solani* (ATCC 36031), *Aspergillus terreus* (CBS 106.25), *Aspergillus fumigatus* (CBS 113.26), *Aspergillus flavus* (CBS 113.32), *Candida albicans* (ATCC 76615), *Candida parapsilosis* (ATCC 22019), *Candida dubliniensis* (CD36), *Candida glabrata* (CBS 138), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (CBS 7779), *Trichosporon*

asahii (CBS 2479), *Trichosporon mucoides* (CBS 7625), *Trichosporon inkin* (CBS 5585), *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and *Escherichia coli* BL-21 (Novagen) were used to extract genomic DNA for standardization of the nPCR methodology. Extraction of DNA from yeast and bacteria was performed as described previously (Ahmad *et al.*, 2002). DNA from the filamentous fungi was also isolated as described previously (Ahmad *et al.*, 2005) with the following modifications. The conidia of the reference *Fusarium* and *Aspergillus* species were inoculated in 6 cm Petri dishes containing 1.5 ml glucose/yeast extract/peptone broth and grown at 30 °C for 72 h. The mycelial mat was removed using a plastic pipette tip and left to dry on Whatman paper for 10 min. The mycelial mat was transferred into 50 ml polypropylene screw-cap tubes containing six glass beads (4 mm diameter). The tubes were immersed in liquid nitrogen for 10 s, thawed and vortexed vigorously for 30 s. A 0.8 ml portion of DNA extraction buffer containing 0.2 M Tris/HCl (pH 7.6), 10 mM EDTA, 0.5 M NaCl and 1 % SDS and 0.8 ml of phenol:chloroform:isoamyl alcohol (25:24:1) was added. The contents were mixed and an aliquot of 0.7 ml was transferred to a 1.5 ml microcentrifuge tube and centrifuged at 14 000 g for 15 min at 4 °C. The supernatant was transferred to a fresh tube and extracted with an equal volume of phenol:chloroform:isoamyl alcohol. The aqueous phase was extracted once with chloroform:isoamyl alcohol. The DNA in the supernatant was precipitated with 0.6 vols 2-propanol and centrifuged at 14 000 g for 15 min at 4 °C. The pelleted DNA was washed with 70 % ethanol, allowed to dry at room temperature and dissolved in 25 μ l distilled water. For the experiments measuring *in vitro* sensitivity, the concentration of DNA was measured by recording the A₂₆₀ and A₂₈₀, and an appropriate amount, after dilution of the stock solution if required, was used in PCR protocols.

DNA extraction from serum and BAL samples. DNA from serum and BAL specimens was extracted as described previously (Ahmad *et al.*, 2004) with the following modifications. Briefly, 200 μ l serum or BAL was mixed with 500 μ l 6 M guanidine isothiocyanate and 500 μ l water-saturated phenol (pH 8.0). The mixture was boiled for 5 min, immersed in liquid nitrogen for 1 min and allowed to thaw at room temperature. The process was repeated three times and then 250 μ l chloroform:isoamyl alcohol (24:1) was added and the sample was mixed and centrifuged at 14 000 g for 10 min. The supernatant (450 μ l) was transferred to a fresh tube and the DNA was precipitated by the addition of 0.7 ml 2-propanol. After mixing well, the tube was kept at –70 °C for 30 min and then centrifuged at 14 000 g for 15 min at 4 °C. The pelleted DNA was washed twice with 0.5 ml 70 % ethanol, dried and resuspended in 25 μ l 10 mM Tris/HCl (pH 8.0).

Primer sequences and PCR assay. The primers for the first round (FOXYF1, 5'-ACTTGTTGCCTCGGCGGATCAG-3'; and FOXYR1, 5'-ACGATTACCAGTAACGACGGTTTA-3') and nested (FOXYF2, 5'-TCAGCCCGCTCCCGGTAAAA-3'; and FOXYR2, 5'-ACGCTATGGAAGCTCGACGTGA-3') PCRs were derived from the internally transcribed spacer (ITS)-1 and ITS-2 region sequences of the rRNA operon. The specificity of the primers for *F. oxysporum* was suggested by BLAST searches. Amplification of target DNA was carried out in thin-walled 0.2 ml PCR tubes in a total volume of 50 μ l containing 1 $\times$ AmpliTaq PCR buffer I, 1 U AmpliTaq DNA polymerase, 4 pmol each of FOXYF1 and FOXYR1 primer, 2 μ l DNA extracted from culture or other specimens and 0.1 mM each dNTP (Ahmad *et al.*, 2002). After first-round amplification, 1 μ l of the product was further amplified using the nested primers FOXYF2 and FOXYR2. PCRs were carried out in a Perkin-Elmer cyler (GeneAmp PCR System 9700) under the following conditions: denaturation at 95 °C for 1 min, annealing at 65 °C for 30 s and extension at 72 °C for 1 min. An initial denaturation step at 95 °C for 5 min and a final extension step at 72 °C for 10 min were also included (Ahmad *et al.*, 2002). Amplification was performed for 40 cycles for the first-round PCR,

followed by 35 cycles for the nPCR. Amplicons were detected by agarose gel electrophoresis as described previously (Ahmad *et al.*, 2004).

Interpretation of a positive test. A cut-off value of 80 pg ml⁻¹ was taken as positive for the BDG test as described in the instruction sheet provided with the kit. The instructions supplied with the kit were also followed to eliminate possible contamination. Negative and positive samples supplied with the kit were included with each test run. Specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using lung culture positivity as evidence of proven infection and values obtained from sera of control mice as negative controls. All doubtful or equivocal diagnostic values (60–79 pg ml⁻¹) were taken as negative.

RESULTS

PCR amplification using genomic DNA from *F. oxysporum*, *F. solani*, *A. fumigatus*, *A. terreus* and *A. flavus* with primers FOXYF1 and FOXYR1 resulted in specific amplification of a single amplicon of approximately 350 bp only from *F. oxysporum* and not from the four other filamentous fungi (Fig. 1a). Similar results were obtained when primers FOXYF2 and FOXYR2 were used, with an amplicon of nearly 300 bp being obtained only from *F. oxysporum* (data not shown). Furthermore, when 1 µl of the first-round PCR product (Fig. 1a) was reamplified with primers FOXYF2 and FOXYR2 by nPCR, again a single amplicon of nearly 300 bp was obtained only from the amplified DNA obtained from *F. oxysporum* and not from *F. solani*, *A. fumigatus*, *A. terreus* or *A. flavus* (Fig. 1b). No amplicon was obtained by PCR amplification with FOXYF1 and FOXYR1 or FOXYF2 and FOXYR2 with genomic DNA obtained from 11 other species of *Candida*, *Trichosporon*, *Cryptococcus* or bacteria (data not shown). These studies established the specificity of the primers used during the first-round and nested steps of amplification for *F. oxysporum* DNA.

The lower limit of sensitivity of nPCR was determined by serially diluting genomic DNA (4.4 µg–44 fg) prepared from the reference strain of *F. oxysporum*. PCR amplification of the ITS region of the rRNA operon of *F. oxysporum* with primers FOXYF1 and FOXYR1 was positive using 440 pg of genomic DNA (data not shown). When the products of the first-round PCR were reamplified with the nested primers FOXYF2 and FOXYR2, the sensitivity was increased by three orders of magnitude, with the minimum amount of genomic DNA required for a positive signal in PCR being reduced from 440 pg to 440 fg (Fig. 2) corresponding to the approximate genomic DNA content of 11 *F. oxysporum* cells (Leišová *et al.*, 2006). This suggested that the established protocol was highly sensitive and specific for *F. oxysporum*, and that it should be able to detect *F. oxysporum* DNA released from a small number of cells.

The cultures of lung tissue and BAL from all 12 control mice were negative for *F. oxysporum* or any other fungal pathogen. Furthermore, none of the serum or BAL samples from control mice were positive for the detection of BDG (mean value 44.23 ± 8.37 pg ml⁻¹) or *F. oxysporum* DNA

Fig. 1. Agarose gels of first-round PCR-amplified products using FOXYF1 and FOXYR1 primers (a) and nPCR products using primers FOXYF2 and FOXYR2 (b) on genomic DNA obtained from *A. fumigatus* (lane 1), *A. terreus* (lane 2), *A. flavus* (lane 3), *F. solani* (lane 4) and *F. oxysporum* (lane 5). M, 100 bp DNA marker; the positions of the 100 and 600 bp fragments are marked.

by nPCR (data not shown). The results of culture, nPCR and BDG detection in 60 mice that were infected intravenously with 1×10^7 *F. oxysporum* conidia and followed for up to 14 days are presented in Tables 1–3. *F. oxysporum* was isolated from lung, liver, spleen, kidneys and heart of all of the infected mice sacrificed every day up to 8 days and then on days 11 and 14 p.i. KOH/Calcofluor mounts of digested lung tissue were positive for *F. oxysporum* in 40/60 mice (67%) (Table 1). However, none of the BAL or blood specimens of 60 mice were positive for the fungus in culture. Using a cut-off value ≥ 80 pg ml⁻¹, sera from 55 mice (92%) were positive for BDG and 43 (75%) for *F. oxysporum* DNA (Tables 1 and 2). In BAL,

Fig. 2. Analytical sensitivity of detection of amplified DNA by nPCR using tenfold serial dilutions of genomic DNA from *F. oxysporum*. Lanes 1–6, amplified DNA obtained using 4.4 ng, 440 pg, 44 pg, 4.4 pg, 440 fg and 44 fg *F. oxysporum* genomic DNA, respectively; lane 7, negative control. The results are representative of two separate experiments.

only nine mice (15%) were positive for BDG and 59 (98%) were positive for *F. oxysporum* DNA (Tables 1 and 2). Whilst BDG positivity in serum remained high throughout the infection period, the nPCR positivity slowly declined. In contrast to the results obtained with serum samples, both the markers exhibited similar kinetics in BAL specimens (Table 1). When the positive results from BDG and nPCR were combined, the sensitivity increased to 98% in serum specimens (Table 3). Taking culture positivity of visceral organs as evidence of infection, the specificity, PPV and NPV of combined detection in serum and BAL specimens were 100, 100 and 92%, respectively (Table 3).

DISCUSSION

Invasive *Fusarium* infections are recognized with increasing frequency in immunocompromised patients, and an early

and accurate diagnosis of this mould infection is crucial for improved therapeutic outcome. In the present study, we established a nPCR protocol that was highly sensitive and *F. oxysporum*-specific with an analytical sensitivity capable of detecting *F. oxysporum* DNA released from as few as 11 cells. Although two nPCR assays have been developed previously for the detection of *Fusarium* species DNA (Hue *et al.*, 1999; Jaeger *et al.*, 2000), they were not specific for *F. oxysporum* and had reduced sensitivity compared with the assay developed in this study. The pan-fungal PCR assays (Chen *et al.*, 2001; Lau *et al.*, 2007) for the detection of *F. oxysporum* DNA are also not suitable as they involve tedious and technically demanding DNA sequencing of the amplicons and are highly prone to erroneous results caused by environmental fungi, particularly *Aspergillus* species, due to the pan-fungal nature of amplification.

After establishing a sensitive and highly specific nPCR assay for the detection of *F. oxysporum* DNA, we evaluated the diagnostic usefulness of this protocol together with another non-specific fungal marker (BDG) in mice intravenously infected with *F. oxysporum* conidia. We used serum instead of whole blood for the detection of *F. oxysporum* DNA so that a direct comparison could be made with the BDG test. Whilst tissue from lungs and other organs from all of the animals yielded *F. oxysporum* in culture, the BAL and blood specimens remained uniformly negative. Taking culture of *F. oxysporum* from visceral organs as evidence of infection, the relative sensitivities of BDG and nPCR were 92 and 75% in serum samples and 15 and 98% in BAL samples, respectively. Our data showed that significant levels of BDG could be detected in serum following intravenous inoculation of *F. oxysporum* conidia on day 1 and also in the sera of successively sacrificed animals at different time points. Whilst the BDG and nPCR results were generally in agreement up to day 6 p.i., there was a notable decline in nPCR positivity (10/21, 48%) in the sera of animals sacrificed on days 7, 8, 11 and 14 p.i. By contrast, *F. oxysporum* DNA was detected in the BAL of 98% of mice,

Table 1. Comparison of KOH/Calcofluor examination, culture, BDG detection and nPCR in *F. oxysporum*-infected mice

Days p.i. (<i>n</i> =6 mice per day)	Lung		Serum		BAL	
	KOH/Calcofluor	Culture	BDG	nPCR	BDG	nPCR
1	5/6	6/6	5/6	6/6	1/6	6/6
2	6/6	6/6	6/6	6/6	0/6	6/6
3	6/6	6/6	6/6	6/6	1/6	6/6
4	6/6	6/6	6/6	5/6	1/6	6/6
5	5/6	6/6	6/6	6/6	1/6	5/6
6	5/6	6/6	5/6	4/6	1/6	6/6
7	2/6	6/6	6/6	3/6	0/6	6/6
8	2/6	6/6	6/6	3/5*	2/6	6/6
11	2/6	6/6	6/6	3/5*	1/6	6/6
14	1/6	6/6	3/6	1/5*	1/6	6/6
Total (%)	40/60 (67)	60/60 (100)	55/60 (92)	43/57 (75)	9/60 (15)	59/60 (98)

*nPCR was not carried out on one animal due to an insufficient quantity of serum sample.

Table 2. BDG levels in serum and BAL specimens of 60 mice infected with *F. oxysporum* and sacrificed at different time points
BDG cut-off values: <60 pg ml⁻¹, negative; 60–79 pg ml⁻¹, equivocal; ≥80 pg ml⁻¹, positive.

Days p.i. (<i>n</i> =6 mice per day)	BDG in serum				BDG in BAL			
	Mean ± SD (pg ml ⁻¹)*	No. of mice at cut-off value			Mean ± SD (pg ml ⁻¹)†	No. of mice at cut-off value		
		<60	60–79	≥80		<60	60–79	≥80
1	129.54 ± 51.36	0	1	5	60.35 ± 21.53	3	2	1
2	155.17 ± 46.02	0	0	6	49.02 ± 15.31	4	2	0
3	166.46 ± 11.24	0	0	6	71.07 ± 50.44	4	1	1
4	150.54 ± 6.70	0	0	6	62.34 ± 21.24	2	3	1
5	114.69 ± 16.83	0	0	6	55.02 ± 17.13	5	0	1
6	112.15 ± 25.72	0	1	5	61.35 ± 22.81	5	0	1
7	257.59 ± 78.63	0	0	6	47.95 ± 11.53	5	1	0
8	206.29 ± 90.11	0	0	6	67.82 ± 34.31	2	2	2
11	283.04 ± 50.66	0	0	6	49.22 ± 56.87	4	1	1
14	87.15 ± 42.37	3	0	3	34.73 ± 32.17	5	0	1
Total	145.92 ± 83.52	3 (5 %)	2 (3 %)	55 (92 %)	51.03 ± 30.42	39 (65 %)	12 (20 %)	9 (15 %)

*Mean BDG in normal mice serum was 44.23 ± 8.37 pg ml⁻¹.

†Mean BDG in normal mice BAL was 26.73 ± 5.91 pg ml⁻¹.

whereas BDG positivity at a cut-off level of 80 pg ml⁻¹ was much lower (15 %). Doubtful results (60–79 pg ml⁻¹) were obtained in another 20 %. The high sensitivity of nPCR on BAL samples reflects the high rate of establishment of pulmonary infection in this model, as evidenced by the 100 % positive culture rate of lung specimens. The different levels of sensitivity for BDG and nPCR in BAL may be due to the fact that different fractions of BAL were used for the two analyses. The use of PBS for lung washings may have resulted in the dilution of BDG levels in lavage samples,

Table 3. Sensitivity, specificity, PPV and NPV of diagnostic markers in serum and BAL of 60 mice infected with *F. oxysporum*

Diagnostic marker	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cumulative				
Microscopy	67	100	100	38
Serum				
BDG	92	100	100	71
nPCR	75	100	100	46
BAL				
BDG	15	100	100	19
nPCR	98	100	100	92
Combined				
Serum				
BDG + <i>F. oxysporum</i> DNA	98	100	100	92
BAL				
BDG + <i>F. oxysporum</i> DNA	98	100	100	92

thus yielding fewer positive results. Alternatively, the higher sensitivity of nPCR may be attributed to the fact that we used BAL sediment, which might have contained viable/non-viable fungal fragments that were lysed during the DNA extraction procedure. It is also probable that the sensitivity of the nPCR might have been different if DNA had been extracted from whole blood rather than serum. The higher BDG positivity in serum may be related to the angioinvasive characteristic of the fungus, which facilitates its release directly into the bloodstream (Boutati & Anaissie, 1997). The kinetics of BDG and *F. oxysporum* DNA were different in serum than in BAL. Whilst BDG positivity in serum remained high throughout the infection period, nPCR positivity declined slowly. In contrast, BDG and nPCR positivity in BAL remained almost the same throughout the infection period. As our data were based on a single specimen per mouse, these preliminary observations need to be examined and confirmed in more detail in further studies.

A limitation of our study was that we used the intravenous route of infection, which may not accurately mimic human pulmonary infection. Although haematogenous acquisition of lung infection is expected to release fungal elements back into the blood supply, the blood culture remained uniformly negative. Experimental data on the detection of *F. oxysporum* DNA and BDG in BAL or serum specimens of mice or other animals are scarce. In an earlier study of four mice infected with *F. solani*, Hue *et al.* (1999) detected DNA in seven (47 %) of the 15 culture-positive tissue samples and in three (23 %) of 13 culture-negative samples obtained from different organs. None of the blood samples were positive by PCR. The authors attributed the low sensitivity of the PCR assay to the poor

efficiency of DNA extraction from the specimens. The diagnostic value of these markers in cases of pulmonary or disseminated *Fusarium* infections is unknown. Although serum specimens contaminated with *Fusarium* and some other mould species cross-react with the EB-A2 monoclonal antibody used in Pastorex *Aspergillus* and Platelia *Aspergillus* kits (Kappe & Schulze-Berge, 1993; Quindós, 2006), the value of these kits in the diagnosis of invasive *Fusarium* infections has not been determined. Thus galactomannan detection may not be used in parallel with BDG detection to decrease the possibility of false-positive or false-negative results. The clinical application of the *F. oxysporum*-specific nPCR assay developed in this study may become apparent in BDG-positive clinical specimens of unknown aetiology. Whilst a positive BDG test may be helpful in ruling out non-fungal aetiology, it is not a species-specific fungal marker; hence BDG-positive specimens from suspected patients need to be subjected to *F. oxysporum*-specific PCR. It may be pertinent to mention that *F. oxysporum* is one of the most common *Fusarium* species reported in our region (Nir-Paz *et al.*, 2004).

In conclusion, this study demonstrates the usefulness of nPCR and Fungitell assays in the early diagnosis of invasive fusariosis. The combined detection of BDG and *F. oxysporum* DNA in serum specimens enhanced the sensitivity to 98 %. The nPCR established in this study specifically detected *F. oxysporum* DNA in serum and BAL specimens, thus minimizing the chances of misdiagnosis from other invasive mould infections that exhibit similar tissue morphology. Further experimental studies are warranted to determine the kinetics of these markers in serially collected serum samples.

ACKNOWLEDGEMENTS

The study was supported by Kuwait University Research grant MI 04/02. The technical assistance of Rachel Chandy and Dr Asha Prasad is acknowledged.

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