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Fusarium-specific monoclonal antibody

Detection of human pathogenic *Fusarium* species in hospital and communal sink biofilms by using a highly specific monoclonal antibody

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Summary

The fungus *Fusarium* is well known as a plant pathogen, but has recently emerged ~~over recent years~~ as an opportunistic pathogen of humans. Habitats providing direct human exposure to infectious propagules are largely unknown, but there is growing evidence that plumbing systems are sources of human pathogenic strains in the *Fusarium solani* species complex (FSSC) and *Fusarium oxysporum* species complex~~es~~ (FOSC), the most common groups infecting humans. Here, we use a newly developed *Fusarium*-specific monoclonal antibody (mAb ED7) to track FSSC and FOSC strains in sink drain biofilms by detecting its target antigen, an extracellular 200kDa ~~heat-stable~~ carbohydrate, in saline swabs. The ~~diagnostic~~ antigen was detectable in 52% of swab samples collected from sinks across a University campus and a tertiary care hospital. The mAb was 100% accurate in detecting FSSC, FOSC and *F. dimerum* species complex (FDSC) strains that were present, as mixed fungal communities, in 83% of sink drain biofilms. Specificity of the ELISA was

confirmed by sequencing of the internally transcribed spacer 1 (ITS1)-5.8S-ITS2 rRNA-encoding regions of culturable yeasts and moulds that were recovered using mycological culture, while translation elongation factor (TEF)-1α analysis of *Fusarium* isolates included FSSC 1-a, FOSC 33 and FDSC ET-gr, the most common clinical pathotypes in each group.

Originality-Significance Statement

- Development of a monoclonal antibody (mAb ED7) specific to *Fusarium*, a fungal genus containing human and plant pathogens
- *Fusarium* diagnostic antigen detected in swabs of sink drain biofilms, with 100% accuracy of mAb-based ELISA confirmed by ITS sequencing of mixed fungal communities comprising human pathogenic yeasts and moulds
- First report describing the use of a highly specifc mAb to track human pathogenic fusaria, demonstrating widespread occurrence of pathogen in communal and hospital sinks with potential for nosocomial and community acquired infections

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Introduction

Species in the fungal genus *Fusarium* are ubiquitous environmental moulds, and pathogens of both plants and animals (Zhang *et al*., 2006; Thornton and Wills, 2015). In immunocompromised humans, such as patients with haematological malignancies and hematopoietic stem cell and solid organ transplant recipients, *Fusarium* species are significant emerging pathogens, causing a frequently fatal disseminated disease known as fusariosis with an associated mortality rate of 50-75% (Girmenia *et al*., 2000; Musa *et al*., 2000; Boutati and Anaissie, 1997; Dignani and Anaissie, 2004; Jensen *et al*., 2004; Nucci and Anaissie, 2007). In some tertiary cancer centres, *Fusarium* has emerged as the second most common mould pathogen after *Aspergillus* (Walsh and Groll, 1999; Muhammed *et al*., 2011). Regardless of human immune status, *Fusarium* species can cause localised nail infections (onychomycosis)(Arrese *et al*., 1996), bone and joint infections (Koehler et al., 2014), infections of burn wounds (Latenser, 2003), skin infections (Nucci and Anaissie, 2002; Gurusidappa and Mamatha, 2011), and are the most frequent cause of mycotic eye infections known as fungal keratitis (Jurkunas *et al*., 2009), leading to progressive corneal destruction and endophthalmitis, with loss of vision or even loss of the affected eye (Dursun *et al*., 2003; Edelstein *et al*., 2012).

A recent multistate outbreak of fungal keratitis in the USA and in Singapore and Hong Kong was associated with contact lens solution contaminated with multiple strains of *Fusarium* and which led to visual loss in many patients and the need for corneal transplantation (Chang *et al*., 2006). While such outbreaks are rare, disseminated *Fusarium* infections and keratomycoses have increased in frequency over the past decade (Koehler *et al*., 2014) and an increasing body of evidence suggests that the main environmental sources of human pathogenic *Fusarium* species are contaminated water systems (Doggett, 2000; Anaissie *et al*., 2002; Anaissie *et al*., 2011; Mehl and

Epstein, 2008). A number of studies have recovered pathogenic *Fusarium* species from plumbing fixtures and it is hypothesised that microbial biofilms on fixtures may serve as important reservoirs of infectious *Fusarium* propagules in hospitals and homes (Mehl and Epstein, 2008; Short *et al*., 2011).

Identification of environmental reservoirs of human pathogenic ~~mould~~molds including *Fusarium* has typically relied on nucleic acid-based technologies following recovery of fungi using selective media (Anaissie *et al*., 2002; Mehl and Epstein, 2008; Short et al., 2011; Anaissie *et al*., 2011; Rougeron *et al*., 2014). Recently, highly specific monoclonal antibodies (mAb) have been used to identify pathogenic species or species complexes in environmental samples containing mixed populations of yeasts and ~~mould~~molds (Thornton, 2009; Davies and Thornton, 2014; Thornton *et al*., 2015). While mAb-based approaches similarly rely on culture for recovery of fungi from environmental samples, detection of diagnostic antigens in crude culture extracts using genus- or species-specific mAbs offers an attractive alternative approach to pathogen detection, particularly when combined with unsophisticated diagnostic modalities such as lateral-flow technology (Thornton, 2008; Thornton, 2012).

In this study, we set out to determine whether a newly developed *Fusarium*-specific mAb (ED7) could be used to track the fungus by detecting a water-soluble diagnostic antigen in swabs of communal and hospital sinks. By using the mAb in an enzyme-linked immunsorbent assay (ELISA), we show that it can differentiate *Fusarium* species from other unrelated yeast and ~~mould~~mold pathogens of humans present in mixed fungal communities encountered in sink biofilms. The ELISA represents a simple method for specific detection of *Fusarium* species in environmental reservoirs and for identifying plumbing systems contaminated with the fungus.

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Results

Production of hybridoma cell lines, isotyping of mAb and specificity

A single fusion was performed and 389 hybridoma cell lines were screened for specificity against a range of clinically relevant yeasts and molds (Table S1)~~mAb production~~. The aim was to identify cell lines secreting mAbs specific to *Fusarium* that could be used to track the fungus in environmental samples containing mixed species of human pathogenic fungi. To this end, a~~A~~ single cell line, ED7, ~~produced~~ was identified that produced mAb~~s~~ belonging to the immunoglobulin class M (IgM), which was genus-specific, reacting in ELISA tests with antigens from *Fusarium* species and with the *F. solani* teleomorph *Haematonectria haematococca* only (Figs. 1A and 1B). It did not cross-react with antigens from a wide range of unrelated ~~mould~~mold and yeast species (Fig. 1A).

Western blotting of the ED7 antigen and epitope ~~antigen~~ characterization

Gel electrophoresis and western blotting studies showed that ~~mAb~~ ED7 binds to a major ~~extracellular~~ antigen with molecular weight of ~200 kDa which is secreted extracellularly by both *F. solani* and *F. oxysporum* (Fig. 2A). *Fusarium solani* antigens were subjected to enzymatic (Fig. 2B and Fig. 2C), heat (Fig. 2D) and chemical (Fig. 2E) modifications in order to characterise the epitope bound by ED7. Reductions in mAb binding following treatment with pronase shows that its epitope consists of protein, while reductions with trypsin indicate a protein epitope containing positively charged lysine and arginine side chains. The lack of reduction in ED7 binding following digestion of immobilized antigen with trypsin (Fig. 2B) and pronase (Fig. 2C) shows that it does not bind to a protein epitope. Reductions in mAb binding following heat treatment shows that an epitope is heat labile. There was no significant reduction in ED7 binding over 70 min of heating, showing that its epitope is heat stable (Fig. 2D). Reductions in mAb binding following chemical

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digestion of an antigen with periodate shows that its epitope is carbohydrate and contains vicinal hydroxyl groups. The pronounced reductions in ED7 binding following periodate oxidation shows that its epitope consists of carbohydrate residues (Fig. 2E). Taken together, ~~Binding of mAb ED7 to its target antigen was unaffected by pronase (Fig. 2B) or trypsin (Fig. 2C) digestion or by heating (Fig. 2D). t~~These results~~, combined with significant reductions in antibody binding following periodate oxidation (Fig. 2E),~~ indicate that ~~mAb~~ ED7 binds to an extracellular antigen and that its epitope is a ~~-~~heat stable carbohydrate moiety~~epitope~~ containing ~~with~~ vicinal hydroxyl groups.

Immunofluorescence and immunogold electron microscopy

Immuno-localisation studies using IF showed that the ED7 antigen was present on the surface of spores and hyphae (Figs. 2F-~~I and 2G~~), while IEM showed that the antigen was present in the spore and hyphal cell wall and in an extracellular fibrillar matrix surrounding both (Figs. 2~~JH~~-L~~J~~). In the TEM image shown in Fig. 2J, 56% of gold particles were distributed in the fibrillar matrix surrounding the cell, while 40% and 4% of gold particles were distributed in the cell wall and cytoplasm respectively. This shows that the ED7 antigen is predominantly extracellular or located within the cell wall.

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Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the ITS regions of the rRNA-encoding gene unit and by Translation Elongation Factor-1α PCR

Monoclonal antibody ED7 was highly specific for the three human pathogenic species of *Fusarium*, *F. solani*, *F. oxysporum* and *F. dimerum*, which were culturable from 75% of the sink swabs (Table

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1 and Table S2)~~2 and summarised in Table 3~~). ELISA tests of the saline sink swabs showed that 52% contained detectable levels of *Fusarium* antigen (Table 1 and Table S2~~3~~), with ELISA absorbance values in the range ≥0.100 (the threshold value for antigen detection) and up to 1.500. In four hospital samples (samples S47, S48 and S49 from ophthalmology and sample S64 from oncology) *Fusarium* strains could not be recovered for identification by ITS sequencing despite detection of the diagnostic antigen in swab samples with absorbance values of 0.264, 0.530, 0.187 and 0.193 respectively (Table 1~~2~~). This was likely due to the *Fusarium* isolates being outgrown in the mixed culture plates by faster growing or more abundant unrelated fungi. Importantly, ~~mAb~~ ED7 was shown not to cross-react with unrelated fungi (axenic culture absorbance values of ≤0.100 in all cases) including the human pathogenic yeast or yeast-like fungi *Candida*, *Exophiala*, *Meyerozyma*, *Rhodotorula*, *Trichosporon*, the human pathogenic hyaline or dematiaceous molds *Aspergillus*, *Phialophora*, *Phoma*, *Trichoderma*, and the human pathogenic mucormycete *Mucor* (Table 1~~2~~). The remaining 93% of samples positive for *Fusarium* antigen, either at the swab stage or following periods of biological amplification in mixed or axenic cultures (Table S2), yielded strains of the three *Fusarium* species. There was 100% concordance between *Fusarium* genus identification by ELISA and species identification by ITS sequencing (Table 1~~3~~). The species of *F. solani* and *F. oxy~~p~~sporum* recovered from sink swabs were subsequently shown by TEF-1α PCR (Supporting Data Set 1) to belong to *F. solani* species complex (FSSC) 1-a, 1-c, 2-a, 2-v, 5-d, 5-k, 9-a, 15-a, 20-d and *F. oxysporum* (FOSC) species complexes 16, 33, 99, 111, 126, 134, 183 (Table S3~~Table 4 and Appendix 1~~). All of the recovered *F. dimerum* isolates belonged to the *F. dimerum* species complex (FDSC) ET-gr (Table S3). ITS analysis of axenic cultures (Table 1~~3~~) showed that a number of sink samples (e.g. S2, S6, S17, S19, S21, S24, S30, S38) contained mixtures of *Fusarium* species, while ITS and TEF-PCR analysis (Table 1 and Table S3~~Tables 3, 4 and~~

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~~Appendix 1~~) showed that others contained mixtures of species complexes of the same species (e.g. S8, S9, S25, S28). Monoclonal antibody ED7 was able to detect all of the *Fusarium* species complexes recovered in this study.

In addition to drain swabs, water samples were collected from the taps of sinks in the hospital haematology and oncology units and from the main water tanks feeding the ophthalmology unit. The ED7 diagnostic antigen could not be detected in any of the water samples directly and, while all of the samples yielded fungi, only two of the tap samples (oncology W57 and W60) contained *Fusarium* strains that belonged to FDSC ET-gr. and which were detectable by ELISA at the mixed culture stage (Table 1~~2~~). The sink biofilms corresponding to these water samples were also positive at the swab ELISA stage (Table 1).

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Discussion

The genus *Fusarium* comprises ubiquitous environmental ~~mould~~molds capable of infecting plants and humans (Zhang *et al*., 2006). Unlike agriculture, where the most economically damaging pathogens are considered to be *F. graminearum* and *F. oxysporum* (Dean *et al*., 2012), the species most commonly cited as human pathogens belong to the *Fusarium solani* species complex (FSSC, responsible for 50% of reported infections in humans), followed by strains in the *Fusarium oxysporum* species complex (FOSC)(Torres and Kontoyiannis, 2011). The *Fusarium dimerum* species complex (FDSC) is less frequently reported as causing human disease, but it is similarly capable of causing disseminated infections in immunocompromised patients (Bigley *et al*., 2004; Schroers *et al*., 2009).

While the natural habitats of plant pathogenic *Fusarium* strains are well characterised as soil and decaying plant material, habitats providing direct human exposure to infectious propagules are largely unexplored. The increasing frequency of opportunistic fungal infections in humans means that improved surveillance methods are needed to identify environmental reservoirs of pathogens to limit the exposure of vulnerable individuals to potentially infective propagules. For *Fusarium*, there is a growing body of evidence to suggest that domestic and municipal water systems are potential reservoirs of human pathogenic strains in the FSSC, FOSC and FDSC groups (Short *et al*., 2011).

Accurate techniques that can be used to identify the fungus in environmental samples containing mixed populations of fungi are currently lacking and, while nucleic acid-based technologies have been developed for the differentiation of *Fusarium* from other human pathogenic species and to identify *Fusarium* species complexes, such techniques have typically been used in retrospective analysis of axenic cultures collected during human and environmental population

studies (Bouchara *et al*., 2009; Steinmann *et al*., 2011; Lackner *et al*., 2012). Furthermore, these studies have often employed *Fusarium*-selective media that eliminate other fungi present in polymicrobial communities (Short *et al*., 2011). While monoclonal antibodies (mAbs) and antibody fragments have been developed for detecting and differentiating *Fusarium* species *in vitro* or *in planta* (Wong *et al*., 1988; Arie *et al*., 1991, 1995; Danks *et al*., 1996; Hayashi *et al*., 1998; Hu *et al*., 2012, 2013), no attempts have been madeused to use mAbs to track human pathogenic strains in environmental samples. Jensen *et al*. (2011) recently reported the development of *Fusarium*-specific mAbs for immunohistochemical diagnosis of fusariosis. The IgM mAbs, which recognise 51 and 63 kDa antigens, reacted strongly with fungal elements in both experimentally infected animals and biopsy samples from patients with fusariosis sepsis and dissemination to the skin.

In this prospective study, we set out to determine whether human pathogenic species of *Fusarium* could be identified in sink drains directly by using crude antigen extracts of biofilms and detection using a genus-specific immunoglobulin M (IgM) mAb, ED7, that binds to an a extracellular ~200kDa carbohydrate antigen present on the surface of spores and hyphae. While the function of the antigen is currently unknowUsing mAb-based ELISAn , we we were able, in Enzyme-Linked Immunosorbent Assay (ELISA) tests, able to detect its presence the diagnostic antigen in 52% of swab samples and, following biological amplification of biofilms on a non-selective mycological medium, were able to identify additional biofilm samples containing pathogenic strains of *Fusarium*. This is the first time, to our knowledge, that a mAb-based detection method has been used to track *Fusarium* in environmental samples. The mAb was able to differentiate *Fusarium* from a wide spectrum of unrelated fungi, including the human pathogens *Aspergillus* (Thornton and Wills, 2015), *Candida*, *Geotrichum*, *Rhodotorula* and *Trichosporon* (Davies and Thornton, 2014; Miceli *et al*., 2011), *Cyphellophora* and *Phialophora* (Feng *et al*.,

2014), *Exophiala* (Zeng *et al*., 2007), *Trichoderma* (Sandoval-Denis *et al*., 2014), *Engyodontium* (Macêdo *et al*., 2007; Thamke *et al*., 2015) and *Mucor* (Petrikkos *et al*., 2012), several of which have been reported previously to inhabit biofilms in water distribution systems (Dogget, 2000). The 100% accuracy of the ED7 ELISA, confirmed by using ITS sequencing and TEF PCR analysis of recovered isolates, demonstrates its robustness in dd~~d~~etecting potentially infectious *Fusarium* species in polymicrobial communities. Importantly, ~~mAb~~ ED7 reacted with all of the species complex strains isolated including the most common clinical pathotypes of *Fusarium,* FSSC 1-a, FOSC 33 and FDSC ET-gr (Schroers *et al*., 2009; Short *et al*., 2011).

While the ED7 ELISA was able to identify *Fusarium* to the level of genus only, the simplicity of the mAb-based approach to detection, even when combined with a standard mycological isolation procedure, means that a recognised environmental niche of this group of pathogenic fungi can be monitored readily. The widespread occurrence of human pathogenic *Fusarium* species in sinks of a tertiary care hospital and sinks of a heavily populated university campus, show that indoor plumbing-associated biofilms and water sources are an unseen source of *Fusarium* infectious propagules for nosocomial and community-acquired infections of vulnerable individuals, an observation consistent with previous studies (Annaisie *et al*., 2011; Short *et al*., 2011). While no cases of fusariosis were reported during the course of this study, t~~T~~he close proximity of the patients to hospital sinks colonised with both pathogenic fusaria and with other opportunistic fungal pathogens is a serious concern given the known vulnerability of immunocompromised individuals to invasive fungal infections.

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Conflicts of Interest

We declare that none of the authors involved in writing this paper have any conflicts of interest with respect to the content of this article.

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Experimental procedures

Ethics statement

All animal work described in this study was conducted under a UK Home Office Project License, and was reviewed by the institution's Animal Welfare Ethical Review Board (AWERB) for approval. The work was carried out in accordance with The Animals (Scientific Procedures) Act 1986 Directive 2010/63/EU, and followed all the Codes of Practice which reinforce this law, including all elements of housing, care, and euthanasia of the animals. Permission for sink sampling at the Royal Devon and Exeter Hospital was granted by the Director of Infection Prevention and Control.

Fungal culture

Fungi (Table S1) were routinely cultured on Potato Dextrose Agar (PDA: 70139; Sigma)~~)~~, Sabouraud Dextrose Agar (SDA: Sabouraud Dextrose Broth (SDB: S3306; Sigma) containing 2% (w/v) agar)~~,A),~~ Malt Yeast extract Agar (MYA: Y3127; Sigma), or Oatmeal Agar (OA: O3506; Sigma), sterilized by autoclaving at 121^{o}C for 15 min. Cultures were grown at 26^{o}C under a 16 h fluorescent light regime.

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Development of mAb, preparation of immunogen, and immunisation regime

BALB~~alb~~/c mice were immunized with soluble antigens prepared from lyophilized mycelium of a human pathogenic strain of *Fusarium solani* species complex 1-a (CBS strain 224.34). Conidia were suspended in water after 10-day old PDA slant cultures were flooded with 5 ml dH_2O and gently agitated with an inoculation loop. Conidial suspensions were then filtered through Miracloth to remove mycelium and transferred to 1.5 ml micro-centrifuge tubes. The conidia were washed three times with dH_2O by repeated vortexing and centrifugation at 14,462 *g* for 5 min and finally suspended in dH_2O to give a concentration of 10^6 conidia ml^{-1} solution. Flasks containing 100 ml of sterilized Potato Dextrose Broth (~~potato dextrose broth (~~PDB; P6685; Sigma) ~~)~~ were inoculated with 200 µl of the conidial suspension and incubated with shaking (75 rpm) for 48 h at 26°C. Hyphal biomass was collected on Miracloth, snap frozen in liquid N_2, and lyophilized. Culture filtrates were retained for gel electrophoresis and western blotting studies and stored at -20°C until required. One mg of lyophilized biomass was suspended in 1 ml phosphate buffered saline (PBS: 0.8% NaCl; 0.02% KCl; 0.115% Na_2HPO_4; 0.02% KH_2PO_4; pH7.2) and the resultant suspension centrifuged for 5 min at 14,462 *g*. The supernatant, containing solubilized antigens, was used as the immunogen and as a source of antigens for hybridoma screening assays. For immunization, 6-wk-old BALB/c female white mice were given four intraperitoneal injections (300 µl per injection) of antigen extract containing 2.3 mg protein ml^{-1} PBS at 2-wk intervals and a single booster injection five days before fusion.

Production and screening of hybridomas and determination of antibody specificity

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Hybridoma cells were produced by the method described elsewhere (Thornton, 2001) and the supernatants were screened by Eenzyme-Llinked Iimmunosorbent Aassay (ELISA) against antigens immobilized to the wells of Maxisorp microtitre plates (442404; Nunc)(50 µl per well). For antibody specificity tests, fungi were grown on replicate agar slopes and surface washings containing water-soluble antigens prepared as described in Thornton (2001). Protein concentrations, determined spectrophotometrically at 280 nm (Nanodrop, Agilent Technologies Limited, Berkshire, UK), were adjusted to 60 µg ml^{-1} buffer. Fifty µl volumes were then used to coat the wells of microtitre plates. After incubating overnight at 4°C, wells were washed four times with PBST (PBS containing Tween-20, 0.05% (v/v)), and once each with PBS and dH_2O and then air-dried at 23°C in a laminar flow hood. The plates were stored in sealed plastic bags at 4°C in preparation for screening of hybridoma supernatants by ELISA as described below.

Plate-Trapped-Antigen-Enzyme-Linked Immunosorbent Assay

Wells containing immobilized antigens were incubated successively with hybridoma tissue culture supernatant (TCS) for 1 h, followed with goat anti-mouse polyvalent (immunoglobulin classes IgG, IgA, and IgM) peroxidase conjugate (A-0412; Sigma Chemical Company, Poole, United Kingdom) diluted 1 in 1000 in PBST for a further hour. Bound antibody was visualized by incubating wells with tetramethyl benzidine (TMB; T-2885; Sigma) substrate solution (Thornton, 2001) for 30 min. The reactions were stopped by the addition of 3 M H_2SO_4 and aAbsorbance values were determined at 450 nm with an MRX automated microplate reader (Dynex Technologies, Billingshurst, UK). Wells were given four 5-min rinses with PBST between incubations and a final rinse with PBS before addition of the substrate solution. Working volumes were 50 µl per well, and control wells were incubated with tissue culture medium (TCM) containing 10% (v/v) fetal

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bovine serum. All incubation steps were performed at 23°C in sealed plastic bags. The threshold for detection of the antigen in ELISA was determined from control means (2 x TCM absorbance values)(Sutula *et al*., 1986). These values were consistently in the range 0.050-0.100. Consequently, absorbance values >0.100 were considered as positive for the detection of antigen.

Determination of Ig subclass and cloning procedure

The Ig class of mAbs was determined by using antigen-mediated ELISA. Wells of microtitre plates coated with *F. solani* CBS224.34 water-soluble antigens from surface washings were incubated successively with ~~hybridoma supernatant~~ ED7 TCS for 1 h, followed with goat anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, or IgA-specific antiserum (ISO-2; Sigma) diluted 1 in 3000 in PBST for 30 min and rabbit anti-goat peroxidase conjugate diluted 1 in 1000 (A-5420; Sigma) for a further 30 min. Bound antibody was visualized with TMB substrate as described above. Hybridoma cells lines were sub-cloned three times by limiting dilution, and cell lines were grown in bulk in a non-selective medium preserved by slowly freezing in fetal bovine serum/dimethyl sulfoxide (92:8 [v/v]), and stored in liquid nitrogen.

Gel electrophoresis and Western blotting

For sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), culture filtrates from 2-d-old PDB shake cultures of *F. solani* CBS224.34 and *F. oxysporum* f.sp. *lycopersici* CBS167.30, ~~–~~prepared as described, were diluted in Laemmli buffer (Laemmli, 1970) and were denatured by heating at 95°C for 10 min. Antigens were separated in 4-20% (w/v) polyacrylamide gradient gels (161-1159; Bio-Rad) for 1.5 h at 23°C (165V) under denaturing conditions, and pre-stained broad range markers (161-0318; Bio-Rad) were used for molecular weight determinations.

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For w~~W~~esterns, separated antigens were transferred electrophoretically to a PVDF membrane (162-0175; Bio-Rad). The membranes were blocked for 16 h at 4°C with PBS containing 1% (w/v) bovine serum albumin (BSA) and incubated with ~~hybridoma supernatant~~ED7 TCS diluted 1 in 2 with PBS containing 0.5% (w/v) BSA (PBSA) for 2 h at 23°C. After washing three times with PBS, membranes were incubated for 1 h with goat anti-mouse IgM (µ-chain specific) alkaline phosphatase conjugate (A-9688; Sigma), diluted 1 in 15,000 in PBSA. After the membranes were washed twice with PBS and once with PBST, the bound antibodies were visualized by incubation in BCIP/NBT substrate solution. Reactions were stopped by immersion in dH_2O and air-dried between sheets of Whatman filter paper.

Characterization of antigen by enzymatic and chemical modifications and by heating

Water-soluble antigens from surface washings of slopes of *F. solani* CBS224.34 were prepared as described. Heat stability studies were conducted by placing tubes of solubilised antigen ~~solubilised antigen from three replicate cultures of *F. solani* CBS224.34~~ in a boiling water bath. At 10 min intervals, samples were removed, centrifuged at 14,462 *g* ~~14,500 rpm~~ for 5 min, and antigens immobilised to the wells of microtitre plates for assay by ELISA as described. For periodate oxidation, microtitre wells containing immobilised antigens from surface washings of the fungus~~antigens~~ were incubated with 50 µl of sodium *meta*-periodate solution (20 mM $NaIO_4$ in 50 mM sodium acetate buffer (pH4.5)) or acetate buffer only (control) at 4°C in sealed plastic bags. Plates were given four 3-min PBS washes before processing by ELISA as described. For protease digestions, microtitre wells containing immobilised antigen were incubated with 50 µl of pronase (protease XIV; 9 mg ml^{-1} in PBS) or trypsin (1 mg ml^{-1} in Milli-Q H_2O) solution or Milli-Q H_2O or PBS only controls respectively for 4 h at 37°C or 4°C. Plates were given four 3-min rinses with

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PBS and then assayed by ELISA with ~~hybridoma supernatant~~ED7 TCS as described.

Immunofluorescence and immunogold electron microscopy

For immunfluorescence (IF), sterilised slides were coated with a washed spore suspensions of *F. solani* CBS224.34 containing 1% (w/v) glucose solution and incubated at 26°C for 16 h to allow spore germination and formation of germ tubes. After air-drying, the ~~slides~~ cells were fixed to the slides as described in Thornton (2001) and incubated with ~~hybridoma supernatant~~ED7 TCS or TCM only (negative control) for 1 h, followed by three 5 min PBS washes. Slides were then incubated with goat anti-mouse polyvalent fluorescein isothiocyanate (FITC) conjugate (diluted 1 in 40 in PBS)(F1010; Sigma) for 30 min. Slides were given three 5 min washes with PBS and mounted in PBS-glycerol mounting medium (F4680; Sigma) before overlaying with coverslips. All incubation steps were performed at 23°C in a humid environment to prevent evaporation and slides were stored in the dark, at 4°C, prior to examination using an epifluorescence microscope (Olympus IX81) fitted with 495 nm (excitation) and 518 nm (emission) filters for FITC. For immunogold electron microscopy (IEM) , ~~the method spores were embedded in LR White resin and immunostained by using hybridoma supernatant or TCM control and anti-mouse polyvalent 20nm gold conjugate according to the technique~~ described in Thornton & Talbot (2001) was used. Spores and hyphae of *F. solani* were prepared by incubating washed conidia in 1% (w/v) glucose solution at 26°C for 16 h to allow spore germination and formation of germ tubes. Cells were embedded in LR White resin (Agar Scientific Ltd.) and ultra thin sections prepared for immunolabeling. Sections immobilized to nickel grids were blocked by immersion in PBST containing 1% (w/v) BSA (PBST-BSA) which had been sterile filtered through a 0.2 µm filter. The grids were washed three times (3 min each) in sterile filtered PBST and then incubated in ED7 TCS or TCM only (negative control) for 1 h. After

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four washes (3 min each) with sterile filtered PBST, the grids were incubated for a further hour in PBST-BSA containing a 1:20 dilution of goat anti-mouse 20 nm gold conjugate (EM.GAF20; BBI Solutions). The grids were washed four times (3 min each) in sterile filtered PBST and then placed on Whatman filter paper to dry. Dried grids were then incubated for 20 min in 2% (w/v) uranyl acetate solution followed by 2% (w/v) lead citrate solution for 4 min. Working volumes were 100 µl and incubation and washing steps were carried out at at 23°C. Immunostained samples were examined using a Jeol JEM 1400 transmission electron microscope fitted with a Gatan ES 100W CCD camera.

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Statistical analysis

Unless otherwise stated, numerical data were analysed using the statistical programme Minitab (Minitab 16, Minitab®, Coventry, UK). Analysis of variance (ANOVA) was used to compare means of more than two data sets and Post-hoc Tukey-Kramer analysis was then performed to distinguish which sets were significantly different from one another.

Sampling from drains

A total of 65 sinks were swabbed, comprising 32 sinks across the ICU, ITU, haematology, oncology and ophthalmology units of the Royal Devon and Exeter tertiary care hospital (Exeter, Devon, UK) and 33 restroom sinks located around the University of Exeter campus (Exeter, Devon, UK). In addition, cold-water samples were collected from taps connected to the sinks in the haematology and oncology unit, and from the two main water tanks feeding the ophthalmology unit. To isolate fungi from sink biofilms, sterile cotton buds (Boots, UK) wetted with PBS were used to scour the inner surfaces of sink drainpipes for approximately 10 s. Swabs with visible detritus were immersed

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in 1.5-ml micro-centrifuge tubes containing 1 ml PBS to dislodge biofilm debris, and the sealed tubes transferred to the laboratory for processing by ELISA and mycological culture.

Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the ITS regions of the rRNA-encoding gene unit and Translation Elongation Factor-1α PCR

Biofilm debris was pelleted by centrifugation at 14,462 *g* for 5 min and 50 µl samples of supernatant transferred to the wells of microtitre plates for assay by ELISA (Table 1~~2; Swab-ELISA~~and Table S2; Swab-ELISA) as described. The biofilm pellet was re-suspended in 1 ml dH_2O, 200 µl samples spread on the surface of PDA containing ~~the~~ 1 µg ml^{-1} of the broad-spectrum antibiotic rifampicin, and the plates incubated for 2 d at 26°C under a 16 h fluorescent light regime. Fungi in these mixed culture plates were separated on the basis of gross morphological characteristics and axenic slope cultures generated following sub-culture on PDA. Crude antigen extracts were prepared as surface washings from mixed cultures and from axenic cultures and assayed by ELISA (Table 1 and Table S2~~2~~; Mixed culture-ELISA and Axenic culture-ELISA, respectively) as described.

Fungal DNA was extracted from axenic culture material by using the CTAB method (Chow & Kafer, 1993) and fungi were identified by sequencing of the ITS1-5.8S-ITS2 region of the rRNA-encoding gene unit (White et al., 1990) according to procedures described elsewhere (Thornton *et al*., 2002), using the primers ITS1ext (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS4ext (5'-TTCTTTTCCTCCGCTTATTGATATGC-3'). Species identity was predicted based on >95% sequence identity (E-value = 0.0)(Altschul et al. 1997) of the ITS1-5.8S-ITS2 region of recovered species to species recorded in GenBank. *Fusarium* species were further identified to species complex level by using the forward primer ef-1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and

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reverse primer ef-2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3'), which amplify an ~700 bp region of Translation Elongation Factor 1-alpha (TEF-1α), the single-locus identification tool in *Fusarium* (Geiser et al., 2004). PCR reactions were carried out in a total volume of 25 µl consisting of 1 µl DNA at a concentration of 30 - 75 ng μl^{-1}, 12.5 µl of GoTaq® Green Master Mix DNA polymerase (Promega, MF7112), 9.5 µl of nuclease free water (Promega) and 1 µl of each primer at 20 pmol. The following cycling parameters were used: an initial denaturation step at 95°C for 8 min; 35 cycles of 15 sec at 95°C (denaturation); 20 s at 54°C (annealing), 1 min at 72°C (extension) followed by a final 5 min extension step at 72°C. Phylogenetic sub-groups of *Fusarium* species were determined by interrogation of the FUSARIUM-ID v. 1.0 database (http://isolate.fusariumdb.org)(O'Donnell et al., 2010), with the newly acquired TEF-1α sequences (Supporting Data Set 1~~Appendix 1~~).

Nucleotide sequence accession numbers

Newly determined ITS sequences were submitted to GenBank and the ITS accession numbers KT876496 to KT876723 were obtained. Species designations of recovered fungi are shown in Table 1~~2~~.

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References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database programs. *Nucleic Acids Res* **25:** 3389-3402.

Anaissie, E.J., Kuchar, R.T., Rex, J.H., Francesconi, A., Kasai, M., Muller, F.M., *et al*. (2011) Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic ~~mould~~mold pathogens. *Clin Infect Dis* **33:** 1871-1878.

Anaissie, E.J., Penzak, S.R., and Dignani, M.C. (2002) The hospital water supply as a source of nosocomial infections: a plea for action. *Arch Intern Med* **162:** 1483-1492.

Arie, T., Hayashi, Y., Nagatani, A., Furuya, M., and Yamaguchi, I. (1991) Production and partial characterization of monoclonal antibodies against *Fusarium oxysporum* 860926a. *Ann Phytopathol Soc Jpn* **57:** 696-701.

Arie, T., Hayashi, Y., Yoneyama K, Nagatani, A., Furuya, M., and Yamaguchi, I. (1995) Detection of *Fusarium* spp. in plants with monoclonal antibody. *Ann Phytopathol Soc Jpn* **61:** 311-317.

Fusarium-specific monoclonal antibody

Arrese, J.E., Piérard-Franchimont, C., and Piérard, G.E. (1996) Fatal hyalohyphomycosis following *Fusarium* onychomycosis in an immunocompromised patient. *Am J Dermatopathol* **18:** 196-198.

Bigley, V.H., Duarte, R.F., Gosling, R.D., Kibbler, C.C., Seaton, S., and Potter, M. (2004) *Fusarium dimerum* infection in a stem cell transplant recipient treated successfully with voriconazole. *Bone Marrow Transplant* **34:** 815-817.

Bouchara, J-P., Hsieh, H.Y., Croquefer, S., Barton, R., Marchais, V., Pihet, M., *et al.* (2009) Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. *J Clin Microbiol* **47:** 142-152.

Boutati, E.I., and Anaissie, E.J. (1997) *Fusarium*, a significant emerging pathogen in patients with haematological malignancies: ten years experience at a cancer centre and implications for management. *Blood* **90:** 999-1008.

Chang, D.C., Grant, G.B., O'Donnell, K., Wannemuehler, K.A., Noble-Wang, J., Rao, C.Y., *et al.* (2006) Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* **296:** 953-963.

Chow, T.Y.K., and Käfer, E. (1993) A rapid method for isolation of total nucleic acids from *Aspergillus nidulans*. *Fungal Genet Newsl* **40:** 25-27.

Danks, J.N., Rizvi, R.H., Barker, I., Turner, J.A., Rahman, S., and Northway, B.J. (1996) Specific monoclonal antibodies to *Fusarium* species and *Michrodochium nivale*. *Food Agric Immunol* **8:** 249-68.

Davies, G., and Thornton, C.R. (2014) Differentiation of the emerging human pathogens *Trichosporon asahii* and *Trichosporon asteroides* from other pathogenic yeasts and ~~mould~~molds by using species-specific monoclonal antibodies. *PLoS ONE* **9:** e84789.

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Fusarium-specific monoclonal antibody

Dignani, M.C., and Anaissie, E. (2004) Human fusariosis. *Clin Microbiol Infect* **10:** 67-75.

Doggett, M.S. (2000) Characterisation of fungal biofilms within a municipal water distribution system. *Appl Env Microbiol* **66:** 1249-1251.

Dursun, D., Fernandez, V., and Miller, D. (2003) Advanced *Fusarium* keratitis progressing to endophthalmitis. *Cornea* **22:** 300-303.

Edelstein, S.L., Akduman, L., Durham, B.H., Fothergill, A.W., and Hsu, H.Y. (2012) Resistant *Fusarium* keratitis progressing to endophthalmitis. *Ophthalmology Contact Lens* **38:** 331-335.

Feng, P., Qiaoyun, L., Najafzadeh, M.J., Gerrits van den Ende, A.H.G., Sun, J., Li, R., *et al*. (2014) *Cyphellophora* and its relatives in *Phialophora*: biodiversity and possible role in human disease. *Fungal Diversity* **65:** 17-45.

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.D., *et al*. (2004) FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. *Eur J Pl Pathol* **110:** 473-479.

Girmenia, C., Pagano, L., Corvatta, L., Mele, L., Del, A., Favero, P., *et al*. (2000) The epidemiology of fusarioses in patients with haematological diseases. *Br J Haematol* **111:** 272-276.

Gurusidappa, S.B., and Mamatha, H.S. (2011) Fusarial skin lesion in immunocompromised. *Ind J Cancer* **48:** 116-117.

Hayashi, Y., Arie, T., Yoneyama, K., and Yamaguchi, I. (1998) Characterisation of the antigenic determinant on *Fusarium oxysporum* recognized by a genus-specific monoclonal antibody. *J Gen Appl Microbiol* **44:** 43-47.

Hu, Z-Q., Liu, J-L., Li, H-P., Xing, S., Xue, S., Zhang, J-B., *et al*. (2012) Generation of a highly reactive chicken-derived single-chain variable fragment against *Fusarium verticillioides* by phage display. *Int J Mol Sci* **13:** 7038-7056.

Hu, Z-Q., Li, H-P., Zhang, J-B., Huang, T., Liu, J-L., Xue, S., *et al*. (2013) A phage-displayed chicken single-chain antibody fused to alkaline phosphatase detects *Fusarium* pathogens and their presence in cereal grains. *Anal Chim Acta* **764:** 84-92.

Jensen, T.G., Gahrn-Hansen, B., Arendrup, M., and Bruun, B. (2004) *Fusarium* fungaemia in immunocompromised patients. *Clin Microbiol Infect* **10:** 499-501.

Jensen, H.M.E., Aalbæk, B., Jungersen, G., Hartvig, T., Moser, C., Rozell, B.L., *et al.* (2011) Immunohistochemical diagnosis of fusariosis with monoclonal antibodies. *Mycoses* **54:** s54-s55.

Jurkunas, U., Behlau, I., and Colby, K. (2009) Fungal keratitis: changing pathogens and risk factors. *Cornea* **28:** 638-643.

Koehler, P., Tacke, D., and Cornely, O.A. (2014) Bone and joint infections by *Mucorales*, *Scedosporium*, *Fusarium* and even rarer fungi. *Crit Rev Microbiol*. doi: 10.3109/1040841X.2014.910749.

Lackner, M., Najafzadeh, M.J., Sun, J., Lu, Q., and de Hoog, G.S. (2012) Rapid identification of *Pseudallescheria* and *Scedosporium* strains by using rolling circle amplification. *Appl Environ Microbiol* **78:** 126-133.

Latenser, B.A. (2003) *Fusarium* infections in burn patients: a case report and review of the literature. *J Burn Care Rehabil* **24:** 285-288.

Macêdo, D.P.C., Neves, R.P., de Souza-Motta, C.M., and Magalhães, O.M.C. (2007) *Engyodontium album* fungaemia: the first reported case. Braz J Microbiol 38: 110-112.

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Fusarium-specific monoclonal antibody

Mehl, H.L., and Epstein, L. (2008) Sewage and community shower drains are environmental reservoirs of *Fusarium* species complex group 1, a human and plant pathogen. *Env Microbiol* **10:** 219-227.

Miceli, M.H., Díaz, J.A., and Lee, S.A. (2011) Emerging opportunistic yeast infections. *Lancet Infect Dis* **11:** 142-151.

Muhammed, M., Carneiro, H., Coleman, J., and Mylonakis, E. (2011) The challenge of managing fusariosis. *Virulence* **2:** 91-96.

Musa, H.O., Al Eisa, A., Halim, M., Sahovic, E., Gyger, M., Chaudhri, N., *et al.* (2000) The spectrum of fusarium infection in immunocompromised patients with haematological malignancies and in non-immunocompromised patients; A single institution experience over 10 years. *Br J Haematol* **108:** 544-548.

Nucci, M., and Anaissie, E. (2002) Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clin Infect Dis* **35:** 909-920.

Nucci, N., and Anaissie, E. (2007) *Fusarium* infections in immuncompromised patients. *Clin Microbiol Rev* **20:** 695-704.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Sarver, B.A.J., Arunmozhi Balajee, S., Schroers, H-J., et *al*. (2010) Internet-accessible DNA sequence database for identifying Fusaria from human and animal infections. *J Clin Microbiol* **48:** 3708-3718.

Petrikkos, G., Skiada, A., Lortholary, O., Roilides, E., Walsh, T.J., and Kontoyiannis, D.P. (2012) Epidemiology and clinical manifestations of mucormycosis. *Clin Infect Dis* **54:** S23-S24.

Fusarium-specific monoclonal antibody

Rougeron, A., Schuliar, G., Leto, J., Sitterle, E., Landry, D., Bougnoux, M.E., *et al*. (2014) Human-impacted areas of France are environmental reservoirs of the *Pseudallescheria boydii/Scedosporium apiospermum* species complex. *Env Microbiol* **17:** 1039-1048.

Sandoval-Denis, M., Sutton, D.A., Cano-Lira, J.F., Gene, J., Fothergill, A.W., Wiederhold, N.P., *et al*. (2014) Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities. *J Clin Microbiol* **52:** 2112-2125.

Schroers, H.-J., O'Donnell, K., Lamprecht, S.C., Kammophthalmologyr, P.L., Johnson, S., Sutton, D.A., et al. (2009) Taxonomy and phylogeny of the *Fusarium dimerum* species group. *Mycologia* **101:** 44-70.

Short, D.P.G., O'Donnell, K., Zhang, N., Juba, J.H., and Geiser, D.M. (2011) Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. *J Clin Microbiol* **49:** 4264-4272.

Steinmann, J., Schmidt, D., Buer, J., and Rath, P-M. (2011) Discrimination of *Scedosporium prolificans* against *Pseudallescheria boydii* and *Scedosporium apiospermum* by semiautomated repetitive sequence-based PCR. *Med Mycol* **49:** 475-483.

Sutula, C.L., Gillett, J.M., Morrisey, S.M., and Ramsdell, D.C. (1986) Interpreting ELISA data and establishing the positive-negative threshold. *Plant Dis* **70:** 722-26.

Thamke, D.C., Mediratta, D.K., Dhabarde, A., and Shukla, A.K. (2015) Mycotic keratitis due to *Engyodontium album*: first case report from India. *Indian J Med Microbiol* **33:** 303-304.

Thornton, C.R. (2001) Immunological methods for fungi. In *Molecular and Cellular Biology of Filamentous Fungi, A Practical Approach*. Talbot, N.J. (ed.). Oxford, UK: University Press, pp. 227-257.

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Fusarium-specific monoclonal antibody

Thornton, C.R. (2008) Development of an immunochromatographic lateral flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vacc Immunol* **15:** 1095-1105.

Thornton, C.R. (2009) Tracking the emerging human pathogen *Pseudallescheria boydii* by using highly specific monoclonal antibodies. *Clin Vacc Immunol* **16:** 756-64.

Thornton, C.R. (2012) Serological techniques for diagnosis. In *Fungal Plant Pathogens*, *Principles and Protocols*. Lane, C., Beales, P., Hughes, K. (eds.). Wallingford, UK: CABI, pp. 159-177.

Thornton, C.R., and Wills, O.E. (2015) Immunodetection of fungal and oomycete pathogens: Established and emerging threats to human health, animal welfare, and global food security. *Crit Rev Microbiol.* **41:** 27-51.

Thornton, C.R., Ryder, L.S., Le Cocq, K., and Soanes, D.M. (2015) Identifying the emerging human pathogen *Scedosporium prolificans* by using a species-specific monoclonal antibody that binds to the melanin biosynthetic enzyme tetrahydroxynaphthalene reductase. *Env Microbiol* **17:** 1023-1048.

Torres, H.J., and Kontoyiannis, D. (2011) Hyalohyphomycosis (hyaline ~~mould~~molds). In *Essentials of Clinical Mycology*, Kauffman, C.A., Pappas, P.G., Sobel, J.D., and Dismukes, W.E. (eds.). New York, USA: Springer Science+Business Media, pp. 281-304.

Walsh, T.J., and Groll, A.H. (1999) Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transpl Infect Dis* **1:**247-261.

Wong, W.C., White, M., and Wright, I.G. (1988) Production of monoclonal antibodies to *Fusarium oxysporum* f.sp. *cubense* race 4. *Lett Appl Microbiol* **6:** 39-42.

Zeng, J.S., Sutton, D.A., Fothergill, A.W., Rinaldi, M.G., Harrak, M.J., and de Hoog, G.S. (2007) Spectrum of clinically relevant *Exophiala* species in the Unites States. *J Clin Microbiol* **45:** 3713-3720.

Fusarium-specific monoclonal antibody

Zhang, N., O'Donnell, K., Sutton, D.A., Nalim, F.A., Summerbell, R.C., Padhye, A.A., et al. (2006) Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *J Clin Microbiol* **44:** 2186-2190.

Table 1. Details of fungi used in mAb ED7 specificity tests.

Organism	Isolate no.	Source[a]
Aspergillus cervinus	537.65	CBS
Aspergillus ficuum	555.65	CBS
Aspergillus flavus	91856iii	IMI
Aspergillus fumigatus	AF293[-]	SK
Aspergillus nidulans	A4	FGSC
Aspergillus niger	102.40	CBS

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Organism	Isolate no.	Source[a]
Aspergillus oryzae	672.92	CBS
Aspergillus restrictus	116.50	CBS
Aspergillus terreus var. *terreus*	601.65	CBS
Botrytis cinerea	R2	CRT
Byssochlamys nivea	153.59	CBS
Candida glabrata	4692	CBS
Candida krusei	5590	CBS
Candida parapsilosis	8836	CBS
Candida tropicalis	1920	CBS
Cryptococcus neoformans (Serotype D)	5728	CBS
Cunninghamella elegans	151.80	CBS
Filobasidiella bacillispora	10865	CBS
Filobasidiella neoformans	10490	CBS

Table 1. *continued*

Organism	Isolate no.	Source[a]
Filobasidiella neoformans	10496	CBS
Fusarium acutatum	402.97	CBS
Fusarium anthophilum	222.76	CBS
Fusarium avenaceum	386.62	CBS
Fusarium cerealis	134.80	CBS
Fusarium chlamydosporium var. *chlamydosporium*	491.77	CBS

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1	*Fusarium culmorum*	256.51	CBS
2	*Fusarium dimerum* var. *dimerum*	108944	CBS
3	*Fusarium incarnatum*	678.77	CBS
4	*Fusarium nygamai*	140.95	CBS
5	*Fusarium oxysporum* f.sp. *cucurbitacearum*	254.52	CBS
6	*Fusarium oxysporum* f.sp. *lycopersici*	167.30	CBS
7	*Fusarium oxysporum* f.sp. *marmaris*	420.80	CBS
8	*Fusarium oxysporum* f.sp. *radicis-lycopersici*	872.95	CBS
9	*Fusarium oxysporum* f.sp. *vasinfectum*	409.90	CBS
10	*Fusarium proliferatum* var. *proliferatum*	181.30	CBS
11	*Fusarium sacchari*	183.32	CBS
12	*Fusarium solani*	224.34	CBS
13	*Fusarium solani*	109696	CBS
14			

15 **Table 1.** *continued*

16			
17	Organism	Isolate no.	Source
18			
19	*Fusarium solani*	188.34	CBS
20	*Fusarium solani*	115659	CBS
21	*Fusarium solani*	117608	CBS
22	*Fusarium solani*	119223	CBS
23	*Fusarium solani* var. *petroliphilum*	102256	CBS
24	*Fusarium verticillioides*	102699	CBS

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Organism	Isolate no.	Source
Geotrichum candidum	115.23	CBS
Haematonectria haematococca	114067	CBS
Haematonectria haematococca	119603	CBS
Haematonectria haematococca	130692	CBS
Lichtheimia corymbifera	T14A (FJ713070)	CRT
Magnusiomyces capitatus	207.83	CBS
Mucor circinelloides f.sp. *circinelloides*	E2A (FJ713065)	CRT
Neosartorya fischeri var. *fischeri*	687.71	CBS
Paecilomyces variotii	339.51	CBS
Penicillium cyclopium	123.14	CBS
Penicillium islandicum	338.48	CBS
Penicillium spinulosum	346.61	CBS
Pichia norvegensis	6564	CBS

Table 1. *continued*

Organism	Isolate no.	Source
Pseudallescheria boydii	835.96	CBS
Pythium insidiosum	673.85	CBS
Rhizomucor miehei	MG4(2) (FJ713069)	CRT
Rhizopus stolonifer	389.95	CBS
Rhodosporidium toruloides	6016	CBS
Rhodotorula mucilaginosa	326	CBS

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1	*Scedosporium apiospermum*	117407	CBS
2	*Scedosporium aurantiacum*	121926	CBS
3	*Scedosporium aurantiacum*	118934	CBS
4	*Scedosporium prolificans*	102176	CBS
5	*Sporidiobolus salmonicolor*	6781	CBS
6	*Trichoderma hamatum*	GD12 (AY247559)	CRT
7	*Trichosporon asahii* var. *asahii*	8973	CBS
8	*Trichosporon asahii* var. *asahii*	5286	CBS
9	*Trichosporon asahii* var. *asahii*	7632	CBS
10	*Trichosporon asahii* var. *asahii*	5599	CBS
11	*Trichosporon asteroides*	6183	CBS
12	*Trichosporon asteroides*	7623	CBS
13	*Trichosporon asteroides*	2481	CBS
14			
15	**Table 1.** *continued*		
16			
17	Organism	Isolate no.	Source
18			
19	*Trichosporon asteroides*	7624	CBS
20	*Trichosporon cutaneum*	2466	CBS
21	*Trichosporon inkin*	7630	CBS
22	*Trichosporon inkin*	7655	CBS
23	*Trichosporon loubieri*	7065	CBS

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~~*Trichosporon ovoides* 7556 CBS~~

~~*Trichosporon mycotoxinovorans* 9756 CBS~~

~~*Wickerhamomyces anomalus* 5759 CBS~~

~~a. CBS = Centraalbureau voor Schimmelcultures, PO Box 85167, 3508 AD Utrecht, The Netherlands; CRT = C.R. Thornton; IMI = International Mycological Institute, Egham, England; SV = S. Krappman, Institute of Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Georg-August-University, Gottingen, Germany.~~

Table 1~~2~~. Locations and identities of sink swabs and water samples and results of ELISA tests and fungal identification based on ITS sequencing.

Location (Source)[a]	Swab ELISA abs (450nm)[b]	Mixed culture ELISA abs (450nm)[b]	Isolate No.	Axenic culture ELISA abs (450nm)[b]	Identification based on ITS sequencing[c]	GenBank Accession No.
University (S1)	0.0763	0.0575	A1-1	1.3736	***Fusarium oxysporum***	KT876668
University (S1)			A1-2	0.7646	***Fusarium oxysporum***	KT876662
University (S1)			A1-3	0.0196	*Penicillium crustosum*	KT876719
University (S1)			A1-4	0.0139	*Penicillium expansum*	KT876718
University (S2)	0.5472	0.0045	A2-1	1.5723	***Fusarium solani***	KT876635
University (S2)			A2-3	0.6554	***Fusarium oxysporum***	KT876690
University (S2)			A2-5	1.1312	***Fusarium solani***	KT876631
University (S3)	0.0053	0.0077	A3-1	0.0082	*Trichosporon domesticum*	KT876717
University (S3)			A3-2	0.0162	*Cadophora fastigiata*	KT876615
University (S3)			A3-3	0.0014	*Cyphellophora oxyspora*	KT876613

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University (S3)			A3-4	0.0093	*Penicillium crustosum*	KT876714
University (S4)	0.0297	0.0049	A4-1	0.0105	*Phoma herbarum*	KT876697
University (S4)			A4-2	0.0079	*Penicillium echinulatum*	KT876710
University (S4)			A4-3	0.0029	*Cytobasidium slooffiae*	KT876704
University (S4)			A4-6	0.0060	*Trichoderma asperellum*	KT876620
University (S5)	0.0206	0.0039	A5-1	1.1815	***Fusarium oxysporum***	KT876692
University (S5)			A5-2	0.0034	*Penicillium crustosum*	KT876715
University (S5)			A5-5	0.0088	*Aspergillus niger*	KT876702
University (S5)			A5-6	1.0630	***Fusarium oxysporum***	KT876667
University (S5)			A5-7	0.0037	*Rhodotorula mucilaginosa*	KT876700
University (S5)			A5-8	0.0088	*Cyphellophora oxyspora*	KT876614
University (S6)	0.0251	0.0412	A6-1	1.1130	***Fusarium oxysporum***	KT876648
University (S6)			A6-2	0.9410	***Fusarium oxysporum***	KT876678
University (S6)			A6-3	0.6377	***Fusarium oxysporum***	KT876688
University (S6)			A6-4	1.0020	***Fusarium solani***	KT876640
University (S7)	0.1716	0.0082	A7-1	0.0098	*Exophiala pisciphila*	KT876618
University (S7)			A7-2	0.0096	*Penicillium brevicompactum*	KT876695
University (S7)			A7-3	1.0556	***Fusarium oxysporum***	KT876684
University (S7)			A7-4	1.0077	***Fusarium oxysporum***	KT876671
University (S8)	0.0431	0.0008	A8-1	1.1045	***Fusarium oxysporum***	KT876672
University (S8)			A8-2	0.9707	***Fusarium oxysporum***	KT876654
University (S8)			A8-3	0.0091	*Trichoderma atroviride*	KT876622
University (S9)	0.3806	0.0052	A9-1	0.9087	***Fusarium solani***	KT876639
University (S9)			A9-2	0.8849	***Fusarium solani***	KT876638
University (S9)			A9-3	0.9004	***Fusarium solani***	KT876632
University (S9)			A9-4	0.8093	***Fusarium solani***	KT876636
University (S10)	0.0034	0.0028	A10-1	0.0188	*Rhodotorula slooffiae*	
University (S10)			A10-2	0.0020	*Exophiala pisciphila*	KT876616
University (S11)	0.0231	0.0005	B1-1	0.6064	***Fusarium dimerum***	KT876625
University (S11)			B1-6	0.8138	***Fusarium dimerum***	KT876628
University (S12)	0.0200	0.0020	B2-1	0.5678	***Fusarium dimerum***	KT876626
University (S12)			B2-5	0.4827	***Fusarium dimerum***	KT876624
University (S13)	0.0091	0.0023	B3-4	0.0026	*Phoma herbarum*	KT876696
University (S14)	0.0163	0.0008	B4-1	0.6992	***Fusarium dimerum***	KT876627
University (S15)	0.0132	0.0003	B5-1	0.8008	***Fusarium oxysporum***	KT876674
University (S15)			B5-2	0.0048	*Mucor circinelloides*	KT876701
University (S15)			B5-3	0.8851	***Fusarium oxysporum***	KT876677
University (S16)	0.0229	0.0002	B6-1	0.8193	***Fusarium oxysporum***	KT876676
University (S16)			B6-2	0.7582	***Fusarium oxysporum***	KT876661
University (S17)	0.0395	0.0014	B7-1	0.8201	***Fusarium oxysporum***	KT876675
University (S17)			B7-6	0.7758	***Fusarium dimerum***	KT876623
University (S18)	0.0133	0.0011	B8-1	0.7347	***Fusarium solani***	KT876637
University (S18)			B8-7	0.0095	*Rhodosporidium babjevae*	KT876706
University (S19)	0.0212	0.0048	B9-1	0.0051	*Meyerozyma guilliermondii*	KT876707
University (S19)			B9-2	0.8104	***Fusarium oxysporum***	KT876657

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Fusarium-specific monoclonal antibody

University (S19)			B9-3	0.6302	***Fusarium solani***	KT876634
University (S19)			B9-5	0.0017	*Penicillium crustosum*	KT876720
University (S20)	0.5467	0.0394	B10-1	0.7467	***Fusarium oxysporum***	KT876680
University (S20)			B10-2	0.7667	***Fusarium oxysporum***	KT876659
University (S20)			B10-6	0.0071	*Candida parapsilosis*	KT876703
University (S20)			B10-7	0.0251	*Meyerozyma guilliermondii*	KT876711
University (S20)			B10-9	0.0031	*Cystobasidium slooffiae*	KT876712
University (S20)			B10-12	0.0251	*Trichosporon asteroides*	KT876713
University (S21)	0.0083	0.0269	C1-1	0.0044	*Clavispora lusitaniae*	KT876708
University (S21)			C1-3	0.8875	***Fusarium oxysporum***	KT876682
University (S21)			C1-4	0.8884	***Fusarium dimerum***	KT876629
University (S21)			C1-7	0.0003	*Exophiala pisciphila*	KT876617
University (S22)	0.2977	0.0047	C2-1	0.9443	***Fusarium oxysporum***	KT876658
University (S22)			C2-4	0.9825	***Fusarium oxysporum***	KT876694
University (S22)			C2-7	0.8677	***Fusarium oxysporum***	KT876687
University (S23)	0.0808	0.0116	C3-1	0.9853	***Fusarium oxysporum***	KT876683
University (S23)			C3-2	1.0160	***Fusarium oxysporum***	KT876693
University (S23)			C3-4	0.7485	***Fusarium oxysporum***	KT876644
University (S24)	0.1113	0.0202	C4-1	0.8930	***Fusarium oxysporum***	KT876670
University (S24)			C4-2	0.0028	*Candida intermedia*	KT876709
University (S24)			C4-5	0.9008	***Fusarium dimerum***	KT876630
University (S25)	0.5741	0.0097	C5-1	0.9874	***Fusarium oxysporum***	KT876652
University (S25)			C5-2	1.0996	***Fusarium oxysporum***	KT876656
University (S25)			C5-3	1.1236	***Fusarium oxysporum***	KT876649
University (S25)			C5-4	1.0355	***Fusarium oxysporum***	KT876669
University (S25)			C5-5	1.0672	***Fusarium oxysporum***	KT876660
University (S26)	0.0603	0.0348	C6-1	1.0135	***Fusarium oxysporum***	KT876665
University (S26)			C6-2	0.9362	***Fusarium oxysporum***	KT876681
University (S26)			C6-3	0.8898	***Fusarium oxysporum***	
University (S26)			C6-4	1.0577	***Fusarium oxysporum***	KT876686
University (S26)			C6-5	0.9445	***Fusarium oxysporum***	KT876646
University (S27)	0.1279	0.0113	C7-1	0.9724	***Fusarium oxysporum***	KT876666
University (S28)	0.0607	0.0035	C8-1	0.8694	***Fusarium oxysporum***	KT876685
University (S28)			C8-2	0.9599	***Fusarium oxysporum***	KT876655
University (S28)			C8-3	0.0031	*Penicillium expansum*	KT876716
University (S28)			C8-4	0.0049	*Phoma herbarum*	KT876698
University (S28)			C8-6	0.9003	***Fusarium oxysporum***	KT876679
University (S29)	0.0310	0.0001	C9-1	1.0488	***Fusarium oxysporum***	KT876651
University (S29)			C9-2	0.9254	***Fusarium oxysporum***	KT876645
University (S29)			C9-3	0.9711	***Fusarium oxysporum***	KT876664
University (S29)			C9-4	1.035	***Fusarium oxysporum***	KT876647
University (S29)			C9-5	0.9604	***Fusarium oxysporum***	KT876663
University (S30)	0.1002	0.0333	C10-1	0.9254	***Fusarium oxysporum***	KT876689
University (S30)			C10-2	1.0198	***Fusarium solani***	KT876642
University (S30)			C10-4	0.9007	***Fusarium solani***	KT876641
University (S30)			C10-7	1.0697	***Fusarium solani***	KT876643
University (S31)	0.6175	0.1129	CRT1-1	0.4639	***Fusarium oxysporum***	KT876691
University (S31)			CRT1-2	0.9086	***Fusarium oxysporum***	KT876650

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University (S31)			CRT1-3	0.7503	***Fusarium oxysporum***	KT876652
University (S32)	0.0303	0.0021	CRT2-1	0.0040	*Trichoderma asperellum*	KT876619
University (S32)			CRT2-2	0.0417	*Trichoderma asperellum*	KT876621
University (S33)	0.0663	0.0028	CRT3-1	0.0256	*Phoma herbarum*	KT876699
ICU (S34)	0.0285	0.3620	R1-1	1.5057	***Fusarium solani***	KT876550
ICU (S34)			R1-2	1.5154	***Fusarium solani***	KT876551
ICU (S34)			R1-3	1.5555	***Fusarium solani***	KT876549
ICU (S35)	0.0071	0.0147	R2-4	0.0096	*Clonostachys rosea*	KT876552
ICU (S35)			R2-5	0.0018	*Clonostachys rosea*	KT876553
ICU (S35)			R2-6	0.0035	*Clonostachys rosea*	KT876554
ICU (S36)	0.0391	0.045	R3-1	0.0006	*Trichoderma asperellum*	KT876548
ITU (S37)	0.7291	0.7724	R5-1	1.5606	***Fusarium dimerum***	KT876561
ITU (S37)			R5-2	1.3481	***Fusarium dimerum***	KT876565
ITU (S37)			R5-3	1.4990	***Fusarium dimerum***	KT876567
ITU (S38)	1.2086	0.3691	R6-1	1.3388	***Fusarium dimerum***	KT876572
ITU (S38)			R6-2	1.4734	***Fusarium dimerum***	KT876563
ITU (S38)			R6-3	1.4312	***Fusarium dimerum***	KT876562
ITU (S38)			R6-9	1.2648	***Fusarium oxysporum***	KT876557
ITU (S39)	0.1121	0.8186	R7-1	1.3266	***Fusarium dimerum***	KT876570
ITU (S39)			R7-2	1.2352	***Fusarium dimerum***	KT876564
ITU (S40)	1.0157	0.3612	R8-1	1.2399	***Fusarium dimerum***	KT876568
ITU (S40)			R8-2	1.1552	***Fusarium dimerum***	KT876566
ITU (S40)			R8-3	1.1856	***Fusarium dimerum***	KT876558
ITU (S41)	0.0391	0.0477	R9-3	0.0113	*Exophiala phaeomuriformis*	KT876555
ITU (S42)	0.0549	0.0097	R10-2	0.0072	*Cadophora fastigiata*	KT876556
ITU (S42)			R10-6	0.0124	*Coniochaeta fasciculata*	KT876721
ITU (S43)	0.0630	1.5751	X2-2	1.1492	***Fusarium dimerum***	KT876571
ITU (S43)			X2-4	1.2505	***Fusarium dimerum***	KT876560
ITU (S43)			X2-5	1.1397	***Fusarium dimerum***	KT876569
ITU (S43)			X2-6	1.1716	***Fusarium dimerum***	KT876559
Ophthalmology Unit (S44)	1.0313	1.5035	X3-1	1.1353	***Fusarium dimerum***	KT876509
Ophthalmology Unit (S44)			X3-2	0.0224	*Candida parapsilosis*	KT876498
Ophthalmology Unit (S44)			X3-3	1.1856	***Fusarium dimerum***	KT876512
Ophthalmology Unit (S45)	1.4582	1.4049	X4-1	1.1322	***Fusarium dimerum***	KT876511
Ophthalmology Unit (S45)			X4-2	1.1523	***Fusarium dimerum***	KT876500
Ophthalmology Unit (S45)			X4-3	0.0622	*Candida parapsilosis*	KT876508
Ophthalmology Unit (S45)			X4-4	0.1245	*Gloeotinia temulenta*	KT876515
Ophthalmology Unit (S46)	0.3325	1.4466	X5-2	1.0654	***Fusarium dimerum***	KT876510
Ophthalmology Unit (S46)			X5-3	0.0599	*Rhodotorula mucilaginosa*	KT876501

Fusarium-specific monoclonal antibody

Ophthalmology Unit (S46)			X5-4	1.0862	***Fusarium dimerum***	KT876513
Ophthalmology Unit (S47)	0.2640	1.3729	X6-1	0.0616	*Candida parapsilosis*	KT876499
Ophthalmology Unit (S47)			X6-2	0.063	*Engyodontium album*	KT876522
Ophthalmology Unit (S47)			X6-3	0.0603	*Engyodontium album*	KT876521
Ophthalmology Unit (S47)			X6-4	0.0657	*Exophiala phaeomuriformis*	KT876504
Ophthalmology Unit (S48)	0.5299	0.0223	X8-2	0.0118	*Cladosporium macrocarpum*	KT876506
Ophthalmology Unit (S48)			X8-3	0.0985	*Gloeotinia temulenta*	KT876514
Ophthalmology Unit (S48)			X8-4	0.0662	*Engyodontium album*	KT876523
Ophthalmology Unit (S49)	0.1872	0.0061	X9-1	0.0686	*Engyodontium album*	KT876520
Ophthalmology Unit (S49)			X9-2	0.0611	*Candida parapsilosis*	KT876496
Ophthalmology Unit (S49)			X9-3	0.0641	*Candida parapsilosis*	KT876497
Ophthalmology Unit (S49)			X9-4	0.0677	*Exophiala dermatitidis*	KT876503
Ophthalmology Unit (S49)			X9-5	0.0705	*Engyodontium album*	KT876519
Ophthalmology Unit (S49)			X9-7	0.0660	*Exophiala pisciphila*	KT876502
Ophthalmology Unit (T1)	0.0132	0.0159	X10-1	0.0868	*Cladosporium sphaerospermum*	KT876507
Ophthalmology Unit (T1)			X10-3	0.0130	*Engyodontium album*	KT876518
Ophthalmology Unit (T2)	0.0117	0.0883	W1-1	0.0114	*Paraconiothyrium fuckelii*	KT876505
Ophthalmology Unit (T2)			W1-2	0.0139	*Engyodontium album*	KT876517
Ophthalmology Unit (T2)			W1-3	0.0108	*Engyodontium album*	KT876516
Haematology Unit (S50)	0.2694	0.0093	K1-1	0.0172	*Trichoderma asperellum*	KT876534
Haematology Unit (S51)	0.7466	1.1746	K2-1	0.0144	*Trichoderma viride*	KT876533
Haematology Unit (S51)			K2-3	1.1385	***Fusarium solani***	KT876543
Haematology Unit (S51)			K2-4	1.2487	***Fusarium solani***	KT876542
Haematology Unit (S52)	1.0621	1.3090	K6-1	1.1544	***Fusarium oxysporum***	KT876541
Haematology			K6-4	0.0071	*Candida parapsilosis*	KT876524

Fusarium-specific monoclonal antibody

Unit (S52)						
Haematology Unit (S53)	1.0120	1.2628	K8-1	1.2376	*Fusarium solani*	KT876722
Haematology Unit (S53)			K8-2	1.2983	*Fusarium solani*	KT876723
Haematology Unit (S53)			K8-6	0.0084	*Cadophora fastigiata*	KT876531
Haematology Unit (S53)			K8-7	0.0051	*Trichoderma asperellum*	KT876535
Haematology Unit (S54)	0.9843	1.2903	K9-1	0.0046	*Candida parapsilosis*	KT876525
Haematology Unit (S54)			K9-2	0.0090	*Rhodotorula dairenensis*	KT876526
Haematology Unit (S54)			K9-4	0.0073	*Cadophora fastigiata*	KT876532
Haematology Unit (S54)			K9-5	1.1652	***Fusarium solani***	KT876547
Haematology Unit (S54)			K9-6	1.0316	***Fusarium solani***	KT876545
Haematology Unit (S55)	0.8638	1.0753	K10-1	1.0684	***Fusarium solani***	KT876546
Haematology Unit (S55)			K10-2	1.0386	***Fusarium solani***	KT876544
Haematology Unit (W50)	0.0082	0.0451	G1-2	0.0097	*Exophiala pisciphila*	KT876529
Haematology Unit (W51)	0.0581	0.0553	G2-1	0.0104	*Engyodontium album*	KT876540
Haematology Unit (W51)			G2-3	0.0062	*Exophiala pisciphila*	KT876530
Haematology Unit (W52)	0.0125	0.0118	G6-2	0.0161	*Exophiala castellanii*	KT876528
Haematology Unit (W53)	0.0075	0.0219	G8-1	0.0065	*Engyodontium album*	KT876538
Haematology Unit (W54)	0.0002	0.0164	G9-2	0.0005	*Gloeotinia temulenta*	KT876537
Haematology Unit (W55)	0.0021	0.0182	G10-1	0.0051	*Engyodontium album*	KT876539
Oncology Unit (S56)	0.4822	1.2287	H1-1	0.9022	***Fusarium dimerum***	KT876595
Oncology Unit (S56)			H1-3	0.0151	*Pichia kudriavzevii*	KT876578
Oncology Unit (S57)	1.0579	1.1650	H2-1	0.9312	***Fusarium dimerum***	KT876590
Oncology Unit (S57)			H2-5	0.0061	*Magnusiomyces capitatus*	KT876611
Oncology Unit (S58)	1.0938	1.2091	H3-1	0.8909	***Fusarium oxysporum***	KT876584
Oncology Unit (S58)			H3-4	0.0075	*Candida palmioleophila*	KT876573
Oncology Unit			H3-5	0.0140	*Rhodotorula glutinis*	KT876598

Fusarium-specific monoclonal antibody

(S58)						
Oncology Unit (S59)	0.0253	1.0856	H4-1	0.8884	***Fusarium dimerum***	KT876596
Oncology Unit (S59)			H4-3	0.0004	*Magnusiomyces capitatus*	KT876612
Oncology Unit (S60)	1.0856	1.0193	H5-1	1.1148	***Fusarium dimerum***	KT876593
Oncology Unit (S60)			H5-3	0.005	*Candida tropicalis*	KT876574
Oncology Unit (S61)	0.5256	1.1898	H6-1	1.1238	***Fusarium dimerum***	KT876594
Oncology Unit (S62)	1.0789	1.1588	H7-1	1.1513	***Fusarium oxysporum***	KT876583
Oncology Unit (S62)			H7-3	0.0045	*Candida tropicalis*	KT876575
Oncology Unit (S63)	0.0953	0.0152	H8-1	0.0063	*Phoma herbarum*	KT876580
Oncology Unit (S63)			H8-3	0.0126	*Candida albicans*	KT876577
Oncology Unit (S63)			H8-4	0.0088	*Rhodotorula mucilaginosa*	KT876599
Oncology Unit (S64)	0.1926	0.0162	H9-1	0.0152	*Exophiala dermatitidis*	KT876581
Oncology Unit (S64)			H9-2	0.0049	*Candida orthopsilosis*	KT876576
Oncology Unit (S64)			H9-3	0.0109	*Pichia kudriavzevii*	KT876579
Oncology Unit (S65)	0.4961	1.1005	H10-1	0.9606	***Fusarium dimerum***	KT876589
Oncology Unit (S65)			H10-4	0.0163	*Trametes ochracea*	KT876608
Oncology Unit (W56)	0.0030	0.0247	P1-2	0.0082	*Exophiala dermatitidis*	KT876582
Oncology Unit (W56)			P1-3	0.0024	*Trametes versicolor*	KT876603
Oncology Unit (W57)	0.0061	1.0891	P2-1	0.8451	***Fusarium dimerum***	KT876587
Oncology Unit (W57)			P2-2	0.9429	***Fusarium dimerum***	KT876597
Oncology Unit (W57)			P2-3	0.8627	***Fusarium dimerum***	KT876588
Oncology Unit (W58)	0.0016	0.0202	P3-1	0.0177	*Trametes ochracea*	KT876607
Oncology Unit (W59)	0.0035	0.0125	P4-1	0.0164	*Beauveria bassiana*	KT876586
Oncology Unit (W59)			P4-2	0.0084	*Gliomastix polychroma*	KT876584
Oncology Unit (W60)	0.0025	1.1746	P5-1	0.8563	***Fusarium dimerum***	KT876591
Oncology Unit			P5-2	0.8066	***Fusarium dimerum***	KT876592

Fusarium-specific monoclonal antibody

(W60)						
Oncology Unit (W60)			P5-3	0.0024	*Piptoporus betulinus*	KT876609
Oncology Unit (W62)	0.0011	0.0417	P7-1	0.0097	*Trametes versicolor*	KT876605
Oncology Unit (W63)	0.0044	0.0060	P8-1	0.0144	*Trametes versicolor*	KT876606
Oncology Unit (W63)			P8-2	0.0106	*Stereum gausapatum*	KT876601
Oncology Unit (W64)	0.0022	0.0163	P9-1	0.0101	*Stereum gausapatum*	KT876600
Oncology Unit (W64)			P9-2	0.0068	*Trametes versicolor*	KT876604
Oncology Unit (W64)			P9-3	0.0140	*Stereum gausapatum*	KT876602
Oncology Unit (W65)	0.0051	0.0280	P10-1	0.0032	*Phaeophlebiopsis peniophoroides*	KT876610

a. S, sink; W, tap water from corresponding sink number; T, water sample from main tank.

b. Threshold absorbance value for detection in ELISA ≥0.100. Shading indicates earliest point in sampling process at which antigen was detectable in ELISA tests with ~~mAb~~ ED7.

c. *Fusarium* strains in bold further characterized by TEF-1α PCR analysis (Table S~~3~~4 and ~~-~~Supporting Data Set 1~~Appendix 1~~).

~~**Table 3**. Summary of ELISA tests and mycological culture of sink swabs.~~

~~Total no. sinks swabbed~~	~~No. swab samples yielding fungi (%)[a]~~	~~No. samples positive for *Fusarium* antigen at swab stage~~	~~No. samples positive for *Fusarium* antigen by mixed culture stage~~	~~No. samples positive for *Fusarium* antigen by axenic culture stage~~	~~No. antigen positive samples yielding *Fusarium* spp.~~	~~No. antigen positive samples not yielding *Fusarium* spp.~~
~~65~~	~~65 (100%)~~	~~34 (52%)~~	~~37 (57%)~~	~~54 (83%)~~	~~50 (93%)~~	~~4 (7%)~~

~~**a.** *Fusarium* spp. and/or unrelated fungi.~~

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Fusarium-specific monoclonal antibody

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~~**Table 4.** Translation Elongation Factor-1α PCR analysis of *Fusarium* isolates recovered from sinks.~~

~~**Isolate No.**~~	~~**Location**~~	~~**TEF-1α ID**~~
~~A1-1~~	~~University~~	~~*F. oxysporum* species complex 33~~

Fusarium-specific monoclonal antibody

A1-2	University	*F. oxysporum* species complex 33
A2-1	University	*F. solani* species complex 1-a
A2-3	University	*F. oxysporum* species complex 33
A2-5	University	*F. solani* species complex 1-a
A5-1	University	*F. oxysporum* species complex 33
A5-6	University	*F. oxysporum* species complex 33
A6-1	University	*F. oxysporum* species complex 33
A6-2	University	*F. oxysporum* species complex 33
A6-3	University	*F. oxysporum* species complex 33
A6-4	University	*F. solani* species complex 9-a
A7-3	University	*F. oxysporum* species complex 183
A7-4	University	*F. oxysporum* species complex 126
A8-1	University	*F. oxysporum* species complex 33
A8-2	University	*F. oxysporum* species complex 33
A9-1	University	*F. solani* species complex 5-d
A9-2	University	*F. solani* species complex 5-d
A9-3	University	*F. solani* species complex 1-e
A9-4	University	*F. solani* species complex 1-a
B1-1	University	*F. dimerum* species complex ET-gr.
B1-6	University	*F. dimerum* species complex ET-gr.
B2-1	University	*F. dimerum* species complex ET-gr.
B2-5	University	*F. dimerum* species complex ET-gr.
B4-1	University	*F. dimerum* species complex ET-gr.
B5-1	University	*F. oxysporum* species complex 16
B5-3	University	*F. oxysporum* species complex 16
B6-1	University	*F. oxysporum* species complex 16
B6-2	University	*F. oxysporum* species complex 16
B7-1	University	*F. oxysporum* species complex 16
B7-6	University	*F. dimerum* species complex ET-gr.
B8-1	University	*F. solani* species complex 15-a
B9-2	University	*F. oxysporum* species complex 99
B9-3	University	*F. solani* species complex 1-a
B10-1	University	*F. oxysporum* species complex 33
B10-2	University	*F. oxysporum* species complex 33
C1-3	University	*F. oxysporum* species complex 33
C1-4	University	*F. dimerum* species complex ET-gr.
C2-1	University	*F. oxysporum* species complex 33
C2-4	University	*F. oxysporum* species complex 33
C2-7	University	*F. oxysporum* species complex 33
C3-1	University	*F. oxysporum* species complex 134
C3-2	University	*F. oxysporum* species complex 134
C3-4	University	*F. oxysporum* species complex 134
C4-1	University	*F. oxysporum* species complex 33
C4-5	University	*F. dimerum* species complex ET-gr.
C5-1	University	*F. oxysporum* species complex 33
C5-2	University	*F. oxysporum* species complex 126
C5-3	University	*F. oxysporum* species complex 33
C5-4	University	*F. oxysporum* species complex 33
C5-5	University	*F. oxysporum* species complex 33

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C6-1	University	*F. oxysporum* species complex 33
C6-2	University	*F. oxysporum* species complex 33
C6-3	University	*F. oxysporum* species complex 33
C6-4	University	*F. oxysporum* species complex 33
C6-5	University	*F. oxysporum* species complex 33
C7-1	University	*F. oxysporum* species complex 134
C8-1	University	*F. oxysporum* species complex 134
C8-2	University	*F. oxysporum* species complex 134
C8-6	University	*F. oxysporum* species complex 33
C9-1	University	*F. oxysporum* species complex 134
C9-2	University	*F. oxysporum* species complex 134
C9-3	University	*F. oxysporum* species complex 134
C9-4	University	*F. oxysporum* species complex 134
C9-5	University	*F. oxysporum* species complex 134
C10-1	University	*F. oxysporum* species complex 111
C10-2	University	*F. solani* species complex 2-v
C10-4	University	*F. solani* species complex 2-v
C10-7	University	*F. solani* species complex 2-v
CRT1-1	University	*F. oxysporum* species complex 33
CRT1-2	University	*F. oxysporum* species complex 33
CRT1-3	University	*F. oxysporum* species complex 33
R1-1	ICU	*F. solani* species complex 1-a
R1-2	ICU	*F. solani* species complex 1-a
R1-3	ICU	*F. solani* species complex 1-a
R5-1	ITU	*F. dimerum* species complex ET-gr.
R5-2	ITU	*F. dimerum* species complex ET-gr.
R5-3	ITU	*F. dimerum* species complex ET-gr.
R6-1	ITU	*F. dimerum* species complex ET-gr.
R6-2	ITU	*F. dimerum* species complex ET-gr.
R6-3	ITU	*F. dimerum* species complex ET-gr.
R6-9	ITU	*F. solani* species complex 20-d
R7-1	ITU	*F. dimerum* species complex ET-gr.
R7-2	ITU	*F. dimerum* species complex ET-gr.
R8-1	ITU	*F. dimerum* species complex ET-gr.
R8-2	ITU	*F. dimerum* species complex ET-gr.
R8-3	ITU	*F. dimerum* species complex ET-gr.
X2-2	ITU	*F. dimerum* species complex ET-gr.
X2-4	ITU	*F. dimerum* species complex ET-gr.
X2-5	ITU	*F. dimerum* species complex ET-gr.
X2-6	ITU	*F. dimerum* species complex ET-gr.
X3-1	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
X3-3	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
X4-1	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
X4-2	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
X5-2	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
X5-4	Ophthalmology Unit	*F. dimerum* species complex ET-gr.
K2-3	Haematology Unit	*F. solani* species complex 5-k
K2-4	Haematology Unit	*F. solani* species complex 5-k
K6-1	Haematology Unit	*F. oxysporum* species complex 33

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K8-1	Haematology Unit	*F. solani* species complex 5-k
K8-2	Haematology Unit	*F. solani* species complex 5-k
K9-5	Haematology Unit	*F. solani* species complex 2-a
K9-6	Haematology Unit	*F. solani* species complex 2-a
K10-1	Haematology Unit	*F. solani* species complex 2-a
K10-2	Haematology Unit	*F. solani* species complex 2-a
H1-1	Oncology Unit	*F. dimerum* species complex ET-gr.
H2-1	Oncology Unit	*F. dimerum* species complex ET-gr.
H3-1	Oncology Unit	*F. oxysporum* species complex 33
H4-1	Oncology Unit	*F. dimerum* species complex ET-gr.
H5-1	Oncology Unit	*F. dimerum* species complex ET-gr.
H6-1	Oncology Unit	*F. dimerum* species complex ET-gr.
H7-1	Oncology Unit	*F. oxysporum* species complex 33
H10-1	Oncology Unit	*F. dimerum* species complex ET-gr.
P2-1	Oncology Unit	*F. dimerum* species complex ET-gr.
P2-2	Oncology Unit	*F. dimerum* species complex ET-gr.
P2-3	Oncology Unit	*F. dimerum* species complex ET-gr.
P5-1	Oncology Unit	*F. dimerum* species complex ET-gr.
P5-2	Oncology Unit	*F. dimerum* species complex ET-gr.

Figure legends

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Figure 1. **Specificity of ED7 determined by Enzyme-Linked Immunosorbent Assay tests of surface washings containing water-soluble antigens from *Fusarium* species and related and unrelated yeasts and molds.** (A) ELISA a~~ELISA a~~bsorbance values at 450 nm for ~~from specificity screening tests using mAb ED7 and~~ antigens from *F. solani* and unrelated yeasts and ~~mould~~molds ~~(A),~~ and (B) for antigens from the *F. solani* teleomorph *Haematonectria*~~-~~ *haematococca* and related *Fusarium* species ~~(B)~~. Wells were coated with 60 µg protein ml^{-1} buffer. Bars are the means of three biological replicates ± standard errors and ~~-~~ ~~t~~The threshold absorbance value for detection of antigen in ELISA is ≥0.100 (indicated by lines on graphs). Numbers in parentheses after species names denote strain numbers with further details of strains provided in Table S1.

Figure 2. **Characterisation ~~C~~~~haracterisation~~ of the ED7 antigen and its epitope and spatial distribution of the antigen in spores and hyphae~~ ~~bound by mAb ED7~~.** (A) Western immunoblot with ~~mAb~~ ED7 using culture fluid from 2-d-old PDB cultures of *F. solani* CBS224.34 (lane 1) and *F. oxysporum* f.sp. *lycopersici* CBS167.30 (lane 2). Wells were loaded with 1.6 µg of protein. M_r denotes molecular weight in kDa. Note the major extracellular antigen with molecular weight of ~200 kDa. (B) Absorbance values from ELISA tests with ~~mAb~~ ED7 using ~~immobilized antigen~~surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and treated with trypsin or PBS only (control) at 4°C and 37°C. Bars are the means of three biological replicates ± standard errors and bars with the same letter are not significantly different at $p<0.001$ (ANOVA and Tukey-Kramer test). (C) Absorbance values from ELISA tests with ~~mAb~~ ED7 using surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and ~~immobilized antigen~~ treated with pronase or Milli-Q H_2O only (control) at 4°C and 37°C. Bars are the means of three biological replicates ± standard errors and bars with the same

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letter are not significantly different at p<0.001 (ANOVA and Tukey-Kramer test). (D) ~~Absorbance values from ELISA tests with mAb ED7 following~~ Stability~~heating~~ of the water-soluble ED7 antigen following heating of surface washings ~~antigen~~ at 100°C over a 70 min period. Treated antigen was subsequently immobilized to the wells of microtitre plates and assayed by ELISA. Bars are the means of three biological replicates ± standard errors and bars with the same letter are not significantly different at p<0.001 (ANOVA and Tukey-Kramer test). (E) Absorbance values from ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and ~~Absorbance values from ELISA tests with mAb ED7 and immobilized antigen~~ treated with periodate (open circles) or with acetate only control (closed circles) at 4°C over a 20 h period. Each point is the mean of three biological replicates ± standard errors. (F to ~~and~~ I~~G~~) Photomicrographs of *F. solani* CBS224.34 immunostained with ~~mAb~~ ED7 or TCM control and goat anti-mouse polyvalent Ig fluorescein isothiocyanate (FITC) conjugate. (F) Brightfield image of germinated conidium with hypha probed with ED7 followed by fluorochrome conjugate (scale bar = 6 µm); (G) Same field of view as panel F but examined under epifluorescence. Note intense staining of the cell wall of microconidium and hypha; ~~. Scale bar = 6 µm.~~ (H) Brightfield image of germinated conidium with hypha probed with TCM (negative control) followed by FITC conjugate (scale bar = 3 µm); (I) Same field of view as panel H but examined under epifluorescence. Note lack of staining, demonstrating specific binding of ED7 to surface antigen. (~~H~~ J-L) Immunogold labeling of sections of conidia and hyphae of *F. solani* CBS224.34. (J~~H~~) Transverse section of conidium incubated with ~~mAb~~ ED7 and anti-mouse immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular fimbrial matrix surrounding the spore (scale bar = 250 nm); (K~~I~~) Longitudinal section of hypha incubated with ~~mAb~~ ED7 and anti-mouse immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular

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matrix surrounding the cell (scale bar = 100 nm); (L~~J~~) Transverse section of a ~~micro~~conidium incubated with TCM~~TCM (~~ (negative control) and anti-mouse immunoglobulin 20 nm gold particles, showing lack of staining by the secondary gold conjugate~~reporter. S~~ (scale bar = 180 nm).

Fusarium-specific monoclonal antibody

Detection of human pathogenic *Fusarium* species in hospital and communal sink biofilms by using a highly specific monoclonal antibody

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Summary

The fungus *Fusarium* is well known as a plant pathogen, but has recently emerged as an opportunistic pathogen of humans. Habitats providing direct human exposure to infectious propagules are largely unknown, but there is growing evidence that plumbing systems are sources of human pathogenic strains in the *Fusarium solani* species complex (FSSC) and *Fusarium oxysporum* species complex (FOSC), the most common groups infecting humans. Here, we use a newly developed *Fusarium*-specific monoclonal antibody (mAb ED7) to track FSSC and FOSC strains in sink drain biofilms by detecting its target antigen, an extracellular 200kDa carbohydrate, in saline swabs. The antigen was detectable in 52% of swab samples collected from sinks across a University campus and a tertiary care hospital. The mAb was 100% accurate in detecting FSSC, FOSC and *F. dimerum* species complex (FDSC) strains that were present, as mixed fungal communities, in 83% of sink drain biofilms. Specificity of the ELISA was confirmed by sequencing

of the internally transcribed spacer 1 (ITS1)-5.8S-ITS2 rRNA-encoding regions of culturable yeasts and molds that were recovered using mycological culture, while translation elongation factor (TEF)-1α analysis of *Fusarium* isolates included FSSC 1-a, FOSC 33 and FDSC ET-gr, the most common clinical pathotypes in each group.

Originality-Significance Statement

- Development of a monoclonal antibody (mAb ED7) specific to *Fusarium*, a fungal genus containing human and plant pathogens
- *Fusarium* diagnostic antigen detected in swabs of sink drain biofilms, with 100% accuracy of mAb-based ELISA confirmed by ITS sequencing of mixed fungal communities comprising human pathogenic yeasts and molds
- First report describing the use of a highly specifc mAb to track human pathogenic fusaria, demonstrating widespread occurrence of pathogen in communal and hospital sinks with potential for nosocomial and community acquired infections

Fusarium-specific monoclonal antibody

Introduction

Species in the fungal genus *Fusarium* are ubiquitous environmental molds, and pathogens of both plants and animals (Zhang *et al*., 2006; Thornton and Wills, 2015). In immunocompromised humans, such as patients with haematological malignancies and hematopoietic stem cell and solid organ transplant recipients, *Fusarium* species are significant emerging pathogens, causing a frequently fatal disseminated disease known as fusariosis with an associated mortality rate of 50-75% (Girmenia *et al*., 2000; Musa *et al*., 2000; Boutati and Anaissie, 1997; Dignani and Anaissie, 2004; Jensen *et al*., 2004; Nucci and Anaissie, 2007). In some tertiary cancer centres, *Fusarium* has emerged as the second most common mold pathogen after *Aspergillus* (Walsh and Groll, 1999; Muhammed *et al*., 2011). Regardless of human immune status, *Fusarium* species can cause localised nail infections (onychomycosis)(Arrese *et al*., 1996), bone and joint infections (Koehler et al., 2014), infections of burn wounds (Latenser, 2003), skin infections (Nucci and Anaissie, 2002; Gurusidappa and Mamatha, 2011), and are the most frequent cause of mycotic eye infections known as fungal keratitis (Jurkunas *et al*., 2009), leading to progressive corneal destruction and endophthalmitis, with loss of vision or even loss of the affected eye (Dursun *et al*., 2003; Edelstein *et al*., 2012).

A recent multistate outbreak of fungal keratitis in the USA and in Singapore and Hong Kong was associated with contact lens solution contaminated with multiple strains of *Fusarium* and which led to visual loss in many patients and the need for corneal transplantation (Chang *et al*., 2006). While such outbreaks are rare, disseminated *Fusarium* infections and keratomycoses have increased in frequency over the past decade (Koehler *et al*., 2014) and an increasing body of evidence suggests that the main environmental sources of human pathogenic *Fusarium* species are contaminated water systems (Doggett, 2000; Anaissie *et al*., 2002; Anaissie *et al*., 2011; Mehl and

Epstein, 2008). A number of studies have recovered pathogenic *Fusarium* species from plumbing fixtures and it is hypothesised that microbial biofilms on fixtures may serve as important reservoirs of infectious *Fusarium* propagules in hospitals and homes (Mehl and Epstein, 2008; Short *et al*., 2011).

Identification of environmental reservoirs of human pathogenic molds including *Fusarium* has typically relied on nucleic acid-based technologies following recovery of fungi using selective media (Anaissie *et al*., 2002; Mehl and Epstein, 2008; Short et al., 2011; Anaissie *et al*., 2011; Rougeron *et al*., 2014). Recently, highly specific monoclonal antibodies (mAb) have been used to identify pathogenic species or species complexes in environmental samples containing mixed populations of yeasts and molds (Thornton, 2009; Davies and Thornton, 2014; Thornton *et al*., 2015). While mAb-based approaches similarly rely on culture for recovery of fungi from environmental samples, detection of diagnostic antigens in crude culture extracts using genus- or species-specific mAbs offers an attractive alternative approach to pathogen detection, particularly when combined with unsophisticated diagnostic modalities such as lateral-flow technology (Thornton, 2008; Thornton, 2012).

In this study, we set out to determine whether a newly developed *Fusarium*-specific mAb (ED7) could be used to track the fungus by detecting a water-soluble diagnostic antigen in swabs of communal and hospital sinks. By using the mAb in an enzyme-linked immunsorbent assay (ELISA), we show that it can differentiate *Fusarium* species from other unrelated yeast and mold pathogens of humans present in mixed fungal communities encountered in sink biofilms. The ELISA represents a simple method for specific detection of *Fusarium* species in environmental reservoirs and for identifying plumbing systems contaminated with the fungus.

Fusarium-specific monoclonal antibody

Results

Production of hybridoma cell lines, isotyping of mAb and specificity

A single fusion was performed and 389 hybridoma cell lines were screened for specificity against a range of clinically relevant yeasts and molds (Table S1). The aim was to identify cell lines secreting mAbs specific to *Fusarium* that could be used to track the fungus in environmental samples containing mixed species of human pathogenic fungi. To this end, a single cell line, ED7, was identified that produced mAb belonging to the immunoglobulin class M (IgM), which was genus-specific, reacting in ELISA tests with antigens from *Fusarium* species and with the *F. solani* teleomorph *Haematonectria haematococca* only (Figs. 1A and 1B). It did not cross-react with antigens from a wide range of unrelated mold and yeast species (Fig. 1A).

Western blotting of the ED7 antigen and epitope characterization

Gel electrophoresis and western blotting studies showed that ED7 binds to a major antigen with molecular weight of ~200 kDa which is secreted extracellularly by both *F. solani* and *F. oxysporum* (Fig. 2A). *Fusarium solani* antigens were subjected to enzymatic (Fig. 2B and Fig. 2C), heat (Fig. 2D) and chemical (Fig. 2E) modifications in order to characterise the epitope bound by ED7. Reductions in mAb binding following treatment with pronase shows that its epitope consists of protein, while reductions with trypsin indicate a protein epitope containing positively charged lysine and arginine side chains. The lack of reduction in ED7 binding following digestion of immobilized antigen with trypsin (Fig. 2B) and pronase (Fig. 2C) shows that it does not bind to a protein epitope. Reductions in mAb binding following heat treatment shows that an epitope is heat labile. There was no significant reduction in ED7 binding over 70 min of heating, showing that its epitope is heat stable (Fig. 2D). Reductions in mAb binding following chemical digestion of an antigen with

periodate shows that its epitope is carbohydrate and contains vicinal hydroxyl groups. The pronounced reductions in ED7 binding following periodate oxidation shows that its epitope consists of carbohydrate residues (Fig. 2E). Taken together, these results indicate that ED7 binds to an extracellular antigen and that its epitope is a heat stable carbohydrate moeity containing vicinal hydroxyl groups.

Immunofluorescence and immunogold electron microscopy

Immuno-localisation studies using IF showed that the ED7 antigen was present on the surface of spores and hyphae (Figs. 2F-I), while IEM showed that the antigen was present in the spore and hyphal cell wall and in an extracellular fibrillar matrix surrounding both (Figs. 2J-L). In the TEM image shown in Fig. 2J, 56% of gold particles were distributed in the fibrillar matrix surrounding the cell, while 40% and 4% of gold particles were distributed in the cell wall and cytoplasm respectively. This shows that the ED7 antigen is predominantly extracellular or located within the cell wall.

Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the ITS regions of the rRNA-encoding gene unit and by Translation Elongation Factor-1α PCR

Monoclonal antibody ED7 was highly specific for the three human pathogenic species of *Fusarium*, *F. solani*, *F. oxysporum* and *F. dimerum*, which were culturable from 75% of the sink swabs (Table 1 and Table S2)). ELISA tests of the saline sink swabs showed that 52% contained detectable levels of *Fusarium* antigen (Table 1 and Table S2), with ELISA absorbance values in the range ≥0.100 (the threshold value for antigen detection) and up to 1.500. In four hospital samples (samples S47, S48 and S49 from ophthalmology and sample S64 from oncology) *Fusarium* strains could not be

recovered for identification by ITS sequencing despite detection of the diagnostic antigen in swab samples with absorbance values of 0.264, 0.530, 0.187 and 0.193 respectively (Table 1). This was likely due to the *Fusarium* isolates being outgrown in the mixed culture plates by faster growing or more abundant unrelated fungi. Importantly, ED7 was shown not to cross-react with unrelated fungi (axenic culture absorbance values of ≤0.100 in all cases) including the human pathogenic yeast or yeast-like fungi *Candida*, *Exophiala*, *Meyerozyma*, *Rhodotorula*, *Trichosporon*, the human pathogenic hyaline or dematiaceous molds *Aspergillus*, *Phialophora*, *Phoma*, *Trichoderma*, and the human pathogenic mucormycete *Mucor* (Table 1). The remaining 93% of samples positive for *Fusarium* antigen, either at the swab stage or following periods of biological amplification in mixed or axenic cultures (Table S2), yielded strains of the three *Fusarium* species. There was 100% concordance between *Fusarium* genus identification by ELISA and species identification by ITS sequencing (Table 1). The species of *F. solani* and *F. oxysporum* recovered from sink swabs were subsequently shown by TEF-1α PCR (Supporting Data Set 1) to belong to *F. solani* species complex (FSSC) 1-a, 1-c, 2-a, 2-v, 5-d, 5-k, 9-a, 15-a, 20-d and *F. oxysporum* (FOSC) species complexes 16, 33, 99, 111, 126, 134, 183 (Table S3). All of the recovered *F. dimerum* isolates belonged to the *F. dimerum* species complex (FDSC) ET-gr (Table S3). ITS analysis of axenic cultures (Table 1) showed that a number of sink samples (e.g. S2, S6, S17, S19, S21, S24, S30, S38) contained mixtures of *Fusarium* species, while ITS and TEF-PCR analysis (Table 1 and Table S3) showed that others contained mixtures of species complexes of the same species (e.g. S8, S9, S25, S28). Monoclonal antibody ED7 was able to detect all of the *Fusarium* species complexes recovered in this study.

In addition to drain swabs, water samples were collected from the taps of sinks in the hospital haematology and oncology units and from the main water tanks feeding the ophthalmology

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unit. The ED7 diagnostic antigen could not be detected in any of the water samples directly and, while all of the samples yielded fungi, only two of the tap samples (oncology W57 and W60) contained *Fusarium* strains that belonged to FDSC ET-gr. and which were detectable by ELISA at the mixed culture stage (Table 1). The sink biofilms corresponding to these water samples were also positive at the swab ELISA stage (Table 1).

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Discussion

The genus *Fusarium* comprises ubiquitous environmental molds capable of infecting plants and humans (Zhang *et al*., 2006). Unlike agriculture, where the most economically damaging pathogens are considered to be *F. graminearum* and *F. oxysporum* (Dean *et al*., 2012), the species most commonly cited as human pathogens belong to the *Fusarium solani* species complex (FSSC, responsible for 50% of reported infections in humans), followed by strains in the *Fusarium oxysporum* species complex (FOSC)(Torres and Kontoyiannis, 2011). The *Fusarium dimerum* species complex (FDSC) is less frequently reported as causing human disease, but it is similarly capable of causing disseminated infections in immunocompromised patients (Bigley *et al*., 2004; Schroers *et al*., 2009).

While the natural habitats of plant pathogenic *Fusarium* strains are well characterised as soil and decaying plant material, habitats providing direct human exposure to infectious propagules are largely unexplored. The increasing frequency of opportunistic fungal infections in humans means that improved surveillance methods are needed to identify environmental reservoirs of pathogens to limit the exposure of vulnerable individuals to potentially infective propagules. For *Fusarium*, there is a growing body of evidence to suggest that domestic and municipal water systems are potential reservoirs of human pathogenic strains in the FSSC, FOSC and FDSC groups (Short *et al*., 2011).

Accurate techniques that can be used to identify the fungus in environmental samples containing mixed populations of fungi are currently lacking and, while nucleic acid-based technologies have been developed for the differentiation of *Fusarium* from other human pathogenic species and to identify *Fusarium* species complexes, such techniques have typically been used in retrospective analysis of axenic cultures collected during human and environmental population studies (Bouchara *et al*., 2009; Steinmann *et al*., 2011; Lackner *et al*., 2012). Furthermore, these

studies have often employed *Fusarium*-selective media that eliminate other fungi present in polymicrobial communities (Short *et al*., 2011). While monoclonal antibodies (mAbs) and antibody fragments have been developed for detecting and differentiating *Fusarium* species *in vitro* or *in planta* (Wong *et al*., 1988; Arie *et al*., 1991, 1995; Danks *et al*., 1996; Hayashi *et al*., 1998; Hu *et al*., 2012, 2013), no attempts have been made to use mAbs to track human pathogenic strains in environmental samples. Jensen *et al*. (2011) recently reported the development of *Fusarium*-specific mAbs for immunohistochemical diagnosis of fusariosis. The IgM mAbs, which recognise 51 and 63 kDa antigens, reacted strongly with fungal elements in both experimentally infected animals and biopsy samples from patients with fusariosis sepsis and dissemination to the skin.

In this prospective study, we set out to determine whether human pathogenic species of *Fusarium* could be identified in sink drains directly by using crude antigen extracts of biofilms and detection using a genus-specific immunoglobulin M (IgM) mAb, ED7, that binds to an extracellular ~200kDa carbohydrate antigen present on the surface of spores and hyphae. While the function of the antigen is currently unknown we were able, in Enzyme-Linked Immunosorbent Assay (ELISA) tests, to detect its presence in 52% of swab samples and, following biological amplification of biofilms on a non-selective mycological medium, were able to identify additional biofilm samples containing pathogenic strains of *Fusarium*. This is the first time, to our knowledge, that a mAb-based detection method has been used to track *Fusarium* in environmental samples. The mAb was able to differentiate *Fusarium* from a wide spectrum of unrelated fungi, including the human pathogens *Aspergillus* (Thornton and Wills, 2015), *Candida*, *Geotrichum*, *Rhodotorula* and *Trichosporon* (Davies and Thornton, 2014; Miceli *et al*., 2011), *Cyphellophora* and *Phialophora* (Feng *et al*., 2014), *Exophiala* (Zeng *et al*., 2007), *Trichoderma* (Sandoval-Denis *et al*., 2014), *Engyodontium* (Macêdo *et al*., 2007; Thamke *et al*., 2015) and *Mucor* (Petrikkos *et al*., 2012),

several of which have been reported previously to inhabit biofilms in water distribution systems (Dogget, 2000). The 100% accuracy of the ED7 ELISA, confirmed by using ITS sequencing and TEF PCR analysis of recovered isolates, demonstrates its robustness in detecting potentially infectious *Fusarium* species in polymicrobial communities. Importantly, ED7 reacted with all of the species complex strains isolated including the most common clinical pathotypes of *Fusarium,* FSSC 1-a, FOSC 33 and FDSC ET-gr (Schroers *et al*., 2009; Short *et al*., 2011).

While the ED7 ELISA was able to identify *Fusarium* to the level of genus only, the simplicity of the mAb-based approach to detection, even when combined with a standard mycological isolation procedure, means that a recognised environmental niche of this group of pathogenic fungi can be monitored readily. The widespread occurrence of human pathogenic *Fusarium* species in sinks of a tertiary care hospital and sinks of a heavily populated university campus, show that indoor plumbing-associated biofilms and water sources are an unseen source of *Fusarium* infectious propagules for nosocomial and community-acquired infections of vulnerable individuals, an observation consistent with previous studies (Annaisie *et al*., 2011; Short *et al*., 2011). While no cases of fusariosis were reported during the course of this study, the close proximity of the patients to hospital sinks colonised with both pathogenic fusaria and with other opportunistic fungal pathogens is a serious concern given the known vulnerability of immunocompromised individuals to invasive fungal infections.

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Conflicts of Interest

We declare that none of the authors involved in writing this paper have any conflicts of interest with respect to the content of this article.

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Experimental procedures

Ethics statement

All animal work described in this study was conducted under a UK Home Office Project License, and was reviewed by the institution's Animal Welfare Ethical Review Board (AWERB) for approval. The work was carried out in accordance with The Animals (Scientific Procedures) Act 1986 Directive 2010/63/EU, and followed all the Codes of Practice which reinforce this law, including all elements of housing, care, and euthanasia of the animals. Permission for sink sampling at the Royal Devon and Exeter Hospital was granted by the Director of Infection Prevention and Control.

Fungal culture

Fungi (Table S1) were routinely cultured on Potato Dextrose Agar (PDA: 70139; Sigma), Sabouraud Dextrose Agar (SDA: Sabouraud Dextrose Broth (SDB: S3306; Sigma) containing 2% (w/v) agar), Malt Yeast extract Agar (MYA: Y3127; Sigma), or Oatmeal Agar (OA: O3506; Sigma), sterilized by autoclaving at 121^{o}C for 15 min. Cultures were grown at 26^{o}C under a 16 h fluorescent light regime.

Development of mAb, preparation of immunogen, and immunisation regime

BALB/c mice were immunized with soluble antigens prepared from lyophilized mycelium of a human pathogenic strain of *Fusarium solani* species complex 1-a (CBS strain 224.34). Conidia were suspended in water after 10-day old PDA slant cultures were flooded with 5 ml dH_2O and gently agitated with an inoculation loop. Conidial suspensions were then filtered through Miracloth

to remove mycelium and transferred to 1.5 ml micro-centrifuge tubes. The conidia were washed three times with dH_2O by repeated vortexing and centrifugation at 14,462 *g* for 5 min and finally suspended in dH_2O to give a concentration of 10^6 conidia ml^{-1} solution. Flasks containing 100 ml of sterilized Potato Dextrose Broth (PDB: P6685; Sigma) were inoculated with 200 µl of the conidial suspension and incubated with shaking (75 rpm) for 48 h at 26°C. Hyphal biomass was collected on Miracloth, snap frozen in liquid N_2, and lyophilized. Culture filtrates were retained for gel electrophoresis and western blotting studies and stored at -20°C until required. One mg of lyophilized biomass was suspended in 1 ml phosphate buffered saline (PBS: 0.8% NaCl; 0.02% KCl; 0.115% Na_2HPO_4; 0.02% KH_2PO_4; pH7.2) and the resultant suspension centrifuged for 5 min at 14,462 *g*. The supernatant, containing solubilized antigens, was used as the immunogen and as a source of antigens for hybridoma screening assays. For immunization, 6-wk-old BALB/c female white mice were given four intraperitoneal injections (300 µl per injection) of antigen extract containing 2.3 mg protein ml^{-1} PBS at 2-wk intervals and a single booster injection five days before fusion.

Production and screening of hybridomas and determination of antibody specificity

Hybridoma cells were produced by the method described elsewhere (Thornton, 2001) and the supernatants were screened by Enzyme-Linked Immunosorbent Assay (ELISA) against antigens immobilized to the wells of Maxisorp microtitre plates (442404; Nunc)(50 µl per well). For antibody specificity tests, fungi were grown on replicate agar slopes and surface washings containing water-soluble antigens prepared as described in Thornton (2001). Protein concentrations, determined spectrophotometrically at 280 nm (Nanodrop, Agilent Technologies Limited, Berkshire, UK), were adjusted to 60 µg ml^{-1} buffer. Fifty µl volumes were then used to coat the wells of

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microtitre plates. After incubating overnight at 4°C, wells were washed four times with PBST (PBS containing Tween-20, 0.05% (v/v)), once each with PBS and dH_2O and then air-dried at 23°C in a laminar flow hood. The plates were stored in sealed plastic bags at 4°C in preparation for screening of hybridoma supernatants by ELISA as described below.

Enzyme-Linked Immunosorbent Assay

Wells containing immobilized antigens were incubated successively with hybridoma tissue culture supernatant (TCS) for 1 h, followed with goat anti-mouse polyvalent (immunoglobulin classes IgG, IgA, and IgM) peroxidase conjugate (A-0412; Sigma Chemical Company, Poole, United Kingdom) diluted 1 in 1000 in PBST for a further hour. Bound antibody was visualized by incubating wells with tetramethyl benzidine (TMB: T-2885; Sigma) substrate solution (Thornton, 2001) for 30 min. The reactions were stopped by the addition of 3 M H_2SO_4 and absorbance values were determined at 450 nm with an MRX automated microplate reader (Dynex Technologies, Billingshurst, UK). Wells were given four 5-min rinses with PBST between incubations and a final rinse with PBS before addition of the substrate solution. Working volumes were 50 µl per well and control wells were incubated with tissue culture medium (TCM) containing 10% (v/v) fetal bovine serum. All incubation steps were performed at 23°C in sealed plastic bags. The threshold for detection of the antigen in ELISA was determined from control means (2 x TCM absorbance values)(Sutula *et al*., 1986). These values were consistently in the range 0.050-0.100. Consequently, absorbance values >0.100 were considered as positive for the detection of antigen.

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Determination of Ig subclass and cloning procedure

The Ig class of mAbs was determined by using antigen-mediated ELISA. Wells of microtitre plates coated with *F. solani* CBS224.34 water-soluble antigens from surface washings were incubated successively with ED7 TCS for 1 h, followed with goat anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, or IgA-specific antiserum (ISO-2; Sigma) diluted 1 in 3000 in PBST for 30 min and rabbit anti-goat peroxidase conjugate diluted 1 in 1000 (A-5420; Sigma) for a further 30 min. Bound antibody was visualized with TMB substrate as described above. Hybridoma cells lines were sub-cloned three times by limiting dilution, and cell lines were grown in bulk in a non-selective medium preserved by slowly freezing in fetal bovine serum/dimethyl sulfoxide (92:8 [v/v]), and stored in liquid nitrogen.

Gel electrophoresis and Western blotting

For sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), culture filtrates from 2-d-old PDB shake cultures of *F. solani* CBS224.34 and *F. oxysporum* f.sp. *lycopersici* CBS167.30, prepared as described, were diluted in Laemmli buffer (Laemmli, 1970) and were denatured by heating at 95°C for 10 min. Antigens were separated in 4-20% (w/v) polyacrylamide gradient gels (161-1159; Bio-Rad) for 1.5 h at 23°C (165V) under denaturing conditions, and pre-stained broad range markers (161-0318; Bio-Rad) were used for molecular weight determinations. For westerns, separated antigens were transferred electrophoretically to a PVDF membrane (162-0175; Bio-Rad). The membranes were blocked for 16 h at 4°C with PBS containing 1% (w/v) bovine serum albumin (BSA) and incubated with ED7 TCS diluted 1 in 2 with PBS containing 0.5% (w/v) BSA (PBSA) for 2 h at 23°C. After washing three times with PBS, membranes were incubated for 1 h with goat anti-mouse IgM (μ-chain specific) alkaline phosphatase conjugate (A-

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9688; Sigma), diluted 1 in 15,000 in PBSA. After the membranes were washed twice with PBS and once with PBST, the bound antibodies were visualized by incubation in BCIP/NBT substrate solution. Reactions were stopped by immersion in dH_2O and air-dried between sheets of Whatman filter paper.

Characterization of antigen by enzymatic and chemical modifications and by heating

Water-soluble antigens from surface washings of slopes of *F. solani* CBS224.34 were prepared as described. Heat stability studies were conducted by placing tubes of solubilised antigen in a boiling water bath. At 10 min intervals, samples were removed, centrifuged at 14,462 *g* for 5 min, and antigens immobilised to the wells of microtitre plates for assay by ELISA as described. For periodate oxidation, microtitre wells containing immobilised antigens from surface washings of the fungus were incubated with 50 µl of sodium *meta*-periodate solution (20 mM $NaIO_4$ in 50 mM sodium acetate buffer (pH4.5)) or acetate buffer only (control) at 4°C in sealed plastic bags. Plates were given four 3-min PBS washes before processing by ELISA as described. For protease digestions, microtitre wells containing immobilised antigen were incubated with 50 µl of pronase (protease XIV; 9 mg ml^{-1} in PBS) or trypsin (1 mg ml^{-1} in Milli-Q H_2O) solution or Milli-Q H_2O or PBS only controls respectively for 4 h at 37°C or 4°C. Plates were given four 3-min rinses with PBS and then assayed by ELISA with ED7 TCS as described.

Immunofluorescence and immunogold electron microscopy

For immunfluorescence (IF), sterilised slides were coated with a washed spore suspensions of *F. solani* CBS224.34 containing 1% (w/v) glucose solution and incubated at 26°C for 16 h to allow spore germination and formation of germ tubes. After air-drying, the cells were fixed to the slides as

described in Thornton (2001) and incubated with ED7 TCS or TCM only (negative control) for 1 h, followed by three 5 min PBS washes. Slides were then incubated with goat anti-mouse polyvalent fluorescein isothiocyanate (FITC) conjugate (diluted 1 in 40 in PBS)(F1010; Sigma) for 30 min. Slides were given three 5 min washes with PBS and mounted in PBS-glycerol mounting medium (F4680; Sigma) before overlaying with coverslips. All incubation steps were performed at 23°C in a humid environment to prevent evaporation and slides were stored in the dark, at 4°C, prior to examination using an epifluorescence microscope (Olympus IX81) fitted with 495 nm (excitation) and 518 nm (emission) filters for FITC. For immunogold electron microscopy (IEM) the method described in Thornton & Talbot (2001) was used. Spores and hyphae of *F. solani* were prepared by incubating washed conidia in 1% (w/v) glucose solution at 26°C for 16 h to allow spore germination and formation of germ tubes. Cells were embedded in LR White resin (Agar Scientific Ltd.) and ultra thin sections prepared for immunolabeling. Sections immobilized to nickel grids were blocked by immersion in PBST containing 1% (w/v) BSA (PBST-BSA) which had been sterile filtered through a 0.2 μm filter. The grids were washed three times (3 min each) in sterile filtered PBST and then incubated in ED7 TCS or TCM only (negative control) for 1 h. After four washes (3 min each) with sterile filtered PBST, the grids were incubated for a further hour in PBST-BSA containing a 1:20 dilution of goat anti-mouse 20 nm gold conjugate (EM.GAF20; BBI Solutions). The grids were washed four times (3 min each) in sterile filtered PBST and then placed on Whatman filter paper to dry. Dried grids were then incubated for 20 min in 2% (w/v) uranyl acetate solution followed by 2% (w/v) lead citrate solution for 4 min. Working volumes were 100 μl and incubation and washing steps were carried out at at 23^{o}C. Immunostained samples were examined using a Jeol JEM 1400 transmission electron microscope fitted with a Gatan ES 100W CCD camera.

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Statistical analysis

Unless otherwise stated, numerical data were analysed using the statistical programme Minitab (Minitab 16, Minitab®, Coventry, UK). Analysis of variance (ANOVA) was used to compare means of more than two data sets and Post-hoc Tukey-Kramer analysis was then performed to distinguish which sets were significantly different from one another.

Sampling from drains

A total of 65 sinks were swabbed, comprising 32 sinks across the ICU, ITU, haematology, oncology and ophthalmology units of the Royal Devon and Exeter tertiary care hospital (Exeter, Devon, UK) and 33 restroom sinks located around the University of Exeter campus (Exeter, Devon, UK). In addition, cold-water samples were collected from taps connected to the sinks in the haematology and oncology unit, and from the two main water tanks feeding the ophthalmology unit. To isolate fungi from sink biofilms, sterile cotton buds (Boots, UK) wetted with PBS were used to scour the inner surfaces of sink drainpipes for approximately 10 s. Swabs with visible detritus were immersed in 1.5-ml micro-centrifuge tubes containing 1 ml PBS to dislodge biofilm debris, and the sealed tubes transferred to the laboratory for processing by ELISA and mycological culture.

Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the ITS regions of the rRNA-encoding gene unit and Translation Elongation Factor-1α PCR

Biofilm debris was pelleted by centrifugation at 14,462 *g* for 5 min and 50 µl samples of supernatant transferred to the wells of microtitre plates for assay by ELISA (Table 1 and Table S2; Swab-ELISA) as described. The biofilm pellet was re-suspended in 1 ml dH_2O, 200 µl samples spread on the surface of PDA containing 1 µg ml^{-1} of the broad-spectrum antibiotic rifampicin, and

the plates incubated for 2 d at 26°C under a 16 h fluorescent light regime. Fungi in these mixed culture plates were separated on the basis of gross morphological characteristics and axenic slope cultures generated following sub-culture on PDA. Crude antigen extracts were prepared as surface washings from mixed cultures and from axenic cultures and assayed by ELISA (Table 1 and Table S2; Mixed culture-ELISA and Axenic culture-ELISA, respectively) as described.

Fungal DNA was extracted from axenic culture material by using the CTAB method (Chow & Kafer, 1993) and fungi were identified by sequencing of the ITS1-5.8S-ITS2 region of the rRNA-encoding gene unit (White et al., 1990) according to procedures described elsewhere (Thornton *et al*., 2002), using the primers ITS1ext (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS4ext (5'-TTCTTTTCCTCCGCTTATTGATATGC-3'). Species identity was predicted based on >95% sequence identity (E-value = 0.0)(Altschul et al. 1997) of the ITS1-5.8S-ITS2 region of recovered species to species recorded in GenBank. *Fusarium* species were further identified to species complex level by using the forward primer ef-1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and reverse primer ef-2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3'), which amplify an ~700 bp region of Translation Elongation Factor 1-alpha (TEF-1α), the single-locus identification tool in *Fusarium* (Geiser et al., 2004). PCR reactions were carried out in a total volume of 25 µl consisting of 1 µl DNA at a concentration of 30 - 75 ng μl^{-1}, 12.5 µl of GoTaq® Green Master Mix DNA polymerase (Promega, MF7112), 9.5 µl of nuclease free water (Promega) and 1 µl of each primer at 20 pmol. The following cycling parameters were used: an initial denaturation step at 95°C for 8 min; 35 cycles of 15 sec at 95°C (denaturation); 20 s at 54°C (annealing), 1 min at 72°C (extension) followed by a final 5 min extension step at 72°C. Phylogenetic sub-groups of *Fusarium* species were determined by interrogation of the FUSARIUM-ID v. 1.0 database (http://isolate.fusariumdb.org)(O'Donnell et al., 2010), with the newly acquired TEF-1α sequences

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(Supporting Data Set 1).

Nucleotide sequence accession numbers

Newly determined ITS sequences were submitted to GenBank and the ITS accession numbers KT876496 to KT876723 were obtained. Species designations of recovered fungi are shown in Table 1.

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References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database programs. *Nucleic Acids Res* **25:** 3389-3402.

Anaissie, E.J., Kuchar, R.T., Rex, J.H., Francesconi, A., Kasai, M., Muller, F.M., *et al*. (2011) Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold pathogens. *Clin Infect Dis* **33:** 1871-1878.

Anaissie, E.J., Penzak, S.R., and Dignani, M.C. (2002) The hospital water supply as a source of nosocomial infections: a plea for action. *Arch Intern Med* **162:** 1483-1492.

Arie, T., Hayashi, Y., Nagatani, A., Furuya, M., and Yamaguchi, I. (1991) Production and partial characterization of monoclonal antibodies against *Fusarium oxysporum* 860926a. *Ann Phytopathol Soc Jpn* **57:** 696-701.

Arie, T., Hayashi, Y., Yoneyama K, Nagatani, A., Furuya, M., and Yamaguchi, I. (1995) Detection of *Fusarium* spp. in plants with monoclonal antibody. *Ann Phytopathol Soc Jpn* **61:** 311-317.

Arrese, J.E., Piérard-Franchimont, C., and Piérard, G.E. (1996) Fatal hyalohyphomycosis following *Fusarium* onychomycosis in an immunocompromised patient. *Am J Dermatopathol* **18:** 196-198.

Bigley, V.H., Duarte, R.F., Gosling, R.D., Kibbler, C.C., Seaton, S., and Potter, M. (2004) *Fusarium dimerum* infection in a stem cell transplant recipient treated successfully with voriconazole. *Bone Marrow Transplant* **34:** 815-817.

Fusarium-specific monoclonal antibody

Bouchara, J-P., Hsieh, H.Y., Croquefer, S., Barton, R., Marchais, V., Pihet, M., *et al*. (2009) Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. *J Clin Microbiol* **47:** 142-152.

Boutati, E.I., and Anaissie, E.J. (1997) *Fusarium*, a significant emerging pathogen in patients with haematological malignancies: ten years experience at a cancer centre and implications for management. *Blood* **90:** 999-1008.

Chang, D.C., Grant, G.B., O'Donnell, K., Wannemuehler, K.A., Noble-Wang, J., Rao, C.Y., *et al.* (2006) Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* **296:** 953-963.

Chow, T.Y.K., and Käfer, E. (1993) A rapid method for isolation of total nucleic acids from *Aspergillus nidulans*. *Fungal Genet Newsl* **40:** 25-27.

Danks, J.N., Rizvi, R.H., Barker, I., Turner, J.A., Rahman, S., and Northway, B.J. (1996) Specific monoclonal antibodies to *Fusarium* species and *Michrodochium nivale*. *Food Agric Immunol* **8:** 249-68.

Davies, G., and Thornton, C.R. (2014) Differentiation of the emerging human pathogens *Trichosporon asahii* and *Trichosporon asteroides* from other pathogenic yeasts and molds by using species-specific monoclonal antibodies. *PLoS ONE* **9:** e84789.

Dignani, M.C., and Anaissie, E. (2004) Human fusariosis. *Clin Microbiol Infect* **10:** 67-75.

Doggett, M.S. (2000) Characterisation of fungal biofilms within a municipal water distribution system. *Appl Env Microbiol* **66:** 1249-1251.

Dursun, D., Fernandez, V., and Miller, D. (2003) Advanced *Fusarium* keratitis progressing to endophthalmitis. *Cornea* **22:** 300-303.

Fusarium-specific monoclonal antibody

Edelstein, S.L., Akduman, L., Durham, B.H., Fothergill, A.W., and Hsu, H.Y. (2012) Resistant *Fusarium* keratitis progressing to endophthalmitis. *Ophthalmology Contact Lens* **38:** 331-335.

Feng, P., Qiaoyun, L., Najafzadeh, M.J., Gerrits van den Ende, A.H.G., Sun, J., Li, R., *et al*. (2014) *Cyphellophora* and its relatives in *Phialophora*: biodiversity and possible role in human disease. *Fungal Diversity* **65:** 17-45.

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.D., *et al*. (2004) FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. *Eur J Pl Pathol* **110:** 473-479.

Girmenia, C., Pagano, L., Corvatta, L., Mele, L., Del, A., Favero, P., *et al*. (2000) The epidemiology of fusarioses in patients with haematological diseases. *Br J Haematol* **111:** 272-276.

Gurusidappa, S.B., and Mamatha, H.S. (2011) Fusarial skin lesion in immunocompromised. *Ind J Cancer* **48:** 116-117.

Hayashi, Y., Arie, T., Yoneyama, K., and Yamaguchi, I. (1998) Characterisation of the antigenic determinant on *Fusarium oxysporum* recognized by a genus-specific monoclonal antibody. *J Gen Appl Microbiol* **44:** 43-47.

Hu, Z-Q., Liu, J-L., Li, H-P., Xing, S., Xue, S., Zhang, J-B., *et al*. (2012) Generation of a highly reactive chicken-derived single-chain variable fragment against *Fusarium verticillioides* by phage display. *Int J Mol Sci* **13:** 7038-7056.

Hu, Z-Q., Li, H-P., Zhang, J-B., Huang, T., Liu, J-L., Xue, S., *et al*. (2013) A phage-displayed chicken single-chain antibody fused to alkaline phosphatase detects *Fusarium* pathogens and their presence in cereal grains. *Anal Chim Acta* **764:** 84-92.

Fusarium-specific monoclonal antibody

Jensen, T.G., Gahrn-Hansen, B., Arendrup, M., and Bruun, B. (2004) *Fusarium* fungaemia in immunocompromised patients. *Clin Microbiol Infect* **10:** 499-501.

Jensen, H.M.E., Aalbæk, B., Jungersen, G., Hartvig, T., Moser, C., Rozell, B.L., *et al.* (2011) Immunohistochemical diagnosis of fusariosis with monoclonal antibodies. *Mycoses* **54:** s54-s55.

Jurkunas, U., Behlau, I., and Colby, K. (2009) Fungal keratitis: changing pathogens and risk factors. *Cornea* **28:** 638-643.

Koehler, P., Tacke, D., and Cornely, O.A. (2014) Bone and joint infections by *Mucorales*, *Scedosporium*, *Fusarium* and even rarer fungi. *Crit Rev Microbiol*. doi: 10.3109/1040841X.2014.910749.

Lackner, M., Najafzadeh, M.J., Sun, J., Lu, Q., and de Hoog, G.S. (2012) Rapid identification of *Pseudallescheria* and *Scedosporium* strains by using rolling circle amplification. *Appl Environ Microbiol* **78:** 126-133.

Latenser, B.A. (2003) *Fusarium* infections in burn patients: a case report and review of the literature. *J Burn Care Rehabil* **24:** 285-288.

Macêdo, D.P.C., Neves, R.P., de Souza-Motta, C.M., and Magalhães, O.M.C. (2007) *Engyodontium album* fungaemia: the first reported case. Braz J Microbiol 38: 110-112.

Mehl, H.L., and Epstein, L. (2008) Sewage and community shower drains are environmental reservoirs of *Fusarium* species complex group 1, a human and plant pathogen. *Env Microbiol* **10:** 219-227.

Miceli, M.H., Díaz, J.A., and Lee, S.A. (2011) Emerging opportunistic yeast infections. *Lancet Infect Dis* **11:** 142-151.

Fusarium-specific monoclonal antibody

Muhammed, M., Carneiro, H., Coleman, J., and Mylonakis, E. (2011) The challenge of managing fusariosis. *Virulence* **2:** 91-96.

Musa, H.O., Al Eisa, A., Halim, M., Sahovic, E., Gyger, M., Chaudhri, N., *et al.* (2000) The spectrum of fusarium infection in immunocompromised patients with haematological malignancies and in non-immunocompromised patients; A single institution experience over 10 years. *Br J Haematol* **108:** 544-548.

Nucci, M., and Anaissie, E. (2002) Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clin Infect Dis* **35:** 909-920.

Nucci, N., and Anaissie, E. (2007) *Fusarium* infections in immuncompromised patients. *Clin Microbiol Rev* **20:** 695-704.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Sarver, B.A.J., Arunmozhi Balajee, S., Schroers, H-J., et *al*. (2010) Internet-accessible DNA sequence database for identifying Fusaria from human and animal infections. *J Clin Microbiol* **48:** 3708-3718.

Petrikkos, G., Skiada, A., Lortholary, O., Roilides, E., Walsh, T.J., and Kontoyiannis, D.P. (2012) Epidemiology and clinical manifestations of mucormycosis. *Clin Infect Dis* **54:** S23-S24.

Rougeron, A., Schuliar, G., Leto, J., Sitterle, E., Landry, D., Bougnoux, M.E., *et al*. (2014) Human-impacted areas of France are environmental reservoirs of the *Pseudallescheria boydii/Scedosporium apiospermum* species complex. *Env Microbiol* **17:** 1039-1048.

Sandoval-Denis, M., Sutton, D.A., Cano-Lira, J.F., Gene, J., Fothergill, A.W., Wiederhold, N.P., *et al*. (2014) Phylogeny of the clinically relevant species of the emerging fungus *Trichoderma* and their antifungal susceptibilities. *J Clin Microbiol* **52:** 2112-2125.

Fusarium-specific monoclonal antibody

Schroers, H.-J., O'Donnell, K., Lamprecht, S.C., Kammophthalmologyr, P.L., Johnson, S., Sutton, D.A., et al. (2009) Taxonomy and phylogeny of the *Fusarium dimerum* species group. *Mycologia* **101:** 44-70.

Short, D.P.G., O'Donnell, K., Zhang, N., Juba, J.H., and Geiser, D.M. (2011) Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. *J Clin Microbiol* **49:** 4264-4272.

Steinmann, J., Schmidt, D., Buer, J., and Rath, P-M. (2011) Discrimination of *Scedosporium prolificans* against *Pseudallescheria boydii* and *Scedosporium apiospermum* by semiautomated repetitive sequence-based PCR. *Med Mycol* **49:** 475-483.

Sutula, C.L., Gillett, J.M., Morrisey, S.M., and Ramsdell, D.C. (1986) Interpreting ELISA data and establishing the positive-negative threshold. *Plant Dis* **70:** 722-26.

Thamke, D.C., Mediratta, D.K., Dhabarde, A., and Shukla, A.K. (2015) Mycotic keratitis due to *Engyodontium album*: first case report from India. *Indian J Med Microbiol* **33:** 303-304.

Thornton, C.R. (2001) Immunological methods for fungi. In *Molecular and Cellular Biology of Filamentous Fungi*, *A Practical Approach*. Talbot, N.J. (ed.). Oxford, UK: University Press, pp. 227-257.

Thornton, C.R. (2008) Development of an immunochromatographic lateral flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vacc Immunol* **15:** 1095-1105.

Thornton, C.R. (2009) Tracking the emerging human pathogen *Pseudallescheria boydii* by using highly specific monoclonal antibodies. *Clin Vacc Immunol* **16:** 756-64.

Thornton, C.R. (2012) Serological techniques for diagnosis. In *Fungal Plant Pathogens*, *Principles and Protocols*. Lane, C., Beales, P., Hughes, K. (eds.). Wallingford, UK: CABI, pp. 159-177.

Fusarium-specific monoclonal antibody

Thornton, C.R., and Wills, O.E. (2015) Immunodetection of fungal and oomycete pathogens: Established and emerging threats to human health, animal welfare, and global food security. *Crit Rev Microbiol.* **41:** 27-51.

Thornton, C.R., Ryder, L.S., Le Cocq, K., and Soanes, D.M. (2015) Identifying the emerging human pathogen *Scedosporium prolificans* by using a species-specific monoclonal antibody that binds to the melanin biosynthetic enzyme tetrahydroxynaphthalene reductase. *Env Microbiol* **17:** 1023-1048.

Torres, H.J., and Kontoyiannis, D. (2011) Hyalohyphomycosis (hyaline molds). In *Essentials of Clinical Mycology*, Kauffman, C.A., Pappas, P.G., Sobel, J.D., and Dismukes, W.E. (eds.). New York, USA: Springer Science+Business Media, pp. 281-304.

Walsh, T.J., and Groll, A.H. (1999) Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transpl Infect Dis* **1:**247-261.

Wong, W.C., White, M., and Wright, I.G. (1988) Production of monoclonal antibodies to *Fusarium oxysporum* f.sp. *cubense* race 4. *Lett Appl Microbiol* **6:** 39-42.

Zeng, J.S., Sutton, D.A., Fothergill, A.W., Rinaldi, M.G., Harrak, M.J., and de Hoog, G.S. (2007) Spectrum of clinically relevant *Exophiala* species in the Unites States. *J Clin Microbiol* **45:** 3713-3720.

Zhang, N., O'Donnell, K., Sutton, D.A., Nalim, F.A., Summerbell, R.C., Padhye, A.A., et al. (2006) Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *J Clin Microbiol* **44:** 2186-2190.

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Table 1. Locations and identities of sink swabs and water samples and results of ELISA tests and fungal identification based on ITS sequencing.

Location (Source)[a]	Swab ELISA abs (450nm)[b]	Mixed culture ELISA abs (450nm)[b]	Isolate No.	Axenic culture ELISA abs (450nm)[b]	Identification based on ITS sequencing[c]	GenBank Accession No.
University (S1)	0.0763	0.0575	A1-1	1.3736	***Fusarium oxysporum***	KT876668
University (S1)			A1-2	0.7646	***Fusarium oxysporum***	KT876662
University (S1)			A1-3	0.0196	*Penicillium crustosum*	KT876719
University (S1)			A1-4	0.0139	*Penicillium expansum*	KT876718
University (S2)	0.5472	0.0045	A2-1	1.5723	***Fusarium solani***	KT876635
University (S2)			A2-3	0.6554	***Fusarium oxysporum***	KT876690
University (S2)			A2-5	1.1312	***Fusarium solani***	KT876631
University (S3)	0.0053	0.0077	A3-1	0.0082	*Trichosporon domesticum*	KT876717
University (S3)			A3-2	0.0162	*Cadophora fastigiata*	KT876615
University (S3)			A3-3	0.0014	*Cyphellophora oxyspora*	KT876613
University (S3)			A3-4	0.0093	*Penicillium crustosum*	KT876714
University (S4)	0.0297	0.0049	A4-1	0.0105	*Phoma herbarum*	KT876697
University (S4)			A4-2	0.0079	*Penicillium echinulatum*	KT876710
University (S4)			A4-3	0.0029	*Cytobasidium slooffiae*	KT876704
University (S4)			A4-6	0.0060	*Trichoderma asperellum*	KT876620
University (S5)	0.0206	0.0039	A5-1	1.1815	***Fusarium oxysporum***	KT876692
University (S5)			A5-2	0.0034	*Penicillium crustosum*	KT876715
University (S5)			A5-5	0.0088	*Aspergillus niger*	KT876702
University (S5)			A5-6	1.0630	***Fusarium oxysporum***	KT876667
University (S5)			A5-7	0.0037	*Rhodotorula mucilaginosa*	KT876700
University (S5)			A5-8	0.0088	*Cyphellophora oxyspora*	KT876614
University (S6)	0.0251	0.0412	A6-1	1.1130	***Fusarium oxysporum***	KT876648
University (S6)			A6-2	0.9410	***Fusarium oxysporum***	KT876678
University (S6)			A6-3	0.6377	***Fusarium oxysporum***	KT876688
University (S6)			A6-4	1.0020	***Fusarium solani***	KT876640
University (S7)	0.1716	0.0082	A7-1	0.0098	*Exophiala pisciphila*	KT876618
University (S7)			A7-2	0.0096	*Penicillium brevicompactum*	KT876695
University (S7)			A7-3	1.0556	***Fusarium oxysporum***	KT876684
University (S7)			A7-4	1.0077	***Fusarium oxysporum***	KT876671
University (S8)	0.0431	0.0008	A8-1	1.1045	***Fusarium oxysporum***	KT876672
University (S8)			A8-2	0.9707	***Fusarium oxysporum***	KT876654
University (S8)			A8-3	0.0091	*Trichoderma atroviride*	KT876622
University (S9)	0.3806	0.0052	A9-1	0.9087	***Fusarium solani***	KT876639
University (S9)			A9-2	0.8849	***Fusarium solani***	KT876638
University (S9)			A9-3	0.9004	***Fusarium solani***	KT876632
University (S9)			A9-4	0.8093	***Fusarium solani***	KT876636

Fusarium-specific monoclonal antibody

University (S10)	0.0034	0.0028	A10-1	0.0188	*Rhodotorula slooffiae*	
University (S10)			A10-2	0.0020	*Exophiala pisciphila*	KT876616
University (S11)	0.0231	0.0005	B1-1	0.6064	***Fusarium dimerum***	KT876625
University (S11)			B1-6	0.8138	***Fusarium dimerum***	KT876628
University (S12)	0.0200	0.0020	B2-1	0.5678	***Fusarium dimerum***	KT876626
University (S12)			B2-5	0.4827	***Fusarium dimerum***	KT876624
University (S13)	0.0091	0.0023	B3-4	0.0026	*Phoma herbarum*	KT876696
University (S14)	0.0163	0.0008	B4-1	0.6992	***Fusarium dimerum***	KT876627
University (S15)	0.0132	0.0003	B5-1	0.8008	***Fusarium oxysporum***	KT876674
University (S15)			B5-2	0.0048	*Mucor circinelloides*	KT876701
University (S15)			B5-3	0.8851	***Fusarium oxysporum***	KT876677
University (S16)	0.0229	0.0002	B6-1	0.8193	***Fusarium oxysporum***	KT876676
University (S16)			B6-2	0.7582	***Fusarium oxysporum***	KT876661
University (S17)	0.0395	0.0014	B7-1	0.8201	***Fusarium oxysporum***	KT876675
University (S17)			B7-6	0.7758	***Fusarium dimerum***	KT876623
University (S18)	0.0133	0.0011	B8-1	0.7347	***Fusarium solani***	KT876637
University (S18)			B8-7	0.0095	*Rhodosporidium babjevae*	KT876706
University (S19)	0.0212	0.0048	B9-1	0.0051	*Meyerozyma guilliermondii*	KT876707
University (S19)			B9-2	0.8104	***Fusarium oxysporum***	KT876657
University (S19)			B9-3	0.6302	***Fusarium solani***	KT876634
University (S19)			B9-5	0.0017	*Penicillium crustosum*	KT876720
University (S20)	0.5467	0.0394	B10-1	0.7467	***Fusarium oxysporum***	KT876680
University (S20)			B10-2	0.7667	***Fusarium oxysporum***	KT876659
University (S20)			B10-6	0.0071	*Candida parapsilosis*	KT876703
University (S20)			B10-7	0.0251	*Meyerozyma guilliermondii*	KT876711
University (S20)			B10-9	0.0031	*Cystobasidium slooffiae*	KT876712
University (S20)			B10-12	0.0251	*Trichosporon asteroides*	KT876713
University (S21)	0.0083	0.0269	C1-1	0.0044	*Clavispora lusitaniae*	KT876708
University (S21)			C1-3	0.8875	***Fusarium oxysporum***	KT876682
University (S21)			C1-4	0.8884	***Fusarium dimerum***	KT876629
University (S21)			C1-7	0.0003	*Exophiala pisciphila*	KT876617
University (S22)	0.2977	0.0047	C2-1	0.9443	***Fusarium oxysporum***	KT876658
University (S22)			C2-4	0.9825	***Fusarium oxysporum***	KT876694
University (S22)			C2-7	0.8677	***Fusarium oxysporum***	KT876687
University (S23)	0.0808	0.0116	C3-1	0.9853	***Fusarium oxysporum***	KT876683
University (S23)			C3-2	1.0160	***Fusarium oxysporum***	KT876693
University (S23)			C3-4	0.7485	***Fusarium oxysporum***	KT876644
University (S24)	0.1113	0.0202	C4-1	0.8930	***Fusarium oxysporum***	KT876670
University (S24)			C4-2	0.0028	*Candida intermedia*	KT876709
University (S24)			C4-5	0.9008	***Fusarium dimerum***	KT876630
University (S25)	0.5741	0.0097	C5-1	0.9874	***Fusarium oxysporum***	KT876652
University (S25)			C5-2	1.0996	***Fusarium oxysporum***	KT876656
University (S25)			C5-3	1.1236	***Fusarium oxysporum***	KT876649
University (S25)			C5-4	1.0355	***Fusarium oxysporum***	KT876669
University (S25)			C5-5	1.0672	***Fusarium oxysporum***	KT876660
University (S26)	0.0603	0.0348	C6-1	1.0135	***Fusarium oxysporum***	KT876665

Fusarium-specific monoclonal antibody

University (S26)			C6-2	0.9362	***Fusarium oxysporum***	KT876681
University (S26)			C6-3	0.8898	***Fusarium oxysporum***	
University (S26)			C6-4	1.0577	***Fusarium oxysporum***	KT876686
University (S26)			C6-5	0.9445	***Fusarium oxysporum***	KT876646
University (S27)	0.1279	0.0113	C7-1	0.9724	***Fusarium oxysporum***	KT876666
University (S28)	0.0607	0.0035	C8-1	0.8694	***Fusarium oxysporum***	KT876685
University (S28)			C8-2	0.9599	***Fusarium oxysporum***	KT876655
University (S28)			C8-3	0.0031	*Penicillium expansum*	KT876716
University (S28)			C8-4	0.0049	*Phoma herbarum*	KT876698
University (S28)			C8-6	0.9003	***Fusarium oxysporum***	KT876679
University (S29)	0.0310	0.0001	C9-1	1.0488	***Fusarium oxysporum***	KT876651
University (S29)			C9-2	0.9254	***Fusarium oxysporum***	KT876645
University (S29)			C9-3	0.9711	***Fusarium oxysporum***	KT876664
University (S29)			C9-4	1.035	***Fusarium oxysporum***	KT876647
University (S29)			C9-5	0.9604	***Fusarium oxysporum***	KT876663
University (S30)	0.1002	0.0333	C10-1	0.9254	***Fusarium oxysporum***	KT876689
University (S30)			C10-2	1.0198	***Fusarium solani***	KT876642
University (S30)			C10-4	0.9007	***Fusarium solani***	KT876641
University (S30)			C10-7	1.0697	***Fusarium solani***	KT876643
University (S31)	0.6175	0.1129	CRT1-1	0.4639	***Fusarium oxysporum***	KT876691
University (S31)			CRT1-2	0.9086	***Fusarium oxysporum***	KT876650
University (S31)			CRT1-3	0.7503	***Fusarium oxysporum***	KT876652
University (S32)	0.0303	0.0021	CRT2-1	0.0040	*Trichoderma asperellum*	KT876619
University (S32)			CRT2-2	0.0417	*Trichoderma asperellum*	KT876621
University (S33)	0.0663	0.0028	CRT3-1	0.0256	*Phoma herbarum*	KT876699
ICU (S34)	0.0285	0.3620	R1-1	1.5057	***Fusarium solani***	KT876550
ICU (S34)			R1-2	1.5154	***Fusarium solani***	KT876551
ICU (S34)			R1-3	1.5555	***Fusarium solani***	KT876549
ICU (S35)	0.0071	0.0147	R2-4	0.0096	*Clonostachys rosea*	KT876552
ICU (S35)			R2-5	0.0018	*Clonostachys rosea*	KT876553
ICU (S35)			R2-6	0.0035	*Clonostachys rosea*	KT876554
ICU (S36)	0.0391	0.045	R3-1	0.0006	*Trichoderma asperellum*	KT876548
ITU (S37)	0.7291	0.7724	R5-1	1.5606	***Fusarium dimerum***	KT876561
ITU (S37)			R5-2	1.3481	***Fusarium dimerum***	KT876565
ITU (S37)			R5-3	1.4990	***Fusarium dimerum***	KT876567
ITU (S38)	1.2086	0.3691	R6-1	1.3388	***Fusarium dimerum***	KT876572
ITU (S38)			R6-2	1.4734	***Fusarium dimerum***	KT876563
ITU (S38)			R6-3	1.4312	***Fusarium dimerum***	KT876562
ITU (S38)			R6-9	1.2648	***Fusarium oxysporum***	KT876557
ITU (S39)	0.1121	0.8186	R7-1	1.3266	***Fusarium dimerum***	KT876570
ITU (S39)			R7-2	1.2352	***Fusarium dimerum***	KT876564
ITU (S40)	1.0157	0.3612	R8-1	1.2399	***Fusarium dimerum***	KT876568
ITU (S40)			R8-2	1.1552	***Fusarium dimerum***	KT876566
ITU (S40)			R8-3	1.1856	***Fusarium dimerum***	KT876558
ITU (S41)	0.0391	0.0477	R9-3	0.0113	*Exophiala phaeomuriformis*	KT876555
ITU (S42)	0.0549	0.0097	R10-2	0.0072	*Cadophora fastigiata*	KT876556
ITU (S42)			R10-6	0.0124	*Coniochaeta fasciculata*	KT876721
ITU (S43)	0.0630	1.5751	X2-2	1.1492	***Fusarium dimerum***	KT876571

Fusarium-specific monoclonal antibody

ITU (S43)			X2-4	1.2505	***Fusarium dimerum***	KT876560
ITU (S43)			X2-5	1.1397	***Fusarium dimerum***	KT876569
ITU (S43)			X2-6	1.1716	***Fusarium dimerum***	KT876559
Ophthalmology Unit (S44)	1.0313	1.5035	X3-1	1.1353	***Fusarium dimerum***	KT876509
Ophthalmology Unit (S44)			X3-2	0.0224	*Candida parapsilosis*	KT876498
Ophthalmology Unit (S44)			X3-3	1.1856	***Fusarium dimerum***	KT876512
Ophthalmology Unit (S45)	1.4582	1.4049	X4-1	1.1322	***Fusarium dimerum***	KT876511
Ophthalmology Unit (S45)			X4-2	1.1523	***Fusarium dimerum***	KT876500
Ophthalmology Unit (S45)			X4-3	0.0622	*Candida parapsilosis*	KT876508
Ophthalmology Unit (S45)			X4-4	0.1245	*Gloeotinia temulenta*	KT876515
Ophthalmology Unit (S46)	0.3325	1.4466	X5-2	1.0654	***Fusarium dimerum***	KT876510
Ophthalmology Unit (S46)			X5-3	0.0599	*Rhodotorula mucilaginosa*	KT876501
Ophthalmology Unit (S46)			X5-4	1.0862	***Fusarium dimerum***	KT876513
Ophthalmology Unit (S47)	0.2640	1.3729	X6-1	0.0616	*Candida parapsilosis*	KT876499
Ophthalmology Unit (S47)			X6-2	0.063	*Engyodontium album*	KT876522
Ophthalmology Unit (S47)			X6-3	0.0603	*Engyodontium album*	KT876521
Ophthalmology Unit (S47)			X6-4	0.0657	*Exophiala phaeomuriformis*	KT876504
Ophthalmology Unit (S48)	0.5299	0.0223	X8-2	0.0118	*Cladosporium macrocarpum*	KT876506
Ophthalmology Unit (S48)			X8-3	0.0985	*Gloeotinia temulenta*	KT876514
Ophthalmology Unit (S48)			X8-4	0.0662	*Engyodontium album*	KT876523
Ophthalmology Unit (S49)	0.1872	0.0061	X9-1	0.0686	*Engyodontium album*	KT876520
Ophthalmology Unit (S49)			X9-2	0.0611	*Candida parapsilosis*	KT876496
Ophthalmology Unit (S49)			X9-3	0.0641	*Candida parapsilosis*	KT876497
Ophthalmology Unit (S49)			X9-4	0.0677	*Exophiala dermatitidis*	KT876503
Ophthalmology Unit (S49)			X9-5	0.0705	*Engyodontium album*	KT876519
Ophthalmology Unit (S49)			X9-7	0.0660	*Exophiala pisciphila*	KT876502

Fusarium-specific monoclonal antibody

Ophthalmology Unit (T1)	0.0132	0.0159	X10-1	0.0868	*Cladosporium sphaerospermum*	KT876507
Ophthalmology Unit (T1)			X10-3	0.0130	*Engyodontium album*	KT876518
Ophthalmology Unit (T2)	0.0117	0.0883	W1-1	0.0114	*Paraconiothyrium fuckelii*	KT876505
Ophthalmology Unit (T2)			W1-2	0.0139	*Engyodontium album*	KT876517
Ophthalmology Unit (T2)			W1-3	0.0108	*Engyodontium album*	KT876516
Haematology Unit (S50)	0.2694	0.0093	K1-1	0.0172	*Trichoderma asperellum*	KT876534
Haematology Unit (S51)	0.7466	1.1746	K2-1	0.0144	*Trichoderma viride*	KT876533
Haematology Unit (S51)			K2-3	1.1385	***Fusarium solani***	KT876543
Haematology Unit (S51)			K2-4	1.2487	***Fusarium solani***	KT876542
Haematology Unit (S52)	1.0621	1.3090	K6-1	1.1544	***Fusarium oxysporum***	KT876541
Haematology Unit (S52)			K6-4	0.0071	*Candida parapsilosis*	KT876524
Haematology Unit (S53)	1.0120	1.2628	K8-1	1.2376	*Fusarium solani*	KT876722
Haematology Unit (S53)			K8-2	1.2983	*Fusarium solani*	KT876723
Haematology Unit (S53)			K8-6	0.0084	*Cadophora fastigiata*	KT876531
Haematology Unit (S53)			K8-7	0.0051	*Trichoderma asperellum*	KT876535
Haematology Unit (S54)	0.9843	1.2903	K9-1	0.0046	*Candida parapsilosis*	KT876525
Haematology Unit (S54)			K9-2	0.0090	*Rhodotorula dairenensis*	KT876526
Haematology Unit (S54)			K9-4	0.0073	*Cadophora fastigiata*	KT876532
Haematology Unit (S54)			K9-5	1.1652	***Fusarium solani***	KT876547
Haematology Unit (S54)			K9-6	1.0316	***Fusarium solani***	KT876545
Haematology Unit (S55)	0.8638	1.0753	K10-1	1.0684	***Fusarium solani***	KT876546
Haematology Unit (S55)			K10-2	1.0386	***Fusarium solani***	KT876544
Haematology Unit (W50)	0.0082	0.0451	G1-2	0.0097	*Exophiala pisciphila*	KT876529
Haematology Unit (W51)	0.0581	0.0553	G2-1	0.0104	*Engyodontium album*	KT876540
Haematology Unit (W51)			G2-3	0.0062	*Exophiala pisciphila*	KT876530

Fusarium-specific monoclonal antibody

Haematology Unit (W52)	0.0125	0.0118	G6-2	0.0161	*Exophiala castellanii*	KT876528
Haematology Unit (W53)	0.0075	0.0219	G8-1	0.0065	*Engyodontium album*	KT876538
Haematology Unit (W54)	0.0002	0.0164	G9-2	0.0005	*Gloeotinia temulenta*	KT876537
Haematology Unit (W55)	0.0021	0.0182	G10-1	0.0051	*Engyodontium album*	KT876539
Oncology Unit (S56)	0.4822	1.2287	H1-1	0.9022	***Fusarium dimerum***	KT876595
Oncology Unit (S56)			H1-3	0.0151	*Pichia kudriavzevii*	KT876578
Oncology Unit (S57)	1.0579	1.1650	H2-1	0.9312	***Fusarium dimerum***	KT876590
Oncology Unit (S57)			H2-5	0.0061	*Magnusiomyces capitatus*	KT876611
Oncology Unit (S58)	1.0938	1.2091	H3-1	0.8909	***Fusarium oxysporum***	KT876584
Oncology Unit (S58)			H3-4	0.0075	*Candida palmioleophila*	KT876573
Oncology Unit (S58)			H3-5	0.0140	*Rhodotorula glutinis*	KT876598
Oncology Unit (S59)	0.0253	1.0856	H4-1	0.8884	***Fusarium dimerum***	KT876596
Oncology Unit (S59)			H4-3	0.0004	*Magnusiomyces capitatus*	KT876612
Oncology Unit (S60)	1.0856	1.0193	H5-1	1.1148	***Fusarium dimerum***	KT876593
Oncology Unit (S60)			H5-3	0.005	*Candida tropicalis*	KT876574
Oncology Unit (S61)	0.5256	1.1898	H6-1	1.1238	***Fusarium dimerum***	KT876594
Oncology Unit (S62)	1.0789	1.1588	H7-1	1.1513	***Fusarium oxysporum***	KT876583
Oncology Unit (S62)			H7-3	0.0045	*Candida tropicalis*	KT876575
Oncology Unit (S63)	0.0953	0.0152	H8-1	0.0063	*Phoma herbarum*	KT876580
Oncology Unit (S63)			H8-3	0.0126	*Candida albicans*	KT876577
Oncology Unit (S63)			H8-4	0.0088	*Rhodotorula mucilaginosa*	KT876599
Oncology Unit (S64)	0.1926	0.0162	H9-1	0.0152	*Exophiala dermatitidis*	KT876581
Oncology Unit (S64)			H9-2	0.0049	*Candida orthopsilosis*	KT876576
Oncology Unit (S64)			H9-3	0.0109	*Pichia kudriavzevii*	KT876579
Oncology Unit (S65)	0.4961	1.1005	H10-1	0.9606	***Fusarium dimerum***	KT876589

Fusarium-specific monoclonal antibody

Oncology Unit (S65)			H10-4	0.0163	*Trametes ochracea*	KT876608
Oncology Unit (W56)	0.0030	0.0247	P1-2	0.0082	*Exophiala dermatitidis*	KT876582
Oncology Unit (W56)			P1-3	0.0024	*Trametes versicolor*	KT876603
Oncology Unit (W57)	0.0061	1.0891	P2-1	0.8451	***Fusarium dimerum***	KT876587
Oncology Unit (W57)			P2-2	0.9429	***Fusarium dimerum***	KT876597
Oncology Unit (W57)			P2-3	0.8627	***Fusarium dimerum***	KT876588
Oncology Unit (W58)	0.0016	0.0202	P3-1	0.0177	*Trametes ochracea*	KT876607
Oncology Unit (W59)	0.0035	0.0125	P4-1	0.0164	*Beauveria bassiana*	KT876586
Oncology Unit (W59)			P4-2	0.0084	*Gliomastix polychroma*	KT876584
Oncology Unit (W60)	0.0025	1.1746	P5-1	0.8563	***Fusarium dimerum***	KT876591
Oncology Unit (W60)			P5-2	0.8066	***Fusarium dimerum***	KT876592
Oncology Unit (W60)			P5-3	0.0024	*Piptoporus betulinus*	KT876609
Oncology Unit (W62)	0.0011	0.0417	P7-1	0.0097	*Trametes versicolor*	KT876605
Oncology Unit (W63)	0.0044	0.0060	P8-1	0.0144	*Trametes versicolor*	KT876606
Oncology Unit (W63)			P8-2	0.0106	*Stereum gausapatum*	KT876601
Oncology Unit (W64)	0.0022	0.0163	P9-1	0.0101	*Stereum gausapatum*	KT876600
Oncology Unit (W64)			P9-2	0.0068	*Trametes versicolor*	KT876604
Oncology Unit (W64)			P9-3	0.0140	*Stereum gausapatum*	KT876602
Oncology Unit (W65)	0.0051	0.0280	P10-1	0.0032	*Phaeophlebiopsis peniophoroides*	KT876610

a. S, sink; W, tap water from corresponding sink number; T, water sample from main tank.

b. Threshold absorbance value for detection in ELISA ≥0.100. Shading indicates earliest point in sampling process at which antigen was detectable in ELISA tests with ED7.

c. *Fusarium* strains in bold further characterized by TEF-1α PCR analysis (Table S3 and Supporting Data Set 1).

Fusarium-specific monoclonal antibody

Figure legends

Figure 1. Specificity of ED7 determined by Enzyme-Linked Immunosorbent Assay tests of surface washings containing water-soluble antigens from *Fusarium* species and related and unrelated yeasts and molds. (A) ELISA absorbance values at 450 nm for antigens from *F. solani* and unrelated yeasts and molds and (B) for antigens from the *F. solani* teleomorph *Haematonectria haematococca* and related *Fusarium* species. Wells were coated with 60 µg protein ml^{-1} buffer. Bars are the means of three biological replicates ± standard errors and the threshold absorbance value for detection of antigen in ELISA is ≥0.100 (indicated by lines on graphs). Numbers in parentheses after species names denote strain numbers with further details of strains provided in Table S1.

Figure 2. Characterisation of the ED7 antigen and its epitope and spatial distribution of the antigen in spores and hyphae. (A) Western immunoblot with ED7 using culture fluid from 2-d-old PDB cultures of *F. solani* CBS224.34 (lane 1) and *F. oxysporum* f.sp. *lycopersici* CBS167.30 (lane 2). Wells were loaded with 1.6 µg of protein. M_r denotes molecular weight in kDa. Note the major extracellular antigen with molecular weight of ~200 kDa. (B) Absorbance values from ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and treated with trypsin or PBS only (control) at 4°C and 37°C. Bars are the means of three biological replicates ± standard errors and bars with the same letter are not significantly different at $p<0.001$ (ANOVA and Tukey-Kramer test). (C) Absorbance values from ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and treated with pronase or Milli-Q H_2O only (control) at 4°C and 37°C. Bars are the means of three biological replicates ± standard errors and bars with the same letter are not significantly different at $p<0.001$ (ANOVA and Tukey-Kramer test). (D) Stability of the water-

soluble ED7 antigen following heating of surface washings at 100°C over a 70 min period. Treated antigen was subsequently immobilized to the wells of microtitre plates and assayed by ELISA. Bars are the means of three biological replicates ± standard errors and bars with the same letter are not significantly different at $p<0.001$ (ANOVA and Tukey-Kramer test). (E) Absorbance values from ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the wells of microtitre plates and treated with periodate (open circles) or with acetate only control (closed circles) at 4°C over a 20 h period. Each point is the mean of three biological replicates ± standard errors. (F to I) Photomicrographs of *F. solani* CBS224.34 immunostained with ED7 or TCM control and goat anti-mouse polyvalent Ig fluorescein isothiocyanate (FITC) conjugate. (F) Brightfield image of germinated conidium with hypha probed with ED7 followed by fluorochrome conjugate (scale bar = 6 μm); (G) Same field of view as panel F but examined under epifluorescence. Note intense staining of the cell wall of microconidium and hypha; (H) Brightfield image of germinated conidium with hypha probed with TCM (negative control) followed by FITC conjugate (scale bar = 3 μm); (I) Same field of view as panel H but examined under epifluorescence. Note lack of staining, demonstrating specific binding of ED7 to surface antigen. (J-L) Immunogold labeling of sections of conidia and hyphae of *F. solani* CBS224.34. (J) Transverse section of conidium incubated with ED7 and anti-mouse immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular fimbrial matrix surrounding the spore (scale bar = 250 nm); (K) Longitudinal section of hypha incubated with ED7 and anti-mouse immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular matrix surrounding the cell (scale bar = 100 nm); (L) Transverse section of a conidium incubated with TCM (negative control) and anti-mouse immunoglobulin 20 nm gold particles, showing lack of staining by the secondary gold conjugate (scale bar = 180 nm).

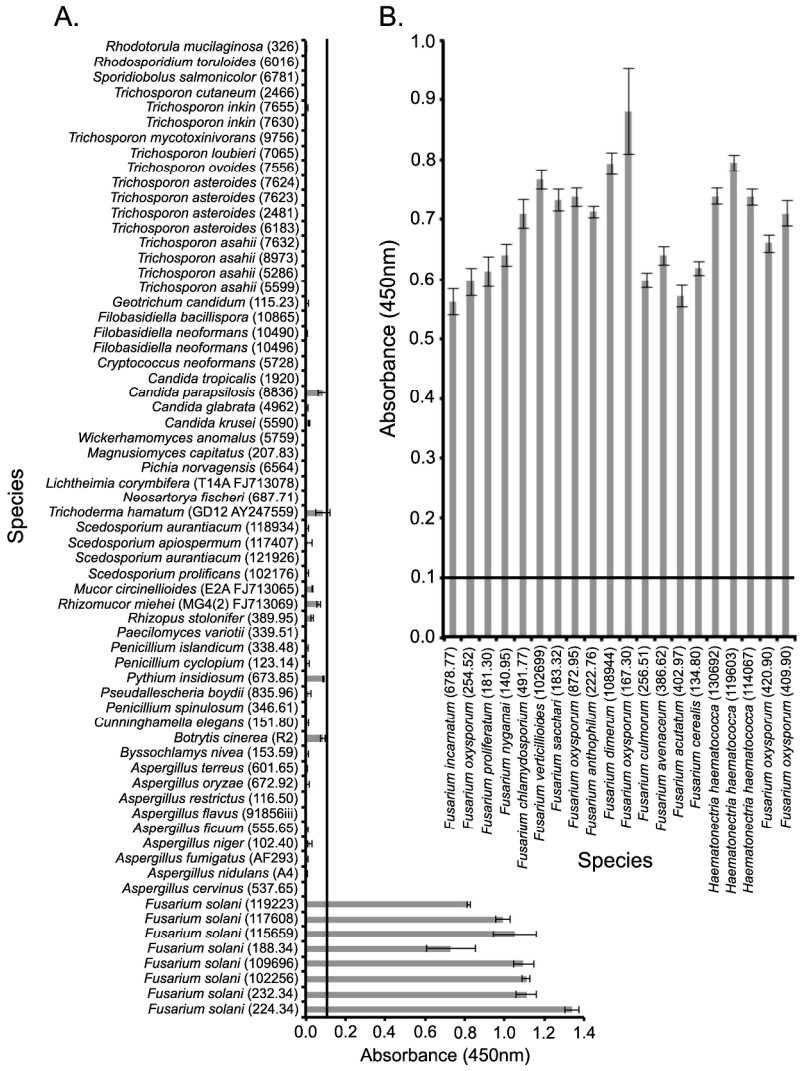

Figure 1. Al-Maqtoofi & Thornton

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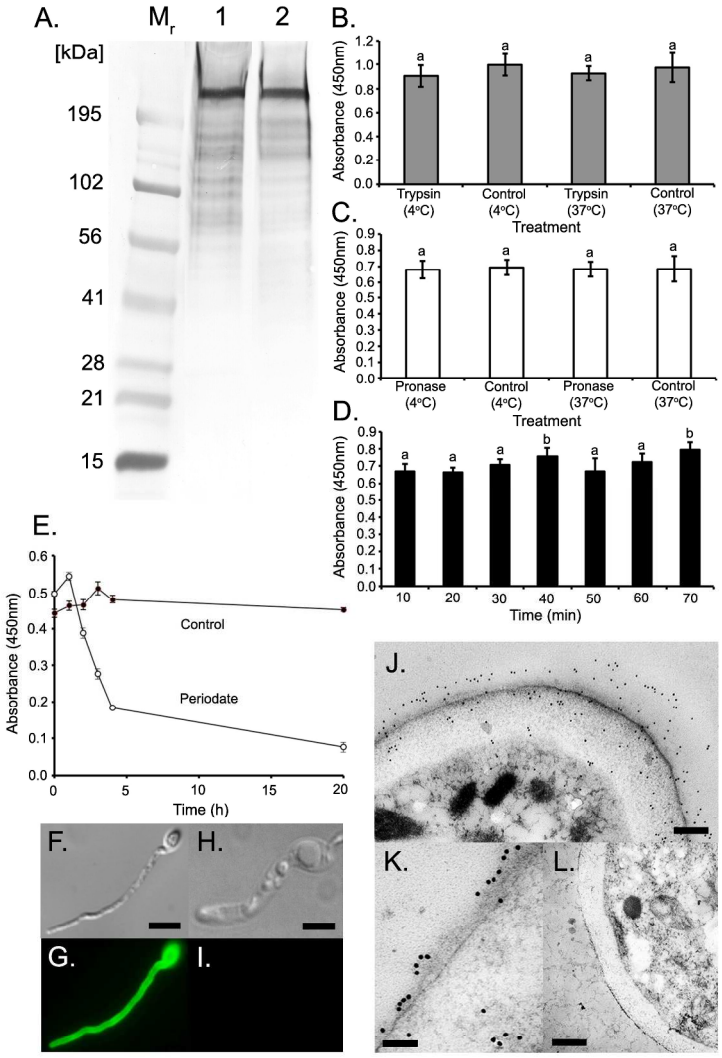

Figure 2. Al-Maqtoofi & Thornton

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