

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

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1	Detection of human pathogenic Fusarium species in hospital and
2	communal sink biofilms by using a highly specific monoclonal
3	antibody
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11	Summary
12	The fungus Fusarium is well known as a plant pathogen, but has recently emerged over recent years
13	as an opportunistic pathogen of humans. Habitats providing direct human exposure to infectious
14	propagules are largely unknown, but there is growing evidence that plumbing systems are sources
15	of human pathogenic strains in the Fusarium solani species complex (FSSC) and Fusarium
16	oxysporum species complexes (FOSC), the most common groups infecting humans. Here, we use a
17	newly developed Fusarium-specific monoclonal antibody (mAb ED7) to track FSSC and FOSC
18	strains in sink drain biofilms by detecting its target antigen, an extracellular 200kDa heat-stable
19	carbohydrate, in saline swabs. The diagnostic-antigen was detectable in 52% of swab samples
20	collected from sinks across a University campus and a tertiary care hospital. The mAb was 100%
21	accurate in detecting FSSC, FOSC and F. dimerum species complex (FDSC) strains that were

1	confirmed by sequencing of the internally transcribed spacer 1 (ITS1)-5.8S-ITS2 rRNA-encoding
2	regions of culturable yeasts and moulds that were recovered using mycological culture, while
3	translation elongation factor (TEF)-1 α analysis of <i>Fusarium</i> isolates included FSSC 1-a, FOSC 33
4	and FDSC ET-gr, the most common clinical pathotypes in each group.
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6	Originality-Significance Statement
7	• Development of a monoclonal antibody (mAb ED7) specific to Fusarium, a fungal genus
8	containing human and plant pathogens
9	• Fusarium diagnostic antigen detected in swabs of sink drain biofilms, with 100% accuracy
10	of mAb-based ELISA confirmed by ITS sequencing of mixed fungal communities
11	comprising human pathogenic yeasts and moulds
12	• First report describing the use of a highly specife mAb to track human pathogenic fusaria,
13	demonstrating widespread occurrence of pathogen in communal and hospital sinks with
14	potential for nosocomial and community acquired infections
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1 Introduction

2 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 3 4 humans, such as patients with haematological malignancies and hematopoietic stem cell and solid 5 organ transplant recipients, Fusarium species are significant emerging pathogens, causing a 6 frequently fatal disseminated disease known as fusariosis with an associated mortality rate of 50-7 75% (Girmenia et al., 2000; Musa et al., 2000; Boutati and Anaissie, 1997; Dignani and Anaissie, 8 2004; Jensen et al., 2004; Nucci and Anaissie, 2007). In some tertiary cancer centres, Fusarium has 9 emerged as the second most common mould pathogen after Aspergillus (Walsh and Groll, 1999; 10 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 11 12 al., 2014), infections of burn wounds (Latenser, 2003), skin infections (Nucci and Anaissie, 2002; 13 Gurusidappa and Mamatha, 2011), and are the most frequent cause of mycotic eye infections known 14 as fungal keratitis (Jurkunas et al., 2009), leading to progressive corneal destruction and 15 endophthalmitis, with loss of vision or even loss of the affected eye (Dursun et al., 2003; Edelstein 16 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).

5 Identification of environmental reservoirs of human pathogenic mouldmolds including Fusarium has typically relied on nucleic acid-based technologies following recovery of fungi using 6 7 selective media (Anaissie et al., 2002; Mehl and Epstein, 2008; Short et al., 2011; Anaissie et al., 8 2011; Rougeron et al., 2014). Recently, highly specific monoclonal antibodies (mAb) have been 9 used to identify pathogenic species or species complexes in environmental samples containing 10 mixed populations of yeasts and mouldmolds (Thornton, 2009; Davies and Thornton, 2014; 11 Thornton et al., 2015). While mAb-based approaches similarly rely on culture for recovery of fungi 12 from environmental samples, detection of diagnostic antigens in crude culture extracts using genus-13 or species-specific mAbs offers an attractive alternative approach to pathogen detection, particularly 14 when combined with unsophisticated diagnostic modalities such as lateral-flow technology 15 (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<u>mold</u> 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.

1 Results

- 2 Production of hybridoma cell lines, isotyping of mAb and specificity
- 3 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 4 5 cell lines secreting mAbs specific to Fusarium that could be used to track the fungus in 6 environmental samples containing mixed species of human pathogenic fungi. To this end, aA single 7 cell line, ED7, produced-was identified that produced mAbs belonging to the immunoglobulin class 8 M (IgM), which was genus-specific, reacting in ELISA tests with antigens from Fusarium species 9 and with the F. solani teleomorph Haematonectria haematococca only (Figs. 1A and 1B). It did not 10 cross-react with antigens from a wide range of unrelated mouldmold and yeast species (Fig. 1A). 11
- 12 Western blotting of the ED7 antigen and epitope antigen characterization—
- 13 Gel electrophoresis and western blotting studies showed that mAb ED7 binds to a major 14 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. 15 2B and Fig. 2C), heat (Fig. 2D) and chemical (Fig. 2E) modifications in order to characterise the 16 17 epitope bound by ED7. Reductions in mAb binding following treatment with pronase shows that its 18 epitope consists of protein, while reductions with trypsin indicate a protein epitope containing 19 positively charged lysine and arginine side chains. The lack of reduction in ED7 binding following 20 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 21 22 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 23

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1	digestion of an antigen with periodate shows that its epitope is carbohydrate and contains vicinal
2	hydroxyl groups. The pronounced reductions in ED7 binding following periodate oxidation shows
3	that its epitope consists of carbohydrate residues (Fig. 2E). Taken together, Binding of mAb ED7 to
4	its target antigen was unaffected by pronase (Fig. 2B) or trypsin (Fig. 2C) digestion or by heating
5	(Fig. 2D)tThese results, combined with significant reductions in antibody binding following
6	periodate oxidation (Fig. 2E), indicate that mAb-ED7 binds to an extracellular antigen and that its
7	epitope is a -heat stable carbohydrate moeityepitope containing with vicinal hydroxyl groups.
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9	Immunofluorescence and immunogold electron microscopy
10	Immuno-localisation studies using IF showed that the ED7 antigen was present on the surface of
11	spores and hyphae (Figs. 2F <u>-1 and 2G</u>), while IEM showed that the antigen was present in the spore
12	and hyphal cell wall and in an extracellular fibrillar matrix surrounding both (Figs. 2JH-LJ). In the
13	TEM image shown in Fig. 2J, 56% of gold particles were distributed in the fibrillar matrix
14	surrounding the cell, while 40% and 4% of gold particles were distributed in the cell wall and
15	cytoplasm respectively. This shows that the ED7 antigen is predominantly extracellular or located
16	within the cell wall.
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20	Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the
21	ITS regions of the rRNA-encoding gene unit and by Translation Elongation Factor-1 α PCR
22	Monoclonal antibody ED7 was highly specific for the three human pathogenic species of Fusarium,

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F. solani, F. oxysporum and F. dimerum, which were culturable from 75% of the sink swabs (Table

1 1 and Table S2)2 and summarised in Table 3). ELISA tests of the saline sink swabs showed that 2 52% contained detectable levels of *Fusarium* antigen (Table 1 and Table S23), with ELISA absorbance values in the range ≥ 0.100 (the threshold value for antigen detection) and up to 1.500. 3 4 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 5 6 detection of the diagnostic antigen in swab samples with absorbance values of 0.264, 0.530, 0.187 7 and 0.193 respectively (Table 12). This was likely due to the Fusarium isolates being outgrown in 8 the mixed culture plates by faster growing or more abundant unrelated fungi. Importantly, mAb 9 ED7 was shown not to cross-react with unrelated fungi (axenic culture absorbance values of ≤ 0.100 10 in all cases) including the human pathogenic yeast or yeast-like fungi Candida, Exophiala, 11 Meyerozyma, Rhodotorula, Trichosporon, the human pathogenic hyaline or dematiaceous molds 12 Aspergillus, Phialophora, Phoma, Trichoderma, and the human pathogenic mucormycete Mucor 13 (Table 12). The remaining 93% of samples positive for Fusarium antigen, either at the swab stage 14 or following periods of biological amplification in mixed or axenic cultures (Table S2), yielded 15 strains of the three Fusarium species. There was 100% concordance between Fusarium genus 16 identification by ELISA and species identification by ITS sequencing (Table 13). The species of F. 17 solani and F. oxypsporum recovered from sink swabs were subsequently shown by TEF-1 α 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, 18 19 9-a, 15-a, 20-d and F. oxysporum (FOSC) species complexes 16, 33, 99, 111, 126, 134, 183 (Table 20 **<u>S3Table 4 and Appendix 1</u>**). All of the recovered *F. dimerum* isolates belonged to the *F. dimerum* 21 species complex (FDSC) ET-gr.(Table S3). ITS analysis of axenic cultures (Table 1-3) showed that 22 a number of sink samples (e.g. S2, S6, S17, S19, S21, S24, S30, S38) contained mixtures of 23 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 <u>1</u>2). The sink biofilms corresponding to these water samples were also positive at the swab ELISA stage (Table 1).

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2 Discussion

The genus Fusarium comprises ubiquitous environmental mould mould capable of infecting plants 3 4 and humans (Zhang et al., 2006). Unlike agriculture, where the most economically damaging 5 pathogens are considered to be F. graminearum and F. oxysporum (Dean et al., 2012), the species 6 most commonly cited as human pathogens belong to the Fusarium solani species complex (FSSC, 7 responsible for 50% of reported infections in humans), followed by strains in the Fusarium 8 oxysporum species complex (FOSC)(Torres and Kontoyiannis, 2011). The Fusarium dimerum 9 species complex (FDSC) is less frequently reported as causing human disease, but it is similarly 10 capable of causing disseminated infections in immunocompromised patients (Bigley et al., 2004; 11 Schroers et al., 2009).

12 While the natural habitats of plant pathogenic Fusarium strains are well characterised as soil 13 and decaying plant material, habitats providing direct human exposure to infectious propagules are 14 largely unexplored. The increasing frequency of opportunistic fungal infections in humans means 15 that improved surveillance methods are needed to identify environmental reservoirs of pathogens to 16 limit the exposure of vulnerable individuals to potentially infective propagules. For *Fusarium*, there 17 is a growing body of evidence to suggest that domestic and municipal water systems are potential 18 reservoirs of human pathogenic strains in the FSSC, FOSC and FDSC groups (Short et al., 2011). 19 Accurate techniques that can be used to identify the fungus in environmental samples 20 containing mixed populations of fungi are currently lacking and, while nucleic acid-based 21 technologies have been developed for the differentiation of Fusarium from other human pathogenic 22 species and to identify Fusarium species complexes, such techniques have typically been used in 23 retrospective analysis of axenic cultures collected during human and environmental population

1 studies (Bouchara et al., 2009; Steinmann et al., 2011; Lackner et al., 2012). Furthermore, these 2 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 3 4 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 5 6 al., 2012, 2013), no attempts have been madeused to use mAbs to track human pathogenic strains in 7 environmental samples. Jensen et al. (2011) recently reported the development of Fusarium-8 specific mAbs for immunohistochemical diagnosis of fusariosis. The IgM mAbs, which recognise 9 51 and 63 kDa antigens, reacted strongly with fungal elements in both experimentally infected 10 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 11 12 Fusarium could be identified in sink drains directly by using crude antigen extracts of biofilms and 13 detection using a genus-specific immunoglobulin M (IgM) mAb, ED7, that binds to an H 14 extracellular ~200kDa carbohydrate antigen present on the surface of spores and hyphae. While the function of the antigen is currently unknow Using mAb based ELISAn, we we were able, in 15 Enzyme-Linked Immunosorbent Assay (ELISA) tests, able-to detect its presence the diagnostic 16 17 antigen-in 52% of swab samples and, following biological amplification of biofilms on a non-18 selective mycological medium, were able to identify additional biofilm samples containing 19 pathogenic strains of Fusarium. This is the first time, to our knowledge, that a mAb-based detection 20 method has been used to track Fusarium in environmental samples. The mAb was able to 21 differentiate Fusarium from a wide spectrum of unrelated fungi, including the human pathogens 22 Aspergillus (Thornton and Wills, 2015), Candida, Geotrichum, Rhodotorula and Trichosporon 23 (Davies and Thornton, 2014; Miceli et al., 2011), Cyphellophora and Phialophora (Feng et al.,

1 2014), Exophiala (Zeng et al., 2007), Trichoderma (Sandoval-Denis et al., 2014), Engvodontium 2 (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 3 100% accuracy of the ED7 ELISA, confirmed by using ITS sequencing and TEF PCR analysis of 4 5 recovered isolates, demonstrates its robustness in detecting potentially infectious Fusarium species 6 in polymicrobial communities. Importantly, mAb-ED7 reacted with all of the species complex 7 strains isolated including the most common clinical pathotypes of Fusarium, FSSC 1-a, FOSC 33 8 and FDSC ET-gr (Schroers et al., 2009; Short et al., 2011).

9 While the ED7 ELISA was able to identify Fusarium to the level of genus only, the 10 simplicity of the mAb-based approach to detection, even when combined with a standard 11 mycological isolation procedure, means that a recognised environmental niche of this group of 12 pathogenic fungi can be monitored readily. The widespread occurrence of human pathogenic 13 Fusarium species in sinks of a tertiary care hospital and sinks of a heavily populated university 14 campus, show that indoor plumbing-associated biofilms and water sources are an unseen source of 15 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., 16 17 2011). While no cases of fusariosis were reported during the course of this study, tThe close 18 proximity of the patients to hospital sinks colonised with both pathogenic fusaria and with other 19 opportunistic fungal pathogens is a serious concern given the known vulnerability of 20 immunocompromised individuals to invasive fungal infections.

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Conflicts of Interest

- .g this paper have any c. 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.

13 Experimental procedures

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15 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.

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24 Fungal culture

Fungi (Table <u>S</u>1) were routinely cultured on Potato Dextrose Agar (PDA<u>: 70139; Sigma</u>)),
Sabouraud Dextrose Agar (SD<u>A: Sabouraud Dextrose Broth (SDB: S3306; Sigma) containing 2%</u>
(w/v) agar),A), Malt Yeast extract Agar (MYA<u>: Y3127; Sigma</u>), or Oatmeal Agar (OA<u>: O3506;</u>
<u>Sigma</u>), sterilized by autoclaving at 121°C for 15 min. Cultures were grown at 26°C under a 16 h
fluorescent light regime.

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2 Development of mAb, preparation of immunogen, and immunisation regime

BALBalb/c mice were immunized with soluble antigens prepared from lyophilized mycelium of a 3 human pathogenic strain of Fusarium solani species complex 1-a (CBS strain 224.34). Conidia 4 were suspended in water after 10-day old PDA slant cultures were flooded with 5 ml dH₂O and 5 6 gently agitated with an inoculation loop. Conidial suspensions were then filtered through Miracloth 7 to remove mycelium and transferred to 1.5 ml micro-centrifuge tubes. The conidia were washed 8 three times with dH₂O by repeated vortexing and centrifugation at 14,462 g for 5 min and finally suspended in dH₂O to give a concentration of 10⁶ conidia ml⁻¹ solution. Flasks containing 100 ml of 9 10 sterilized Potato Dextrose Broth (potato dextrose broth (PDB: P6685; Sigma))-were inoculated 11 with 200 μ l of the conidial suspension and incubated with shaking (75 rpm) for 48 h at 26°C. 12 Hyphal biomass was collected on Miracloth, snap frozen in liquid N₂, and lyophilized. Culture 13 filtrates were retained for gel electrophoresis and western blotting studies and stored at -20°C until 14 required. One mg of lyophilized biomass was suspended in 1 ml phosphate buffered saline (PBS: 15 0.8% NaCl; 0.02% KCl; 0.115% Na₂HPO₄; 0.02% KH₂PO₄; pH7.2) and the resultant suspension 16 centrifuged for 5 min at 14,462 g. The supernatant, containing solubilized antigens, was used as the 17 immunogen and as a source of antigens for hybridoma screening assays. For immunization, 6-wk-18 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 19 20 five days before fusion.

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22 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 1 2 supernatants were screened by Eenzyme-Llinked limmunosorbent Aassay (ELISA) against antigens immobilized to the wells of Maxisorp microtitre plates (442404; Nunc)(50 µl per well). For 3 antibody specificity tests, fungi were grown on replicate agar slopes and surface washings 4 containing water-soluble antigens prepared as described in Thornton (2001). Protein concentrations, 5 6 determined spectrophotometrically at 280 nm (Nanodrop, Agilent Technologies Limited, Berkshire, UK), were adjusted to 60 μ g ml⁻¹ buffer. Fifty μ l volumes were then used to coat the wells of 7 microtitre plates. After incubating overnight at 4°C, wells were washed four times with PBST (PBS 8 containing Tween-20, 0.05% (v/v)), and once each with PBS and dH₂O and then air-dried at 23°C 9 10 in a laminar flow hood. The plates were stored in sealed plastic bags at 4°C in preparation for 11 screening of hybridoma supernatants by ELISA as described below.

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13 Plate-Trapped-Antigen-Enzyme-Linked Immunosorbent Assay

14 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, 15 IgA, and IgM) peroxidase conjugate (A-0412; Sigma Chemical Company, Poole, United Kingdom) 16 17 diluted 1 in 1000 in PBST for a further hour. Bound antibody was visualized by incubating wells 18 with tetramethyl benzidine (TMB: T-2885; Sigma) substrate solution (Thornton, 2001) for 30 min. 19 The reactions were stopped by the addition of 3 M H₂SO₄ and -aAbsorbance values were 20 determined at 450 nm with an MRX automated microplate reader (Dynex Technologies, 21 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 μ l per well₅ 22 23 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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6 Determination of Ig subclass and cloning procedure

7 The Ig class of mAbs was determined by using antigen-mediated ELISA. Wells of microtitre plates 8 coated with F. solani CBS224.34 water-soluble antigens from surface washings were incubated 9 successively with hybridoma supernatant-ED7 TCS for 1 h, followed with goat anti-mouse IgG₁, 10 IgG_{2a}, IgG_{2b}, IgG₃, IgM, or IgA-specific antiserum (ISO-2; Sigma) diluted 1 in 3000 in PBST for 30 11 min and rabbit anti-goat peroxidase conjugate diluted 1 in 1000 (A-5420; Sigma) for a further 30 12 min. Bound antibody was visualized with TMB substrate as described above. Hybridoma cells lines 13 were sub-cloned three times by limiting dilution, and cell lines were grown in bulk in a non-14 selective medium preserved by slowly freezing in fetal bovine serum/dimethyl sulfoxide (92:8 15 [v/v]), and stored in liquid nitrogen.

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17 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 prestained broad range markers (161-0318; Bio-Rad) were used for molecular weight determinations.

For wWesterns, separated antigens were transferred electrophoretically to a PVDF membrane (162-1 2 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 supernatantED7 TCS diluted 1 in 2 3 with PBS containing 0.5% (w/v) BSA (PBSA) for 2 h at 23°C. After washing three times with PBS, 4 membranes were incubated for 1 h with goat anti-mouse IgM (µ-chain specific) alkaline 5 phosphatase conjugate (A-9688; Sigma), diluted 1 in 15,000 in PBSA. After the membranes were 6 7 washed twice with PBS and once with PBST, the bound antibodies were visualized by incubation in 8 BCIP/NBT substrate solution. Reactions were stopped by immersion in dH₂O and air-dried between 9 sheets of Whatman filter paper.

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11 Characterization of antigen by enzymatic and chemical modifications and by heating

12 Water-soluble antigens from surface washings of slopes of F. solani CBS224.34 were prepared as 13 described. Heat stability studies were conducted by placing tubes of solubilised antigen solubilised 14 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 15 16 immobilised to the wells of microtitre plates for assay by ELISA as described. For periodate 17 oxidation, microtitre wells containing immobilised antigens from surface washings of the fungusantigens were incubated with 50 μ l of sodium *meta*-periodate solution (20 mM NaIO₄ in 50 18 19 mM sodium acetate buffer (pH4.5)) or acetate buffer only (control) at 4°C in sealed plastic bags. 20 Plates were given four 3-min PBS washes before processing by ELISA as described. For protease 21 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 H₂O) solution or Milli-Q H₂O or 22 23 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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2 3 Immunofluorescence and immunogold electron microscopy 4 For immunfluorescence (IF), sterilised slides were coated with a washed spore suspensions of F. 5 solani CBS224.34 containing 1% (w/v) glucose solution and incubated at 26°C for 16 h to allow 6 spore germination and formation of germ tubes. After air-drying, the slides cells were fixed to the 7 slides as described in Thornton (2001) and incubated with hybridoma supernatantED7 TCS or TCM 8 only (negative control) for 1 h, followed by three 5 min PBS washes. Slides were then incubated 9 with goat anti-mouse polyvalent fluorescein isothiocyanate (FITC) conjugate (diluted 1 in 40 in 10 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 11 12 steps were performed at 23°C in a humid environment to prevent evaporation and slides were stored 13 in the dark, at 4°C, prior to examination using an epifluorescence microscope (Olympus IX81) 14 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 15 16 using hybridoma supernatant or TCM control and anti-mouse polyvalent 20nm gold conjugate 17 according to the technique described in Thornton & Talbot (2001) was used. Spores and hyphae of 18 F. solani were prepared by incubating washed conidia in 1% (w/v) glucose solution at 26°C for 16 19 h to allow spore germination and formation of germ tubes. Cells were embedded in LR White resin 20 (Agar Scientific Ltd.) and ultra thin sections prepared for immunolabeling. Sections immobilized to 21 nickel grids were blocked by immersion in PBST containing 1% (w/v) BSA (PBST-BSA) which 22 had been sterile filtered through a 0.2 µm filter. The grids were washed three times (3 min each) in 23 sterile filtered PBST and then incubated in ED7 TCS or TCM only (negative control) for 1 h. After

PBS and then assayed by ELISA with hybridoma supernatantED7 TCS as described.

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1	four washes (3 min each) with sterile filtered PBST, the grids were incubated for a further hour in
2	PBST-BSA containing a 1:20 dilution of goat anti-mouse 20 nm gold conjugate (EM.GAF20; BBI
3	Solutions). The grids were washed four times (3 min each) in sterile filtered PBST and then placed
4	on Whatman filter paper to dry. Dried grids were then incubated for 20 min in 2% (w/v) uranyl
5	acetate solution followed by 2% (w/v) lead citrate solution for 4 min. Working volumes were 100μ
6	and incubation and washing steps were carried out at at 23°C. Immunostained samples were
7	examined using a Jeol JEM 1400 transmission electron microscope fitted with a Gatan ES 100W
8	CCD camera.
9	

10 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.

15

16 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 Formatted: Font: Symbol

1 in 1.5-ml micro-centrifuge tubes containing 1 ml PBS to dislodge biofilm debris, and the sealed 2 tubes transferred to the laboratory for processing by ELISA and mycological culture. 3 Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the 4 ITS regions of the rRNA-encoding gene unit and Translation Elongation Factor-1a PCR 5 6 Biofilm debris was pelleted by centrifugation at 14,462 g for 5 min and 50 µl samples of 7 supernatant transferred to the wells of microtitre plates for assay by ELISA (Table 12; Swab-ELISA and Table S2; Swab-ELISA) as described. The biofilm pellet was re-suspended in 1 ml 8 dH₂O, 200 µl samples spread on the surface of PDA containing-the 1 µg ml⁻¹ of the broad-spectrum 9 10 antibiotic rifampicin, and the plates incubated for 2 d at 26°C under a 16 h fluorescent light regime. 11 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 12 13 extracts were prepared as surface washings from mixed cultures and from axenic cultures and 14 assayed by ELISA (Table 1 and Table S22; Mixed culture-ELISA and Axenic culture-ELISA, 15 respectively) as described. 16 Fungal DNA was extracted from axenic culture material by using the CTAB method (Chow 17 & Kafer, 1993) and fungi were identified by sequencing of the ITS1-5.8S-ITS2 region of the rRNA-18 encoding gene unit (White et al., 1990) according to procedures described elsewhere (Thornton et 19 al., 2002), using the primers ITS1ext (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS4ext (5'-20 TTCTTTTCCTCCGCTTATTGATATGC-3'). Species identity was predicted based on >95% 21 sequence identity (E-value = 0.0)(Altschul et al. 1997) of the ITS1-5.8S-ITS2 region of recovered 22 species to species recorded in GenBank. Fusarium species were further identified to species 23 complex level by using the forward primer ef-1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and

1	reverse primer ef-2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3'), which amplify an \sim 700 bp
2	region of Translation Elongation Factor 1-alpha (TEF-1 α), the single-locus identification tool in
3	Fusarium (Geiser et al., 2004). PCR reactions were carried out in a total volume of 25 μ l consisting
4	of 1 μ l DNA at <u>a</u> concentration of 30 - 75 ng μ l ⁻¹ , 12.5 μ l of GoTaq® Green Master Mix DNA
5	polymerase (Promega, MF7112), 9.5 μ l of nuclease free water (Promega) and 1 μ l of each primer at
6	20 pmol. The following cycling parameters were used: an initial denaturation step at 95-°C for 8
7	min; 35 cycles of 15 sec at 95-°C (denaturation); 20 s at 54-°C (annealing), 1 min at 72-°C
8	(extension) followed by a final 5 min extension step at 72-°C. Phylogenetic sub-groups of Fusarium
9	species were determined by interrogation of the FUSARIUM-ID v. 1.0 database
10	(<u>http://isolate.fusariumdb.org</u>)(O'Donnell et al., 2010), with the newly acquired TEF-1 α sequences
11	(Supporting Data Set 1Appendix 1).
12	
13	Nucleotide sequence accession numbers
14	Newly determined ITS sequences were submitted to GenBank and the ITS accession numbers
15	KT876496 to KT876723 were obtained. Species designations of recovered fungi are shown in Table
16	12.
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plants are common in the envir	onment. J Clin Microbiol 44: 2186-2	190.
•		
Table 1. Details of fungi used in m	Ab ED7 specificity tests.	
Organism	Isolate no.	Source
Aspergillus cervinus	537.65	
Aspergillus ficuum	555.65	
Aspergillus flavus	<u>91856iii</u>	<u>——IMI</u>
Aspergillus fumigatus	AF293 ⁻	<u>SK</u>
Aspergillus fumigatus Aspergillus nidulans		SK FGSC
Aspergillus fumigatus Aspergillus nidulans Aspergillus niger	AF293 ⁻ A4 102.40	FGSC CBS
	Zhang, N., O'Donnell, K., Sutton, I Members of the Fusarium sol plants are common in the envir Table 1. Details of fungi used in m Organism Aspergillus cervinus Aspergillus ficuum Interview	Zhang, N., O'Donnell, K., Sutton, D.A., Nalim, F.A., Summerbell, R.C., Members of the Fusarium solani species complex that cause infe plants are common in the environment. J Clin Microbiol 44: 2186-2 Table 1. Details of fungi used in mAb ED7 specificity tests. Organism Isolate no. Aspergillus cervinus 537.65 Aspergillus fieuum 555.65 Long Tille, F. C.,

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	<u> </u>	
Cryptococcus neoformans (Serotype D)	5728	<u> </u>	
Cunninghamella elegans	151.80	CBS	
Filobasidiella bacillispora	10865	CBS	
Filobasidiella neoformans	10490	CBS	
Table 1. <i>continued</i>		0	
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	

1	Fusarium culmorum	-256.51	
2	Fusarium dimerum var. dimerum	108944	
3	Fusarium incarnatum	678.77	
4	Fusarium nygamai		
5	Fusarium oxysporum f.sp. cucurbitacearum	- 254.52	
6	Fusarium oxysporum f.sp. lycopersici	167.30	
7	Fusarium oxysporum f.sp. marmaris	420.80	
8	Fusarium oxysporum f.sp. radicis-lycopersici	872.95	CBS
9	Fusarium oxysporum f.sp. vasinfectum	409.90	
10	Fusarium proliferatum var. proliferatum		
11	Fusarium sacchari	183.32	
12	Fusarium solani	-224.34	
13	Fusarium solani	109696	CBS
14			•
15	Table 1. continued		
10		Techter and	0.
17 18		Isolate no.	
19	Fusarium solani		-CBS-
20	Fusarium solani	<u></u>	
21	Fusarium solani		
22	Fusarium solani		
23	Fusarium solani var. petroliphilum		
24	Fusarium verticillioides	102699	

. 4	Geotrichum candidum	115.23	
2 4	Haematonectria haematococca	114067	
4	Haematonectria haematococca	119603	
ł	Haematonectria haematococca	130692	
ł	Lichtheimia corymbifera	<u> </u>	
ł	Magnusiomyces capitatus	207.83	
4	Mucor circinellioides f.sp. circinellioides	E2A (FJ713065)	
ł	Neosartorya fischeri var. fischeri	687.71	— CBS —
ł	Paccilomyces variotii	339.51	
ł	Penicillium cyclopium	123.14	— CBS
ł	Penicillium islandicum	338.48	
ł	Penicillium spinulosum	346.61	
4	Pichia norvegensis		
=			
Ę	Fable 1. continued		
-			
Ģ	Organism	Isolate no.	
=			
ł	Pseudallescheria boydii	835.96	
4	Pythium insidiosum	673.85	
+ +	Pythium insidiosum Rhizomucor miehei	— 673.85 — MG4(2) (FJ713069)	— CBS — CRT —
+ + +	Pythium insidiosum Rhizomucor michei Rhizopus stolonifer	— 673.85 — MG4(2) (FJ713069) — 389.95	— CBS — CRT — CBS ———
+ + + +	Pythium insidiosum Rhizomucor miehei Rhizopus stolonifer Rhodosporidium toruloides	<u>673.85</u> <u>MG4(2) (FJ713069)</u> <u>389.95</u> <u>6016</u>	CBS CRT CBS CBS

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

1	Trichosporon ovoides	7556	
2	Trichosporon mycotoxinovorans	9756	CBS
3	Wickerhamomyces anomalus	5759	CBS
4			
5	a. CBS – Centraalbureau voor Schimmelcultu	ures, PO Box 85167	, 3508 AD Utrecht, The Netherlands;
6	CRT = C.R. Thornton; IMI = International M	ycological Institute,	Egham, England; SV = S. Krappman,
7	Institute of Microbiology and Genetics, Depa	artment of Molecula	r Microbiology and Genetics, Georg-
8	August-University, Gottingen, Germany.		
9	(
10			
11			
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13			
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15			

16 Table 12. Locations and identities of sink swabs and water samples and results of ELISA tests and fungal

17 identification based on ITS sequencing.

identification base	ed on ITS seq	uencing.				
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	Cvtobasidium 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	W705(500
			150	0.0000	mucilaginosa	K18/6/00
University (S5)			A5-8	0.0088	Cyphellophora oxyspora	K18/6614
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 oxvsporum	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 oxvsporum	KT876674
University (S15)			B5-2	0.0048	Mucor circinelloides	KT876701
University (S15)			B5-3	0.8851	Fusarium oxvsporum	KT876677
University (S16)	0.0229	0.0002	B6-1	0.8193	Fusarium oxysporum	KT876676
University (S16)	0.0222	0.0002	B6-2	0.7582	Fusarium oxysporum	KT876661
University (S17)	0.0395	0.0014	B7-1	0.8201	Fusarium oxysporum	KT876675
University (S17)	0.0270	0.0011	B7-6	0.7758	Fusarium dimerum	KT876623
University (S18)	0.0133	0.0011	B8-1	0 7347	Fusarium solani	KT876637
University (S18)	0.0155	0.0011	B8-7	0.0095	Rhodosporidium habievae	KT876706
Chiversity (510)			10-7	0.0075	Meyerozyma	1110/0/00
University (S19)	0.0212	0.0048	B9-1	0.0051	guilliermondii	KT876707
University (S19)			B9-2	0.8104	Fusarium oxysporum	KT876657

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Linimonaites (S10)			D0 2	0 (202	E anti a al trat	VT076624
University (S19)			B9-3	0.0302	Fusarium solani	K18/0034
University (S19)	0 5467	0.0204	D9-3	0.0017		K18/0/20
University (S20)	0.340/	0.0394	B10-1	0.7467	Fusarium oxysporum	K18/0080
University (S20)			D10-2	0.7007	Fusarium oxysporum	K18/0039
University (320)			D10-0	0.0071	Canalaa parapsilosis	K18/0/05
University (S20)			B10-7	0.0251	Meyerozyma avilliorm on dii	VT076711
University (\$20)			D10.0	0.0021	Custobasidium slooffiaa	K18/0/11 KT876712
University (S20)			D10-9	0.0031	Trichosporon astanoidas	K1870712 KT876712
University (S20)	0.0082	0.0260	C1 1	0.0231	Classianona husitanian	K1870713
University (S21)	0.0085	0.0209	C1-1	0.0044		K10/0/00 VT076602
University (S21)			C1-3	0.8873	Fusarium oxysporum	KT876620
University (S21)			C1-4	0.0002	Fusarium aimerum	K1870029
University (S21)	0 2077	0.0047	C^{1-7}	0.0003		K18/001/ VT876658
University (S22)	0.2977	0.0047	C2-1	0.9443	Fusarium oxysporum	KT876604
University (S22)			C2-4	0.9823	Fusarium oxysporum	VT076607
University (S22)	0.0808	0.0116	C_{2-1}	0.8077	Fusarium oxysporum	K18/008/ VT876682
University (S23)	0.0808	0.0110	C3-1	0.9833	Fusarium oxysporum	K18/0085
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

University (S31)			CRT1-3	0.7503	Fusarium oxvsporum	KT876652
University (S32)	0.0303	0.0021	CRT2-1	0.0040	Trichoderma asperellum	KT876619
University (S32)		1	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 (\$35)	0.0071	0.0147	R2-4	0.0096	Clonostachys rosea	KT876552
ICU (\$35)			R2-5	0.0018	Clonostachys rosea	KT876553
ICU (\$35)			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
	0.0201	0.0477	DO 2	0.0112	Exophiala	
110 (841)	0.0391	0.0477	K9-3	0.0113	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	1 0313	1 5035	X3-1	1 1353	Fusarium dimorum	
Unit (S44)	1.0515	1.5055	AJ-1	1.1555	Tusurtum atmerum	KT876509
Ophthalmology			X3-2	0.0224	Candida parapsilosis	
Unit (S44)				0.022.		KT876498
Ophthalmology			X3-3	1.1856	Fusarium dimerum	
Unit (S44)		1				K1876512
Ophthalmology	1.4582	1.4049	X4-1	1.1322	Fusarium dimerum	WEDDER CELL
Unit (S45)						K1876511
Ophthalmology			X4-2	1.1523	Fusarium dimerum	1/107/ 500
Unit (S45)						K18/6500
Ophthalmology			X4-3	0.0622	Candida parapsilosis	1/107/500
Unit (845)					1 1	K18/6508
Uphthalmology			X4-4	0.1245	Gloeotinia temulenta	KT07(c1c
Unit (S45)		1				K18/6515
Uphthalmology	0.3325	1.4466	X5-2	1.0654	Fusarium dimerum	VT076510
Unit (S46)					D_{l-1} , 1	K18/0010
Upit (\$46)			X5-3	0.0599	Knoaotorula	VT976501
Unit (846)					mucuaginosa	K10/0001

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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

			1			
Unit (S52)		1				
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			K8-6	0.0084	Cadophora fastigiata	KT876531
Haematology			K8-7	0.0051	Trichoderma asperellum	KT07(525
Haematology	0.9843	1.2903	K9-1	0.0046	Candida paransilosis	K18/0535
Unit (S54)						KT876525
Unit (S54)			К9-2	0.0090	Rhodotorula dairenensis	KT876526
Haematology Unit (S54)			К9-4	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	КТ876529
Haematology Unit (W51)	0.0581	0.0553	G2-1	0.0104	Engyodontium album	KT876540
Haematology Unit (W51)		<u> </u>	G2-3	0.0062	Exophiala pisciphila	КТ876530
Haematology Unit (W52)	0.0125	0.0118	G6-2	0.0161	Exophiala castellanii	КТ876528
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	1.0938	1.2091	H3-1	0.8909	Fusarium oxysporum	KT876584
Oncology Unit		1	H3-4	0.0075	Candida palmioleophila	VT076572
(538) Oncology Unit			H3-5	0.0140	Rhodotorula alutinis	KT876598
Shoology Onit			115-5	0.0110	Thousier and grannis	1110/03/0

Fusarium-s	necific	monoc	lonal	antibody
				and the owned of the second se

					1	
(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)			Н5-3	0.005	Candida tropicalis	КТ876574
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)			Н7-3	0.0045	Candida tropicalis	KT876575
Oncology Unit (S63)	0.0953	0.0152	H8-1	0.0063	Phoma herbarum	KT876580
Oncology Unit (S63)		I	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)			Н9-2	0.0049	Candida orthopsilosis	KT876576
Oncology Unit (S64)			Н9-3	0.0109	Pichia kudriavzevii	KT876579
Oncology Unit (S65)	0.4961	1.1005	H10-1	0.9606	Fusarium dimerum	KT876589
Oncology Unit (S65)		1	H10-4	0.0163	Trametes ochracea	KT876608
Oncology Unit (W56)	0.0030	0.0247	P1-2	0.0082	Exophiala dermatitidis	KT876582
Oncology Unit (W56)		1	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

(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)			Р9-2	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

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

2 b. Threshold absorbance value for detection in ELISA ≥0.100. Shading indicates earliest point in

3 sampling process at which antigen was detectable in ELISA tests with mAb-ED7.

4 c Fusarium strains in bold further characterized by TEF-1α PCR analysis (Table S34 and -Supporting

5 <u>Data Set 1</u>Appendix 1).

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

ata Set 1 <mark>Ap</mark>	pendix 1).					
Total no.	nmary of ELIS. No. swab	A tests and m	ycological cultur No. samples	e of sink swabs. No. samples	No. antigen	No. antigen
swabbed	samples yielding- fungi (%) *	samples positive for Fusarium antigen at swab stage	Fusarium Antigen by mixed culture stage	Fusarium Antigen by axenic culture- stage-	samples yielding- Fusarium spp.	samples not yielding Fusarium spp.
65	65 (100%)	34 (52%)	37 (57%)	54 (83%)	50 (93%)	4 (7%)

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11 *ium* spp. and/or unrelated fungi.

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21 22	Table 4. Translation Florention Factor 1 or PCP analysis of Eugenium isolates recovered from sinks	
22	Table 4. Translation Elongation Factor-10. PCK analysis of <i>PusaFium</i> isolates recovered from sinks.	
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1	Figure 1. Specificity of ED7 determined by Enzyme-Linked Immunosorbent Assay tests of
2	surface washings containing water-soluble antigens from <i>Fusarium</i> species and related and
3	unrelated yeasts and molds. (A) ELISA aELISA absorbance values at 450 nm for from specificity
4	screening tests using mAb ED7 and antigens from F. solani and unrelated yeasts and mouldmolds
5	(A), and (B) for antigens from the F. solani teleomorph Haematonectria- haematococca and related
6	<i>Fusarium</i> species (B). Wells were coated with 60 μ g protein ml ⁻¹ buffer. Bars are the means of three
7	biological replicates \pm standard errors and $-\frac{1}{2}$ the threshold absorbance value for detection of antigen
8	in ELISA is ≥0.100 (indicated by lines on graphs). Numbers in parentheses after species names
9	denote strain numbers with further details of strains provided in Table S1.
10	
11	Figure 2. Characterisation Characterisation of the ED7 antigen and its epitope and spatial
12	distribution of the antigen in spores and hyphae bound by mAb ED7. (A) Western immunoblot
13	with mAb-ED7 using culture fluid from 2-d-old PDB cultures of F. solani CBS224.34 (lane 1) and
14	F. oxysporum f.sp. lycopersici CBS167.30 (lane 2). Wells were loaded with 1.6 μ g of protein. M _r
15	denotes molecular weight in kDa. Note the major extracellular antigen with molecular weight of
16	~200_kDa. (B) Absorbance values from ELISA tests with mAb_ED7 using immobilized
17	antigensurface washings containing water-soluble antigens immobilized to the wells of microtitre
18	plates and treated with trypsin or PBS only (control) at 4°C and 37°C. Bars are the means of three
19	biological replicates ± standard errors and bars with the same letter are not significantly different at
20	p<0.001 (ANOVA and Tukey-Kramer test). (C) Absorbance values from ELISA tests with-mAb
21	ED7 using surface washings containing water-soluble antigens immobilized to the wells of
22	microtitre plates and immobilized antigen treated with pronase or Milli-Q H ₂ O only (control) at 4°C
23	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 1 2 values from ELISA tests with mAb ED7 following Stabilityheating of the water-soluble ED7 antigen following heating of surface washings antigen at 100°C over a 70 min period. Treated 3 antigen was subsequently immobilized to the wells of microtitre plates and assayed by ELISA. Bars 4 5 are the means of three biological replicates ± standard errors and bars with the same letter are not 6 significantly different at p<0.001 (ANOVA and Tukey-Kramer test). (E) Absorbance values from 7 ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the 8 wells of microtitre plates and Absorbance values from ELISA tests with mAb ED7 and immobilized 9 antigen treated with periodate (open circles) or with acetate only control (closed circles) at 4°C over 10 a 20 h period. Each point is the mean of three biological replicates ± standard errors. (F to and IG) 11 Photomicrographs of F. solani CBS224.34 immunostained with mAb-ED7 or TCM control and goat 12 anti-mouse polyvalent Ig fluorescein isothiocyanate (FITC) conjugate. (F) Brightfield image of 13 germinated conidium with hypha probed with ED7 followed by fluorochrome conjugate (scale bar 14 $= 6 \mu m$; (G) Same field of view as panel F but examined under epifluorescence. Note intense 15 staining of the cell wall of microconidium and hypha;- Scale bar = 6 µm. (H) Brightfield image of 16 germinated conidium with hypha probed with TCM (negative control) followed by FITC conjugate 17 (scale bar = 3 μ m); (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 18 19 labeling of sections of conidia and hyphae of F. solani CBS224.34. (JH) Transverse section of 20 conidium incubated with mAb-ED7 and anti-mouse immunoglobulin 20 nm gold particles, showing 21 antigen in the cell wall and in an extracellular fimbrial matrix surrounding the spore (scale bar = 22 250 nm); (KI) Longitudinal section of hypha incubated with mAb ED7 and anti-mouse 23 immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular

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1	matrix surrounding the cell (scale bar = 100 nm); (LJ) Transverse section of a mieroconidium
2	incubated with TCMTCM ((negative control) and anti-mouse immunoglobulin 20 nm gold
3	particles, showing lack of staining by the secondary gold <u>conjugatereporter. S (s</u> cale bar = 180 nm).
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5	
6	

1 Detection of human pathogenic *Fusarium* species in hospital and 2 communal sink biofilms by using a highly specific monoclonal 3 antibody

4

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11 Summary

12 The fungus *Fusarium* is well known as a plant pathogen, but has recently emerged as an 13 opportunistic pathogen of humans. Habitats providing direct human exposure to infectious 14 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 15 oxvsporum species complex (FOSC), the most common groups infecting humans. Here, we use a 16 newly developed *Fusarium*-specific monoclonal antibody (mAb ED7) to track FSSC and FOSC 17 18 strains in sink drain biofilms by detecting its target antigen, an extracellular 200kDa carbohydrate, 19 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, 20 FOSC and F. dimerum species complex (FDSC) strains that were present, as mixed fungal 21 22 communities, in 83% of sink drain biofilms. Specificity of the ELISA was confirmed by sequencing

1 of the internally transcribed spacer 1 (ITS1)-5.8S-ITS2 rRNA-encoding regions of culturable yeasts 2 and molds that were recovered using mycological culture, while translation elongation factor (TEF)-1a analysis of Fusarium isolates included FSSC 1-a, FOSC 33 and FDSC ET-gr, the most 3 4 common clinical pathotypes in each group. 5 **Originality-Significance Statement** 6 7 • Development of a monoclonal antibody (mAb ED7) specific to *Fusarium*, a fungal genus 8 containing human and plant pathogens 9 • *Fusarium* diagnostic antigen detected in swabs of sink drain biofilms, with 100% accuracy 10 of mAb-based ELISA confirmed by ITS sequencing of mixed fungal communities 11 comprising human pathogenic yeasts and molds 12 • 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 13 14 potential for nosocomial and community acquired infections 15 16 17 18 19 20 21 22 23

1 Introduction

Species in the fungal genus *Fusarium* are ubiquitous environmental molds, and pathogens of both 2 3 plants and animals (Zhang et al., 2006; Thornton and Wills, 2015). In immunocompromised 4 humans, such as patients with haematological malignancies and hematopoietic stem cell and solid organ transplant recipients, Fusarium species are significant emerging pathogens, causing a 5 6 frequently fatal disseminated disease known as fusariosis with an associated mortality rate of 50-7 75% (Girmenia et al., 2000; Musa et al., 2000; Boutati and Anaissie, 1997; Dignani and Anaissie, 8 2004; Jensen et al., 2004; Nucci and Anaissie, 2007). In some tertiary cancer centres, Fusarium has 9 emerged as the second most common mold pathogen after Aspergillus (Walsh and Groll, 1999; 10 Muhammed et al., 2011). Regardless of human immune status, Fusarium species can cause 11 localised nail infections (onvchomycosis)(Arrese et al., 1996), bone and joint infections (Koehler et al., 2014), infections of burn wounds (Latenser, 2003), skin infections (Nucci and Anaissie, 2002; 12 Gurusidappa and Mamatha, 2011), and are the most frequent cause of mycotic eye infections known 13 14 as fungal keratitis (Jurkunas et al., 2009), leading to progressive corneal destruction and 15 endophthalmitis, with loss of vision or even loss of the affected eye (Dursun et al., 2003; Edelstein 16 *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).

5 Identification of environmental reservoirs of human pathogenic molds including *Fusarium* 6 has typically relied on nucleic acid-based technologies following recovery of fungi using selective 7 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 8 9 identify pathogenic species or species complexes in environmental samples containing mixed 10 populations of yeasts and molds (Thornton, 2009; Davies and Thornton, 2014; Thornton et al., 11 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 12 13 species-specific mAbs offers an attractive alternative approach to pathogen detection, particularly 14 when combined with unsophisticated diagnostic modalities such as lateral-flow technology 15 (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.

1 Results

2 Production of hybridoma cell lines, isotyping of mAb and specificity

3 A single fusion was performed and 389 hybridoma cell lines were screened for specificity against a 4 range of clinically relevant yeasts and molds (Table S1). The aim was to identify cell lines secreting 5 mAbs specific to *Fusarium* that could be used to track the fungus in environmental samples 6 containing mixed species of human pathogenic fungi. To this end, a single cell line, ED7, was 7 identified that produced mAb belonging to the immunoglobulin class M (IgM), which was genusspecific, reacting in ELISA tests with antigens from *Fusarium* species and with the *F. solani* 8 9 teleomorph Haematonectria haematococca only (Figs. 1A and 1B). It did not cross-react with 10 antigens from a wide range of unrelated mold and yeast species (Fig. 1A).

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12 Western blotting of the ED7 antigen and epitope characterization

Gel electrophoresis and western blotting studies showed that ED7 binds to a major antigen with 13 14 molecular weight of ~200 kDa which is secreted extracellularly by both F. solani and F. oxysporum 15 (Fig. 2A). Fusarium solani antigens were subjected to enzymatic (Fig. 2B and Fig. 2C), heat (Fig. 16 2D) and chemical (Fig. 2E) modifications in order to characterise the epitope bound by ED7. 17 Reductions in mAb binding following treatment with pronase shows that its epitope consists of 18 protein, while reductions with trypsin indicate a protein epitope containing positively charged lysine 19 and arginine side chains. The lack of reduction in ED7 binding following digestion of immobilized 20 antigen with trypsin (Fig. 2B) and pronase (Fig. 2C) shows that it does not bind to a protein epitope. 21 Reductions in mAb binding following heat treatment shows that an epitope is heat labile. There was 22 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 23

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.

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7 Immunofluorescence and immunogold electron microscopy

8 Immuno-localisation studies using IF showed that the ED7 antigen was present on the surface of 9 spores and hyphae (Figs. 2F-I), while IEM showed that the antigen was present in the spore and 10 hyphal cell wall and in an extracellular fibrillar matrix surrounding both (Figs. 2J-L). In the TEM 11 image shown in Fig. 2J, 56% of gold particles were distributed in the fibrillar matrix surrounding 12 the cell, while 40% and 4% of gold particles were distributed in the cell wall and cytoplasm 13 respectively. This shows that the ED7 antigen is predominantly extracellular or located within the 14 cell wall.

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16 Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the
17 ITS regions of the rRNA-encoding gene unit and by Translation Elongation Factor-1 α 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 1 2 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 3 4 more abundant unrelated fungi. Importantly, ED7 was shown not to cross-react with unrelated fungi 5 (axenic culture absorbance values of <0.100 in all cases) including the human pathogenic veast or 6 yeast-like fungi Candida, Exophiala, Meyerozyma, Rhodotorula, Trichosporon, the human 7 pathogenic hyaline or dematiaceous molds Aspergillus, Phialophora, Phoma, Trichoderma, and the 8 human pathogenic mucormycete *Mucor* (Table 1). The remaining 93% of samples positive for 9 *Fusarium* antigen, either at the swab stage or following periods of biological amplification in mixed 10 or axenic cultures (Table S2), yielded strains of the three Fusarium species. There was 100% 11 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 12 13 subsequently shown by TEF-1 α PCR (Supporting Data Set 1) to belong to F. solani species 14 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 15 complexes 16, 33, 99, 111, 126, 134, 183 (Table S3). All of the recovered F. dimerum isolates 16 belonged to the F. dimerum species complex (FDSC) ET-gr (Table S3). ITS analysis of axenic 17 cultures (Table 1) showed that a number of sink samples (e.g. S2, S6, S17, S19, S21, S24, S30, 18 S38) contained mixtures of *Fusarium* species, while ITS and TEF-PCR analysis (Table 1 and Table 19 S3) showed that others contained mixtures of species complexes of the same species (e.g. S8, S9, 20 S25, S28). Monoclonal antibody ED7 was able to detect all of the Fusarium species complexes 21 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

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

2 The genus Fusarium comprises ubiquitous environmental molds capable of infecting plants and 3 humans (Zhang *et al.*, 2006). Unlike agriculture, where the most economically damaging pathogens 4 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, 5 6 responsible for 50% of reported infections in humans), followed by strains in the *Fusarium* 7 oxysporum species complex (FOSC)(Torres and Kontoyiannis, 2011). The Fusarium dimerum 8 species complex (FDSC) is less frequently reported as causing human disease, but it is similarly 9 capable of causing disseminated infections in immunocompromised patients (Bigley et al., 2004; 10 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 1 2 polymicrobial communities (Short et al., 2011). While monoclonal antibodies (mAbs) and antibody 3 fragments have been developed for detecting and differentiating *Fusarium* species in vitro or in 4 planta (Wong et al., 1988; Arie et al., 1991, 1995; Danks et al., 1996; Hayashi et al., 1998; Hu et 5 al., 2012, 2013), no attempts have been made to use mAbs to track human pathogenic strains in 6 environmental samples. Jensen et al. (2011) recently reported the development of Fusarium-7 specific mAbs for immunohistochemical diagnosis of fusariosis. The IgM mAbs, which recognise 8 51 and 63 kDa antigens, reacted strongly with fungal elements in both experimentally infected 9 animals and biopsy samples from patients with fusariosis sepsis and dissemination to the skin.

10 In this prospective study, we set out to determine whether human pathogenic species of 11 Fusarium could be identified in sink drains directly by using crude antigen extracts of biofilms and 12 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 13 14 the antigen is currently unknown we were able, in Enzyme-Linked Immunosorbent Assay (ELISA) 15 tests, to detect its presence in 52% of swab samples and, following biological amplification of 16 biofilms on a non-selective mycological medium, were able to identify additional biofilm samples 17 containing pathogenic strains of *Fusarium*. This is the first time, to our knowledge, that a mAb-18 based detection method has been used to track *Fusarium* in environmental samples. The mAb was 19 able to differentiate *Fusarium* from a wide spectrum of unrelated fungi, including the human 20 pathogens Aspergillus (Thornton and Wills, 2015), Candida, Geotrichum, Rhodotorula and 21 Trichosporon (Davies and Thornton, 2014; Miceli et al., 2011), Cyphellophora and Phialophora 22 (Feng et al., 2014), Exophiala (Zeng et al., 2007), Trichoderma (Sandoval-Denis et al., 2014), Engvodontium (Macêdo et al., 2007; Thamke et al., 2015) and Mucor (Petrikkos et al., 2012), 23

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Fusarium-specific monoclonal antibody

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).

7 While the ED7 ELISA was able to identify *Fusarium* to the level of genus only, the 8 simplicity of the mAb-based approach to detection, even when combined with a standard 9 mycological isolation procedure, means that a recognised environmental niche of this group of 10 pathogenic fungi can be monitored readily. The widespread occurrence of human pathogenic 11 *Fusarium* species in sinks of a tertiary care hospital and sinks of a heavily populated university 12 campus, show that indoor plumbing-associated biofilms and water sources are an unseen source of 13 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., 14 15 2011). While no cases of fusariosis were reported during the course of this study, the close 16 proximity of the patients to hospital sinks colonised with both pathogenic fusaria and with other 17 opportunistic fungal pathogens is a serious concern given the known vulnerability of 18 immunocompromised individuals to invasive fungal infections.

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RD&E hospital for allowing us to sample patient sinks.

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.

1 Experimental procedures

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3 *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.

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12 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°C for 15 min. Cultures were grown at 26°C under a 16 h fluorescent light regime.

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19 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 dH₂O 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 1 three times with dH₂O by repeated vortexing and centrifugation at 14,462 g for 5 min and finally 2 suspended in dH₂O to give a concentration of 10⁶ conidia ml⁻¹ solution. Flasks containing 100 ml of 3 sterilized Potato Dextrose Broth (PDB: P6685; Sigma) were inoculated with 200 µl of the conidial 4 suspension and incubated with shaking (75 rpm) for 48 h at 26°C. Hyphal biomass was collected on 5 Miracloth, snap frozen in liquid N₂, and lyophilized. Culture filtrates were retained for gel 6 7 electrophoresis and western blotting studies and stored at -20°C until required. One mg of 8 lyophilized biomass was suspended in 1 ml phosphate buffered saline (PBS: 0.8% NaCl; 0.02% 9 KCl; 0.115% Na₂HPO₄; 0.02% KH₂PO₄; 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 10 11 source of antigens for hybridoma screening assays. For immunization, 6-wk-old BALB/c female 12 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 13 14 fusion.

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16 Production and screening of hybridomas and determination of antibody specificity

17 Hybridoma cells were produced by the method described elsewhere (Thornton, 2001) and the 18 supernatants were screened by Enzyme-Linked Immunosorbent Assay (ELISA) against antigens 19 immobilized to the wells of Maxisorp microtitre plates (442404; Nunc)(50 μ l per well). For 20 antibody specificity tests, fungi were grown on replicate agar slopes and surface washings 21 containing water-soluble antigens prepared as described in Thornton (2001). Protein concentrations, 22 determined spectrophotometrically at 280 nm (Nanodrop, Agilent Technologies Limited, Berkshire, 23 UK), were adjusted to 60 μ g ml⁻¹ buffer. Fifty μ l 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)), once each with PBS and dH₂O 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.

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6 Enzyme-Linked Immunosorbent Assay

7 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. 8 9 IgA, and IgM) peroxidase conjugate (A-0412; Sigma Chemical Company, Poole, United Kingdom) 10 diluted 1 in 1000 in PBST for a further hour. Bound antibody was visualized by incubating wells 11 with tetramethyl benzidine (TMB: T-2885; Sigma) substrate solution (Thornton, 2001) for 30 min. 12 The reactions were stopped by the addition of 3 M H₂SO₄ and absorbance values were determined 13 at 450 nm with an MRX automated microplate reader (Dynex Technologies, Billingshurst, UK). 14 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 15 16 were incubated with tissue culture medium (TCM) containing 10% (v/v) fetal bovine serum. All 17 incubation steps were performed at 23°C in sealed plastic bags. The threshold for detection of the 18 antigen in ELISA was determined from control means (2 x TCM absorbance values)(Sutula et al., 19 1986). These values were consistently in the range 0.050-0.100. Consequently, absorbance values 20 >0.100 were considered as positive for the detection of antigen.

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1 Determination of Ig subclass and cloning procedure

2 The Ig class of mAbs was determined by using antigen-mediated ELISA. Wells of microtitre plates 3 coated with F. solani CBS224.34 water-soluble antigens from surface washings were incubated 4 successively with ED7 TCS for 1 h, followed with goat anti-mouse IgG₁, IgG_{2a}, IgG_{2b}, IgG₃, IgM, or IgA-specific antiserum (ISO-2; Sigma) diluted 1 in 3000 in PBST for 30 min and rabbit anti-goat 5 6 peroxidase conjugate diluted 1 in 1000 (A-5420; Sigma) for a further 30 min. Bound antibody was 7 visualized with TMB substrate as described above. Hybridoma cells lines were sub-cloned three 8 times by limiting dilution, and cell lines were grown in bulk in a non-selective medium preserved 9 by slowly freezing in fetal bovine serum/dimethyl sulfoxide (92:8 [v/v]), and stored in liquid 10 nitrogen.

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12 Gel electrophoresis and Western blotting

For sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), culture filtrates 13 from 2-d-old PDB shake cultures of F. solani CBS224.34 and F. oxysporum f.sp. lycopersici 14 15 CBS167.30, prepared as described, were diluted in Laemmli buffer (Laemmli, 1970) and were 16 denatured by heating at 95° C for 10 min. Antigens were separated in 4-20% (w/v) polyacrylamide 17 gradient gels (161-1159; Bio-Rad) for 1.5 h at 23°C (165V) under denaturing conditions, and pre-18 stained broad range markers (161-0318; Bio-Rad) were used for molecular weight determinations. 19 For westerns, separated antigens were transferred electrophoretically to a PVDF membrane (162-20 0175; Bio-Rad). The membranes were blocked for 16 h at 4°C with PBS containing 1% (w/v) 21 bovine serum albumin (BSA) and incubated with ED7 TCS diluted 1 in 2 with PBS containing 22 0.5% (w/v) BSA (PBSA) for 2 h at 23°C. After washing three times with PBS, membranes were 23 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 dH₂O and air-dried between sheets of Whatman
 filter paper.

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6 Characterization of antigen by enzymatic and chemical modifications and by heating

7 Water-soluble antigens from surface washings of slopes of F. solani CBS224.34 were prepared as 8 described. Heat stability studies were conducted by placing tubes of solubilised antigen in a boiling 9 water bath. At 10 min intervals, samples were removed, centrifuged at 14,462 g for 5 min, and 10 antigens immobilised to the wells of microtitre plates for assay by ELISA as described. For 11 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 NaIO₄ in 50 mM 12 13 sodium acetate buffer (pH4.5)) or acetate buffer only (control) at 4°C in sealed plastic bags. Plates 14 were given four 3-min PBS washes before processing by ELISA as described. For protease 15 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-O H₂O) solution or Milli-O H₂O or 16 17 PBS only controls respectively for 4 h at 37°C or 4°C. Plates were given four 3-min rinses with 18 PBS and then assayed by ELISA with ED7 TCS as described.

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20 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

1 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 2 3 fluorescein isothiocyanate (FITC) conjugate (diluted 1 in 40 in PBS)(F1010; Sigma) for 30 min. 4 Slides were given three 5 min washes with PBS and mounted in PBS-glycerol mounting medium 5 (F4680; Sigma) before overlaying with coverslips. All incubation steps were performed at 23°C in a 6 humid environment to prevent evaporation and slides were stored in the dark, at 4°C, prior to 7 examination using an epifluorescence microscope (Olympus IX81) fitted with 495 nm (excitation) 8 and 518 nm (emission) filters for FITC. For immunogold electron microscopy (IEM) the method 9 described in Thornton & Talbot (2001) was used. Spores and hyphae of F. solani were prepared by 10 incubating washed conidia in 1% (w/v) glucose solution at 26°C for 16 h to allow spore 11 germination and formation of germ tubes. Cells were embedded in LR White resin (Agar Scientific 12 Ltd.) and ultra thin sections prepared for immunolabeling. Sections immobilized to nickel grids 13 were blocked by immersion in PBST containing 1% (w/v) BSA (PBST-BSA) which had been 14 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 15 16 washes (3 min each) with sterile filtered PBST, the grids were incubated for a further hour in PBST-17 BSA containing a 1:20 dilution of goat anti-mouse 20 nm gold conjugate (EM.GAF20; BBI 18 Solutions). The grids were washed four times (3 min each) in sterile filtered PBST and then placed 19 on Whatman filter paper to dry. Dried grids were then incubated for 20 min in 2% (w/v) uranyl 20 acetate solution followed by 2% (w/v) lead citrate solution for 4 min. Working volumes were 100 µl 21 and incubation and washing steps were carried out at at 23°C. Immunostained samples were 22 examined using a Jeol JEM 1400 transmission electron microscope fitted with a Gatan ES 100W 23 CCD camera.

1 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.

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7 Sampling from drains

8 A total of 65 sinks were swabbed, comprising 32 sinks across the ICU, ITU, haematology, oncology 9 and ophthalmology units of the Royal Devon and Exeter tertiary care hospital (Exeter, Devon, UK) 10 and 33 restroom sinks located around the University of Exeter campus (Exeter, Devon, UK). In 11 addition, cold-water samples were collected from taps connected to the sinks in the haematology 12 and oncology unit, and from the two main water tanks feeding the ophthalmology unit. To isolate 13 fungi from sink biofilms, sterile cotton buds (Boots, UK) wetted with PBS were used to scour the 14 inner surfaces of sink drainpipes for approximately 10 s. Swabs with visible detritus were immersed 15 in 1.5-ml micro-centrifuge tubes containing 1 ml PBS to dislodge biofilm debris, and the sealed 16 tubes transferred to the laboratory for processing by ELISA and mycological culture.

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18 Immunodetection of Fusarium species in sink swabs and identification of fungi by analysis of the
19 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 μ l 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 dH₂O, 200 μ l samples spread on the surface of PDA containing 1 μ g 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.

6 Fungal DNA was extracted from axenic culture material by using the CTAB method (Chow 7 & Kafer, 1993) and fungi were identified by sequencing of the ITS1-5.8S-ITS2 region of the rRNA-8 encoding gene unit (White et al., 1990) according to procedures described elsewhere (Thornton et 9 al., 2002), using the primers ITS1ext (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS4ext (5'-10 TTCTTTTCCTCCGCTTATTGATATGC-3'). Species identity was predicted based on >95% 11 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 12 complex level by using the forward primer ef-1 (5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and 13 reverse primer ef-2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3'), which amplify an ~700 bp 14 region of Translation Elongation Factor 1-alpha (TEF-1 α), the single-locus identification tool in 15 16 *Fusarium* (Geiser et al., 2004). PCR reactions were carried out in a total volume of 25 µl consisting of 1 μ l DNA at a concentration of 30 - 75 ng μ l⁻¹, 12.5 μ l of GoTaq® Green Master Mix DNA 17 18 polymerase (Promega, MF7112), 9.5 µl of nuclease free water (Promega) and 1 µl of each primer at 19 20 pmol. The following cycling parameters were used: an initial denaturation step at 95°C for 8 20 min; 35 cycles of 15 sec at 95°C (denaturation); 20 s at 54°C (annealing), 1 min at 72°C (extension) 21 followed by a final 5 min extension step at 72°C. Phylogenetic sub-groups of *Fusarium* species 22 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-1 α sequences 23

1	(Supporting Data Set 1).
2	
3	Nucleotide sequence accession numbers
4	Newly determined ITS sequences were submitted to GenBank and the ITS accession numbers
5	KT876496 to KT876723 were obtained. Species designations of recovered fungi are shown in Table
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1 Table 1. Locations and identities of sink swabs and water samples and results of ELISA tests and fungal

2 identification based on ITS sequencing.

		Mixed		Axenic		
Location	Swab	culture	Isolate	culture	Identification based on	GenBank
(Source) ^a	ELISA abs	ELISA	No	ELISA	ITS sequencing ^c	Accession No
(Bource)	(450nm) ^b	abs	110.	abs	115 sequeneing	
		(450nm) ^b		(450nm) ^b		
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	Cvtobasidium 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
	-			0.0007	Rhodotorula	
University (S5)			A5-7	0.0037	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	КТ876678
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
	0.1710	0.0002	11/ 1	0.0090	Penicillium	1110/0010
University (S7)			A7-2	0.0096	brevicompactum	KT876695
University (S7)	-		A7-3	1.0556	Fusarium oxysporum	KT876684
University (S7)			A7-4	1.0077	Fusarium oxysporum	КТ876671
University (S8)	0.0431	0.0008	A8-1	1 1045	Fusarium oxysporum	KT876672
University (S8)			A8-2	0.9707	Fusarium oxysportum	KT876654
University (S8)	1		A8-3	0.0091	Trichoderma atroviride	KT876622
University (S0)	0 3806	0.0052	A9_1	0.9087	Fusarium solani	KT876639
University (S0)	0.5000	0.0052	Δ9_2	0.8849	Fusarium solani	KT876638
University (S9)	-		A9_3	0.004	Fusarium solani	KT876632
University (S)	1			0.2004	Fusarium solani	KT876636
University (39)			<i>∩.</i> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	0.0075	rusurum solum	IX 1070030

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)		I.	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)		1	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 babievae	KT876706
				0.0050	Meyerozyma	1110,0,00
University (S19)	0.0212	0.0048	B9-1	0.0051	guilliermondii	KT876707
University (S10)			D0 2	0.9104	Eugenium annen amm	1110/0/07
University (S19)			B9-2	0.8104	Fusarium oxysporum	KT876657
University (S19)			B9-3	0.6302	Fusarium solani	K18/6634
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	
			210 /	0.0201	guilliermondii	KT876711
University (S20)			B10-9	0.0031	Cystobasidium slooffiae	KT876712
University (S20)		ſ	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
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)		1	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)		ſ	R8-3	1.1856	Fusarium dimerum	KT876558
ITU (S41)	0.0391	0.0477	R9-3	0.0113	Exophiala	
	0.0540	0.0007	D10.0	0.0070	phaeomuriformis	K18/6555
ITU (S42)	0.0549	0.0097	R10-2	0.0072	Cadophora fastigiata	K18/6556
ITU (S42)	0.0700	1	K10-6	0.0124	Coniochaeta fasciculata	K18/6/21
ITU (S43)	0.0630	1.5751	X2-2	1.1492	Fusarium dimerum	K18/6571

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$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	ITU (S43)			X2-6	1.1716	Fusarium dimerum	KT876559
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Ophthalmology						
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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) 0.3325 1.4466 X5-2 1.0654 Fusarium dimerum KT876510 Ophthalmology Unit (S46) 0.2640 1.3729 X6-1 0.0616 Candida parapsilosis KT876513 Ophthalmology Unit (S47) 0.2640 1.3729 X6-2 0.063 Engyodontium album KT876522 Ophthalmology Unit (S47) X6-3 0.0603 Engyodontium album KT876504 Ophthalmology Unit (S47) 0.5299 0.0223 X8-2 0.0118 Cadosporium macrocarpum KT876504 Ophthalmology Unit (S48) 0.1872 0.0061 X9-1 0.0686 Engyodontium album MCR0523 KT876503 Ophthalmology Unit (S49) 0.1872 0.0061 <td>Onhthalmology</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>IX1070511</td>	Onhthalmology						IX1070511
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Ophthalmology Unit (\$48)X8-30.0985Gloeotinia temulentaKT876514Ophthalmology Unit (\$48)X8-40.0662Engyodontium albumKT876523Ophthalmology Unit (\$49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (\$49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (\$49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (\$49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (\$49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (\$49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (\$49)X9-70.0660Exophiala pisciphilaKT876503	Unit (S48)	0.3299	0.0225	A0-2	0.0118	macrocarpum	KT876506
Unit (S48)X8-30.0985Gloeotinia temulentaKT876514Ophthalmology Unit (S48)X8-40.0662Engyodontium albumKT876523Ophthalmology Unit (S49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (S49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876519	Ophthalmology			VO 2	0.0095		
Ophthalmology Unit (S48)X8-40.0662Engyodontium albumKT876523Ophthalmology Unit (S49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (S49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876502	Unit (S48)			X8-3	0.0985	Gloeotinia temulenta	KT876514
Unit (S48)X8-40.0662Engyodontium albumKT876523Ophthalmology Unit (S49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (S49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876503	Ophthalmology			VO 4	0.0((2		
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Unit (S49)0.18720.0061X9-10.0686Engyodontium albumKT876520Ophthalmology Unit (S49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876502	Ophthalmology	0.1072	0.00(1	WO 1	0.000		
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Unit (S49)X9-20.0611Candida parapsilosisKT876496Ophthalmology Unit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876502	Ophthalmology		1		0.0(11		
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Vinit (S49)X9-30.0641Candida parapsilosisKT876497Ophthalmology Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876502	Ophthalmology						111070190
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Unit (S49)X9-40.0677Exophiala dermatitidisKT876503Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphilaKT876502	Onhthalmology				ļ		
Ophthalmology Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphila	I Init (S10)			X9-4	0.0677	Exophiala dermatitidis	KT876503
Unit (S49)X9-50.0705Engyodontium albumKT876519Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphila	Onhthalmology						1110/0303
Ophthalmology Unit (S49) X9-7 0.0660 Exophiala pisciphila	$\frac{\text{Unit}(S10)}{\text{Unit}(S10)}$			X9-5	0.0705	Engyodontium album	KT876510
Ophthalmology Unit (S49)X9-70.0660Exophiala pisciphila	01111 (347)						IX10/0317
Unit (S49)	Ophthalmology			X0_7	0.0660	Fronhiala niscinhila	
	Unit (S49)			X)-/	0.0000	Елорници різстрінни	KT876502

Ophthalmology Unit (T1)	0.0132	0.0159	X10-1	0.0868	Cladosporium sphaerospermum	KT876507
Ophthalmology Unit (T1)		I	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)			К9-2	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 (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)			Н5-3	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)			Н7-3	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)			Н9-2	0.0049	Candida orthopsilosis	KT876576
Oncology Unit (S64)			Н9-3	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 (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)			Р9-2	0.0068	Trametes versicolor	KT876604
Oncology Unit (W64)			Р9-3	0.0140	Stereum gausapatum	KT876602
Oncology Unit (W65)	0.0051	0.0280	P10-1	0.0032	Phaeophlebiopsis peniophoroides	KT876610

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

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

4 c. *Fusarium* strains in bold further characterized by TEF-1 α PCR analysis (Table S3 and Supporting 5 Data Set 1).

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1 Figure legends

Figure 1. Specificity of ED7 determined by Enzyme-Linked Immunosorbent Assay tests of 2 3 surface washings containing water-soluble antigens from *Fusarium* species and related and 4 unrelated veasts and molds. (A) ELISA absorbance values at 450 nm for antigens from F. solani 5 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 6 7 are the means of three biological replicates \pm standard errors and the threshold absorbance value for 8 detection of antigen in ELISA is ≥ 0.100 (indicated by lines on graphs). Numbers in parentheses 9 after species names denote strain numbers with further details of strains provided in Table S1.

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11 Figure 2. Characterisation of the ED7 antigen and its epitope and spatial distribution of the 12 antigen in spores and hyphae. (A) Western immunoblot with ED7 using culture fluid from 2-d-old 13 PDB cultures of F. solani CBS224.34 (lane 1) and F. oxysporum f.sp. lycopersici CBS167.30 (lane 14 2). Wells were loaded with 1.6 μ g 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 15 16 with ED7 using surface washings containing water-soluble antigens immobilized to the wells of 17 microtitre plates and treated with trypsin or PBS only (control) at 4°C and 37°C. Bars are the means 18 of three biological replicates \pm standard errors and bars with the same letter are not significantly 19 different at p<0.001 (ANOVA and Tukey-Kramer test). (C) Absorbance values from ELISA tests 20 with ED7 using surface washings containing water-soluble antigens immobilized to the wells of 21 microtitre plates and treated with pronase or Milli-Q H₂O only (control) at 4°C and 37°C. Bars are 22 the means of three biological replicates \pm 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-23

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Fusarium-specific monoclonal antibody

1 soluble ED7 antigen following heating of surface washings at 100°C over a 70 min period. Treated 2 antigen was subsequently immobilized to the wells of microtitre plates and assayed by ELISA. Bars 3 are the means of three biological replicates \pm standard errors and bars with the same letter are not 4 significantly different at p<0.001 (ANOVA and Tukey-Kramer test). (E) Absorbance values from 5 ELISA tests with ED7 using surface washings containing water-soluble antigens immobilized to the 6 wells of microtitre plates and treated with periodate (open circles) or with acetate only control 7 (closed circles) at 4°C over a 20 h period. Each point is the mean of three biological replicates ± 8 standard errors. (F to I) Photomicrographs of F. solani CBS224.34 immunostained with ED7 or 9 TCM control and goat anti-mouse polyvalent Ig fluorescein isothiocyanate (FITC) conjugate. (F) 10 Brightfield image of germinated conidium with hypha probed with ED7 followed by fluorochrome conjugate (scale bar = $6 \mu m$); (G) Same field of view as panel F but examined under 11 12 epifluorescence. Note intense staining of the cell wall of microconidium and hypha; (H) Brightfield 13 image of germinated conidium with hypha probed with TCM (negative control) followed by FITC 14 conjugate (scale bar = $3 \mu m$); (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-15 16 L) Immunogold labeling of sections of conidia and hyphae of F. solani CBS224.34. (J) Transverse 17 section of conidium incubated with ED7 and anti-mouse immunoglobulin 20 nm gold particles, 18 showing antigen in the cell wall and in an extracellular fimbrial matrix surrounding the spore (scale 19 bar = 250 nm; (K) Longitudinal section of hypha incubated with ED7 and anti-mouse 20 immunoglobulin 20 nm gold particles, showing antigen in the cell wall and in an extracellular 21 matrix surrounding the cell (scale bar = 100 nm); (L) Transverse section of a conidium incubated 22 with TCM (negative control) and anti-mouse immunoglobulin 20 nm gold particles, showing lack 23 of staining by the secondary gold conjugate (scale bar = 180 nm).



Figure 1. Al-Maqtoofi & Thornton

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 μ g protein ml-1 buffer. Bars are the means of three biological replicates \pm 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.

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Figure 2. Al-Maqtoofi & Thornton

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. Mr 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 watersoluble antigens immobilized to the wells of microtitre plates and treated with trypsin or PBS only (control) at 4oC and 37oC. 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 watersoluble antigens immobilized to the wells of microtitre plates and treated with pronase or Milli-Q H2O only (control) at 4oC and 37oC. Bars are the means of three biological replicates ± standard errors) at 4oC and 37oC. 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 100oC 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 \pm 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 4oC 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 μ m); (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 antimouse immunoglobulin 20 nm g

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