# Evaluation of Monoclonal Antibody MC3 as a Diagnostic Antibody for Invasive Candidiasis

Submitted by Hassan Oliver James Morad to the University of Exeter as a thesis for the degree of Masters by Research in Biological Sciences, September 2015



I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: Hassan Oliver James Morad .....

## Abstract

*Candida* species are the most common cause of invasive fungal infections in immunocompromised humans. Invasive candidiasis (IC) has an associated mortality rate of around 50%, with the diploid, pleomorphic yeast, *Candida albicans* responsible for up to 60% of cases. Current immunodiagnostic tests for IC are based on the detection of circulating mannan antigen (Mn) and anti-mannan antibody (A-Mn) in a patient's serum, using an enzyme-linked immunosorbent assay (ELISA) format. The high rate of false negative results in immunocompromised patients unable to produce sufficient levels of antibody, and false positive results in patients with superficial, non-invasive colonisation has encouraged the development of new non-invasive immunodiagnostic tests that rapidly and more accurately detect IC. In this study, hybridoma technology was used to produce a murine monoclonal antibody (MAb), MC3, which binds to a carbohydrate epitope present on an extracellular mannan-containing antigen in *C. albicans* yeast and hyphal morphotypes. The MAb is highly specific, reacting with antigens from several *Candida* species known to cause IC, but not with antigens from a large number of clinically important fungi, including the invasive human pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus*. The MAb was used in combination with a standard mycological growth medium to develop an ELISA for differentiation of *C. albicans* from other pathogenic yeasts in single and mixed species cultures, substantially improving *in vitro* detection methods using mycological culture. In future, the MAb will be incorporated into the rapid, point-of-care lateral-flow device (LFD) diagnostic format, previously used successfully in the detection of invasive aspergillosis (IA). The use of MAb MC3 in these diagnostic tests would allow high risk patient groups to be routinely monitored for IC.

# Table of Contents

## Table of Figures

## Keywords

*Candida*, Invasive, Candidiasis, Diagnostic, Monoclonal

## Abbreviations

GI, gastrointestinal tract; OPC, oropharyngeal candidiasis; VVC, vulvovaginal candidiasis; IC, Invasive candidiasis; WT, wild-type; MAb, monoclonal antibody; Ig, immunoglobin; Mn, mannan antigen; A-Mn, anti-mannan antibody; LFD, lateral-flow device; ELISA, enzyme-linked immunosorbent assay; PTA, plate trapped antigen; IA, invasive aspergillosis; ICU, intensive care unit; SDA, sabouraud dextrose agar; GPYA, glucose-peptone-yeast extract agar; PDA, potato dextrose agar; PBS, phosphate buffered saline; BSA, Bovine Serum Albumin; TCM, tissue culture medium; FBS foetal bovine serum; TMB, tetramethyl benzidine; PLM, phospholipomannan

## Declaration

The contents of this document are entirely the work of the author, Hassan O.J. Morad, unless otherwise stated and referenced.

# Introduction

*Candida* species have naturally co-evolved with humans to colonise the skin (Gow et al., 2012) and mucosal surfaces, including the oral cavity (Martin et al., 2011), gastrointestinal tract (GI) (Cole et al., 1996) and genitourinary tract (Ang et al., 1993). Their interaction with the host and other members of the microflora represents a delicate relationship and any imbalance may facilitate fungal overgrowth and lead to superficial infections, such as oropharyngeal candidiasis (OPC) (de Repentigny et al., 2004) or vulvovaginal candidiasis (VVC) (Ringdahl, 2000), commonly known as thrush. While these infections represent a significant cause of discomfort to the patient, they are generally non-life threatening and relatively simple to treat (Pappas et al., 2009). The majority of cases occur in immunocompetent individuals, often with pre-disposed risk factors, including diabetes, antibiotic use and hormonal shifts caused by contraception or pregnancy (Calderone & Clancy, 2011). However, OPC is the most common opportunist infection in HIV and AIDS patients (Fidel, 2006).

Invasive candidiasis (IC) is a systemic infection and encompasses both candidemia (infection of the blood) and deep-seated candidiasis (infection of the tissue sites beneath mucosal surfaces) (Clancy and Nguyen, 2013). IC has only become a significant health concern in the last fifty years, as medical advancements have led to a greater number of solid-organ transplants, cancer therapies, invasive surgeries, prosthetics and the wider use of immunomodulating drugs and broad-spectrum antimicrobials (Ascioglu et al., 2002; Bodey et al., 2002; Kojic and Darouiche, 2004). During this half-century, the number of cases of IC has risen dramatically and, despite the use of effective antifungal drugs, mortality rates remain around 50% (Bassetti et al., 2014; Gudlaugsson et al., 2003). *Candida albicans* is the most common causative agent of IC worldwide, responsible for up to 60% of cases and thus of the upmost clinical significance (Diekema et al., 2012; Guinea, 2014); however, other species, such as *C. glabrata, C. parapsilosis, C. tropicalis*, *C. lusitaniae* and *C. krusei* are emerging as more frequent pathogens of immunocompromised individuals (Chi et al., 2011; Pfaller et al., 2014).

*Candida* species are found in a wide variety of environments, as well as on and in the human body, so exogenous and endogenous infections both pose a significant threat to high risk patient groups (Ang et al., 1993; Cole et al., 1996; Gow et al., 2012; Martin et al., 2011). Central nervous catheters and parenteral nutrition lines provide direct access to the blood

and offer skin-colonising and exogenous *Candida* species a route of entry across the normally impenetrable skin barrier (Kojic and Darouiche, 2004). Once in the bloodstream and allowed to disseminate, they are able to infect almost every organ in the body, leading to death if undiagnosed and untreated (Ylipalosaari et al., 2012). Endogenous *Candida* can cause IC when damages to the normal anatomical barrier of the GI mucosa, as a result of GI surgery or aggressive chemotherapy, allow the yeast to penetrate deeper tissues and/or the blood (Vincent et al., 2014). Chemotherapy may also induce neutropenia, while immunosuppression of solid-organ or haematological stem cell transplant patients reduces the ability of the immune system to recognise and clear *Candida* cells, increasing the chances and ease of infection (Ascioglu et al., 2002; Bodey et al., 2002; Safdar and Armstrong, 2011).

IC is the third most frequent nosocomial infection and the fourth most common bloodstream infection, behind staphylococci and enterococci, although it carries much higher mortality rates (Arendrup et al., 2005; Lewis, 2009; Marchetti et al., 2004; Richet et al., 2002; Wisplinghoff et al., 2004). In the US there are a reported 63,000 new cases of IC each year, with an estimated financial cost of $1.7 billion (Calderone & Clancy, 2011; Morgan et al., 2005). *Candida albicans* is the most common causative agent of IC, but efficient diagnosis remains a barrier to rapid treatment (Diekema et al., 2012; Guinea, 2014). The current "gold-standard" for diagnosis of IC relies on the routine testing of blood cultures, which, despite being highly specific, have well documented insensitivity (Jones, 1990; Pfaller and Diekema, 2007). Viable *Candida* cells are rapidly eliminated from the circulation and so the concentration in the sample may be too low for a positive identification (Pfeiffer et al., 2011; Schell et al., 2012). Culture sensitivity for candidemia alone ranges from 63% - 83%, but deep-seated infections, not associated with candidemia, are rarely detected (Ness et al., 1989). Taking all forms of IC into account, the overall sensitivity of cultures is estimated around 50% (Clancy and Nguyen, 2013). A further limitation is that, on average, cultures require between 2-3 days to become positive, whereas for improved patient outcome, treatment should begin within 24 h of a culture being drawn (Garey et al., 2006; Morrell et al., 2005).

The rapid detection of IC by immunodiagnostic methods has largely focussed on the detection of *Candida* mannan antigen (Mn) and anti-mannan antibodies (A-Mn) in patient sera (Bailey et al., 1985; Kedzierska et al., 2007; Sendid et al., 1999). Mannan is a major component of *Candida* cell walls, composing up to 7% of the dry weight and is one of the main antigens that

circulate during infection (Klis, 1994). Many different Mn and A-Mn tests have been developed, including latex agglutination (Bailey et al., 1985), but the combined measurement of Mn and A-Mn using the ELISA format has become an important aid in the monitoring of patients at risk of IC (Mikulska et al., 2010; Sendid et al., 2002; Yera et al., 2001). The tests are marketed as Platelia *Candida* Mn and A-Mn ELISAs and incorporate rat monoclonal antibody (MAb) EBCA-1 and goat anti-human polyclonal immunoglobin, respectively (Jacquinot et al., 1998; Sendid et al., 1999). The specificities and sensitivities of the individual assays are improved when in combination, but specificity still ranges from 21% - 95% (Ellis et al., 2009; Sendid et al., 1999) and the improved sensitivity, of >71%, decreases significantly for many *Candida* species known to cause IC (Mikulska et al., 2010; Verduyn Lunel et al., 2009). Moreover, false negative results have been reported as many immunocompromised patients are unable to produce sufficient levels of antibody, and false positive results in patients with superficial, non-invasive colonisation (Ellis et al., 2009; Sendid et al., 1999).

A "pan-fungal" test successfully detects fungal (1→3)-β-D-glucan, but lacks specificity to *Candida* and cross-reacts with a number of yeasts and moulds, including *Trichosporon* and *Aspergillus* species (Karageorgopoulos et al., 2011; Pickering et al., 2005). False-positives can also occur due to human blood products (albumin and coagulation factors) and several antibiotics (Alexander et al., 2010; Jaijakul et al., 2012; Obayashi et al., 2008). Nucleic-acid based methods, such as PCR, for the detection of IC, have been shown to have sensitivity and specificity of up to 95% and 92% (Avni et al., 2011), but their use in diagnostics is limited by a lack of methodological standardisation, the prohibitive costs of complex laboratory equipment and the need for trained professionals to conduct the procedure (Lucignano et al., 2011; McMullan et al., 2008).

Thus, there is a niche for the development of a non-invasive immunodiagnostic test that is rapid, inexpensive and accurately detects IC. This paper reports the development of a murine hybridoma cell line secreting a *Candida* mannan-specific MAb (MAb MC3) and evaluates its potential use as a diagnostic antibody for pathogen detection. The accuracy of the MAb in differentiating *C. albicans* from other pathogenic yeast species in single and mixed cultures is demonstrated using a highly specific ELISA in combination with a standard mycological culture method. In future, the MAb will be incorporated into a lateral-flow device (LFD), a rapid detection platform which has proved a successful tool for the diagnosis of the fungal infection

invasive aspergillosis (IA) (Held et al., 2013; Thornton et al., 2012; Thornton, 2008; White et al., 2013). The use of MAb MC3 in these diagnostic tests would allow the routine monitoring of vulnerable patients who have an elevated risk of infection, such as those with haematological malignancies, hematopoietic stem cell transplant recipients, recipients of solid-organ transplants and ICU patients.

# Materials and Methods

## Ethics Statement

All animal work described in this study was conducted under a UK Home Office Project Licence, 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.

## Fungal Cultures

Fungal cultures were grown on slopes of Sabouraud dextrose agar (SDA) (SD broth (Difco), agar 20 $gL^{-1}$); glucose-peptone-yeast extract agar (GPYA) (glucose 40 $gL^{-1}$, bacteriological peptone 5 $gL^{-1}$, yeast extract 5 $gL^{-1}$, agar 15 $gL^{-1}$) or potato dextrose agar (PDA) (PDB (Sigma), agar 20 $gL^{-1}$). All media were autoclaved at 121 $^{o}$C for 15 min prior to use and samples were grown at 26 $^{o}$C under a 16 h fluorescent light regime.

## Preparation of Immunogen and Immunisation Regime

Three-day-old GPYA Petri dish cultures of *Candida albicans* SC5314 were flooded with 20 mL of sterile Milli-Q water (MQ-$H_2O$) and the suspended cells snap frozen in liquid $N_2$, lyophilised and placed at -20 $^{o}$C for long term storage. Immunogen was prepared by re-suspending lyophilised cells in sterile filtered phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM $Na_2HPO_4$, and 1.5 mM $KH_2PO_4$ [pH 7.2]) and 2 mg/mL suspensions heat-inactivated by placing at 55 $^{o}$C for 45 min. The immunogen was stored at -20 $^{o}$C before animal immunisations. For immunisations, 6-week-old BALB/c white mice were each given four intraperitoneal injections (300 µL per injection) of immunogen at 2-week intervals and a single booster injection was given five days before fusion.

## Production and Screening of Hybridomas and Determination of Antibody Specificities

Hybridoma cells were produced as described previously (Thornton, 2001). The supernatants were screened by ELISA against *C. albicans* SC5314 antigens immobilised to Maxisorp microtitre plate wells (442404; Nunc) at 50 µl per well. Monoclonal antibody cell lines were tested for specificity against surface washings from replicate slopes of fungal samples (Table 1). Protein concentrations, determined spectrophotometrically at 280 nm (Nanodrop; Agilent Technologies Limited, Berkshire, United Kingdom), were adjusted to 60 µg/mL and 50 µL volumes were used to coat the wells of microtitre plates, which were incubated overnight at 4 $^{o}$C. Wells were washed three times with PBST (PBS containing 0.05% Tween-20), once with PBS and once with $dH_2O$ before being air-dried at 23 $^{o}$C in a laminar flow hood. The plates were sealed in plastic bags and stored at 4 $^{o}$C in preparation for screening of hybridoma supernatants by ELISA.

*Table 1 Details of fungal cultures*

| **Organism** | **Isolate Number** | **Source**[a] |
|---|---|---|
| *Candida albicans* | ATCC90028 | CBS |
| *Candida albicans* | NGY152 | CBS |
| *Candida albicans* | SC5314 | SB |
| *Candida dubiniensis* **var.** *dubliniensis* | 8500 | CBS |
| *Candida glabrata* | 4962 | CBS |
| *Candida guilliermondii* | B9-1 | CRT |
| *Candida intermedia* | C4-2' | CRT |
| *Candida krusei* | 5590 | CBS |
| *Candida lusitaniae* | C1-1 | CRT |
| *Candida orthopsilosis* | H5-3 | CRT |
| *Candida palmioleophila* | H3-4 | CRT |
| *Candida parapsilosis* | X9-2 | CRT |
| *Candida parapsilosis* **var.** *parapsilosis* | 8836 | CBS |
| *Candida pseudotropicalis* | NCPF3234 | CBS |
| *Candida sake* | NCPF3860 | CBS |
| *Candida* ***tropicalis*** **var.** *tropicalis* | 1920 | CBS |
| *Candida xylopsoci* | H1-3 | CRT |
| *Aspergillus ficuum* | 555.65 | CBS |

| | | |
|---|---|---|
| *Aspergillus flavus* | 91856iii | IMI |
| *Aspergillus fumigatus* | AF293 | SK |
| *Aspergillus niger* | 102.4 | CBS |
| *Aspergillus oryzae* | AO1 | CRT |
| *Aspergillus restrictus* | 116.5 | CBS |
| *Aspergillus nidulans* | A4 | FGSC |
| *Aspergillus terreus* **var.** *terreus* | 601.65 | CBS |
| *Cryptococcus neoformans* **(serotype D)** | 5728 | CBS |
| *Cryptococcus neoformans* **var.** *neoformans* | 7779 | CBS |
| *Exophiala castellanii* | G7-2 | CRT |
| *Exophiala dermatitidis* | P1-2 | CRT |
| *Exophiala heteromorpha* | X9-4 | CRT |
| *Exophiala lecanii-corni* | X9-7 | CRT |
| *Exophiala phaeomuriformis* | R9-3 | CRT |
| *Exophiala pisciphila* | A10-2 | CRT |
| *Filobasidella baccillispora* | 10865 | CBS |
| *Filobasidella neoformans* | 10490 | CBS |
| *Galactomyces candidum* | H7-2 | CRT |
| *Kluyveromyces marxianus* | 3073 | CBS |
| *Lichtheimia corymbifera* | TJAFJ713070 | CRT |
| *Magnusiomyces capitatus* | 207.83 | CBS |
| *Neosartorya fischeri* **var.** *fischeri* | 681.71 | CBS |
| *Paecilomyces variotii* | 10.1 | CRT |
| *Penicillium cyclopium* | 123.14 | CBS |
| *Penicillium islandicum* | 338.48 | CBS |
| *Pichia norvegensis* | 6564 | CBS |
| *Pseudallescheria boydii* | 100393 | CBS |
| *Rhizopus oryzae* | 112.09 | CBS |
| *Rhizopus stolonifer* **var.** *stolonifer* | 389.95 | CBS |
| *Rhodosporidium toruloides* | 6016 | CBS |
| *Rhodotorula glutinis* | H3-5 | CRT |
| *Rhodotorula mucilaginosa* | X5-3' | CRT |
| *Rhodotorula slooffiae* | A4-3 | CRT |

| | | |
|---|---|---|
| *Rhodotorula mucilaginosa* **var.** *mucilaginosa* | 326 | CBS |
| *Scedosporium apiospermum* | 117407 | CBS |
| *Scedosporium aurantiacum* | 121926 | CBS |
| *Scedosporium prolificans* | 467.74 | CBS |
| *Sporidiobolus salmonicolor* | 6781 | CBS |
| *Sporidiobolus salmonicolor* | 6832 | CBS |
| *Trichosporon asahii* | 2479 | CBS |
| *Trichosporon asahii* **var.** *asahii* | 5286 | CBS |
| *Trichosporon asahii* **var.** *asahii* | 8972 | CBS |
| *Trichosporon asahii* **var.** *asahii* | 8973 | CBS |
| *Trichosporon asteroides* | 7624 | CBS |
| *Trichosporon asteroides* | B10-12 | CRT |
| *Trichosporon dermatitis* | 2043 | CBS |
| *Trichosporon domesticum* | A3-1 | CRT |
| *Trichosporon inkin* | 7630 | CBS |
| *Trichosporon inkin* | 7655 | CBS |
| *Trichosporon loubieri* | 7065 | CBS |
| *Trichosporon mycotoxinivorans* | 9756 | CBS |
| *Trichosporon ovoides* | 7556 | CBS |
| *Wickerhamomyces anomalus* | 5759 | CBS |

[a] CBS; Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. SK; S. Krappman, Institute of Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Georg-August University, Gottingen, Germany. IMI; International Mycological Institute, Egham, England. FGSC; Fungal Genetics Stock Centre, University of Missouri, Kansas City. CRT; C.R. Thornton, University of Exeter, UK. SB; S. Bates, University of Exeter, UK.

## Enzyme-Linked Immunosorbent Assay

The wells containing immobilised antigens were incubated for 15 min with 200 µl of PBS containing 1.0% Bovine Serum Albumin (BSA) as a blocker. After a rinse with PBS, each well was incubated with 50 µL hybridoma supernatant for 1 h, after which all wells were washed three times, for 5 min each, with PBST. Goat anti-mouse polyvalent (immunoglobulin classes IgG, IgA and IgM) peroxidase conjugate (A-0412; Sigma Chemical Company, Poole, United Kingdom) was diluted 1 in 1000 in PBST containing 0.5% BSA and added to the wells for 1 h. Bound antibody was visualised by incubating wells with tetramethyl benzidine (TMB) (T-2885;

Sigma) substrate solution for 30 min, following three PBST and one PBS 5 min wash. The reactions were stopped by the addition of 3 M $H_2SO_4$. Absorbance values were determined at 450 nm using a microplate reader (Tecan GENios, Tecan Austria GmbH). Control wells were incubated with tissue culture medium (TCM) containing 10% foetal bovine serum. All incubation steps were performed at 23 $^{o}$C in sealed plastic bags. The threshold level for detection of the antigen was determined from controls (2×TCM Abs) (Thornton, 2009), which were consistently 0.050 - 0.100 and so absorbance values above 0.100 were considered as positive for the detection of antigen.

## Determination of Ig Subclass and Cloning Procedure

The Ig class of MAbs was determined by using Plate-Trapped-Antigen (PTA)-ELISA. Wells of antigen-coated microtitre plates were incubated with hybridoma supernatant for 1 h, followed by goat anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgM or IgA-specific antiserum (ISO-2; Sigma) diluted 1 in 3000 in PBST for 30 min and rabbit anti-goat peroxidase conjugate diluted 1 in 1000 (A-5420; Sigma) for a further 30 min. Bound antibody was visualised with TMB substrate as described above. Hybridoma cell lines were sub-cloned three times by limiting dilution, and cell lines were grown in bulk in a non-selective medium preserved by slowly freezing in foetal bovine serum/dimethyl sulfoxide (92:8), and stored in liquid nitrogen.

## Purified *Candida albicans* Cell Wall Mannans

*Candida albicans* cell wall mannans, purified by the method of Longbottom et al. (1976), were obtained from the National Institute for Biological Standards and Control (NIBSC; 76/515). The lyophilised material was resuspended in MQ-$H_2O$ to create a 1 mg/mL stock solution, which was stored at -20 $^{o}$C prior to use. A sample of the mannan stock was diluted 1:2 in MQ-$H_2O$ and then double diluted across the wells of microtitre plates at 50 µL per well. The ELISA protocol previously described was then followed. For Western blotting studies, mannan stock was diluted and denatured in Laemmli buffer by heating at 95 $^{o}$C for 10 min and tested as described below.

## Polyacrylamide Gel Electrophoresis and Western Blotting

SDS-PAGE was carried out using 4–20% gradient polyacrylamide gels under denaturing conditions. Cultures grown on SDA slopes were surface washed with 3 mL of sterile MQ-$H_2O$. Washings were centrifuged for 5 min at 14,500 rpm and the supernatants were denatured in

Laemmli buffer by heating at 95$^{o}$C for 10 min. Proteins were separated electrophoretically at 165 V and pre-stained marker ladders (Bio-Rad Laboratories Limited, Hemel Hempstead, UK) were used for molecular weight comparisons. For Western blotting, separated proteins were transferred electrophoretically on to a PVDF membrane for 2 h at 75 V and the membrane was blocked for 16 h at 4 $^{o}$C, with PBS containing 1% BSA. Blocked membranes were incubated with MAb supernatant diluted 1 in 2 with PBS containing 0.5% BSA (PBSA) for 2 h at 23 $^{o}$C. After washing three times with PBS, membranes were incubated for 1 h with goat anti-mouse alkaline phosphatase conjugate, diluted 1 in 15,000 in PBSA (IgG whole molecule, Sigma; A3562). Membranes were washed three times with PBS, once with PBST and bound antibody visualised by incubation in substrate solution. Reactions were stopped by immersing membranes in d$H_2O$, and membranes were then air dried between sheets of Whatman filter paper.

## *Candida albicans* Mannan Mutants

*Candida albicans* mannan mutants Δ*pmr1*, Δ*och1*, Δ*mnn4* and Δ*mnt1,2* were grown on SDA slopes and surface washed with 3 mL of sterile MQ-$H_2O$. Washings were centrifuged for 5 min at 14,500 rpm and the supernatant protein concentrations, determined spectrophotometrically at 280 nm (Nanodrop; Agilent Technologies Limited, Berkshire, United Kingdom), were adjusted to 60 µg/mL. Fifty µL volumes were used to coat the wells of microtitre plates and assayed by ELISA with MAb MC3 as described. For their use in Western blotting studies, supernatants were diluted and denatured in Laemmli buffer by heating at 95 $^{o}$C for 10 min and tested as described.

## Extracellular Antigen Detection

To investigate release and detection of extracellular antigens, wild-type and mutant strains of *C. albicans* were grown on SDA plates and surface washed to generate yeast cell suspensions. After washing with sterile MQ-$H_2O$, cell suspensions were adjusted to $10^3$ cells/mL, before 1 mL was added to 100 mL autoclaved GPY media (glucose 40 $gL^{-1}$, bacteriological peptone 5 $gL^{-1}$, yeast extract 5 $gL^{-1}$). The cultures were shaken at 125 rpm at 26 $^{o}$C and 1 mL samples taken every 24 h for 5 days, centrifuged at 14,500 rpm and supernatants frozen at -20 $^{o}$C. Microtitre plates were coated with 50 µL of defrosted supernatant and tested by ELISA with MAb MC3 as described. For Western blotting, defrosted

supernatants were diluted and denatured in Laemmli buffer by heating at 95 $^{o}$C for 10 min and the previously described procedure was followed.

## Antigen Characterisation by Heat Treatment, $\beta$ -elimination (NaOH treatment), Periodate Oxidation and Protease Digestion

Heat stability of the epitopes was investigated by heating solubilised antigen in a boiling water bath. At 10 min intervals over a 60 min period, 1 mL samples were removed and centrifuged at 14,500 rpm for 5 min. Fifty µL samples of supernatants were immobilised to the wells of microtitre plates for assay by ELISA as described. To determine whether the epitope was present on O- or N-linked glycans, microtitre wells containing immobilised antigens were incubated with either 50 µL of 50 mM NaOH or 50 µL $H_2O$ for 24h at room temperature in sealed plastic bags. Plates were given three 3 min PBS washes before assaying by ELISA as described. For periodate oxidation, microtitre wells containing immobilised antigens were incubated with 50 µL of sodium meta-periodate solution (20 mM $NaIO_4$ in 50 mM sodium acetate buffer [pH4.5]) or acetate buffer only (control) for 24, 4, 3, 2, 1 or 0 h at 4 $^{o}$C in sealed plastic bags. Plates were given four 3 min PBS washes before processing by ELISA as described. For protease digestions, microtitre wells containing immobilised antigens were incubated with 50 µL of pronase solution (protease XIV; 9 mg/mL in PBS) or trypsin solution (1 mg/mL in MQ-$H_2O$) or PBS and MQ-$H_2O$ only (controls) for 4 h at 37 $^{o}$C or 4 $^{o}$C. Plates were given four 3 min rinses with PBS and then assayed by ELISA as described.

## Immunofluorescence

Microscope slides were sterilised and coated with washed *Candida albicans* yeast cells suspended in 1% D-glucose and 3% FBS and incubated at 26 $^{o}$C for 16 h. After air-drying, the slides were fixed (Thornton, 2001) and incubated with hybridoma supernatant for 1 h, followed by three 5 min PBS washes. Slides were then incubated with goat anti-mouse polyvalent fluorescein isothiocyanate conjugate (diluted 1 in 40 in PBS) (Sigma; F1010) for 30 min. Slides were given three 5 min washes with PBS and mounted in PBS-glycerol mounting medium before overlaying with coverslips. All incubation steps were performed in a humid environment to prevent evaporation and slides were stored in the dark, at 4 $^{o}$C, prior to examination using an epifluorescence microscope (Olympus IX81).

### Differentiation of *Candida albicans* from *Candida glabrata* and Other Yeasts Grown in Mixed Species Cultures

Petri dish culture plates containing SDA were inoculated with cell suspensions of *C. albicans*, *C. glabrata*, *T. asahii* or *C. neoformans*, either as single species cultures or as mixed species cultures. After 24 h incubation at 26 $^{o}$C, antigen solutions were prepared by flooding the plates with 10 mL PBS, suspending cells using sterile L-shaped spreaders, and pelleting of cells by centrifugation at 14,500 rpm for 5 min. Protein concentrations of solutions were adjusted to 60 µg/mL and used to coat the wells of microtitre plates for assay by ELISA.

### Data Analysis

Differences in means were analysed by one-way analysis of variance (ANOVA) and Tukey-Kramer tests were used to determine statistical significance.

## Results

### Production of Hybridoma Cell Lines and Isotyping of MAbs

A single fusion was performed. The cell line MC3 was selected for further testing based on the strength of its reaction in ELISA and was sub-cloned three times. Isotyping showed that MAb MC3 belonged to immunoglobulin class G3 (IgG3).

### MAb Specificity Testing

MAb MC3 was tested by ELISA for specificity, using a wide range of clinically relevant yeasts and moulds (Table 1). It reacted with antigens from several *Candida* species, but did not cross-react with a wide range of other clinically relevant fungi, including the invasive human pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus* (Figure 1) (Dagenais and Keller, 2009; Eisenman et al., 2008). The only cross-reactivity was noted with the yeast *Rhodosporidium toruloides*, which has not been reported as a cause of invasive fungal infection in humans (Figure 1).

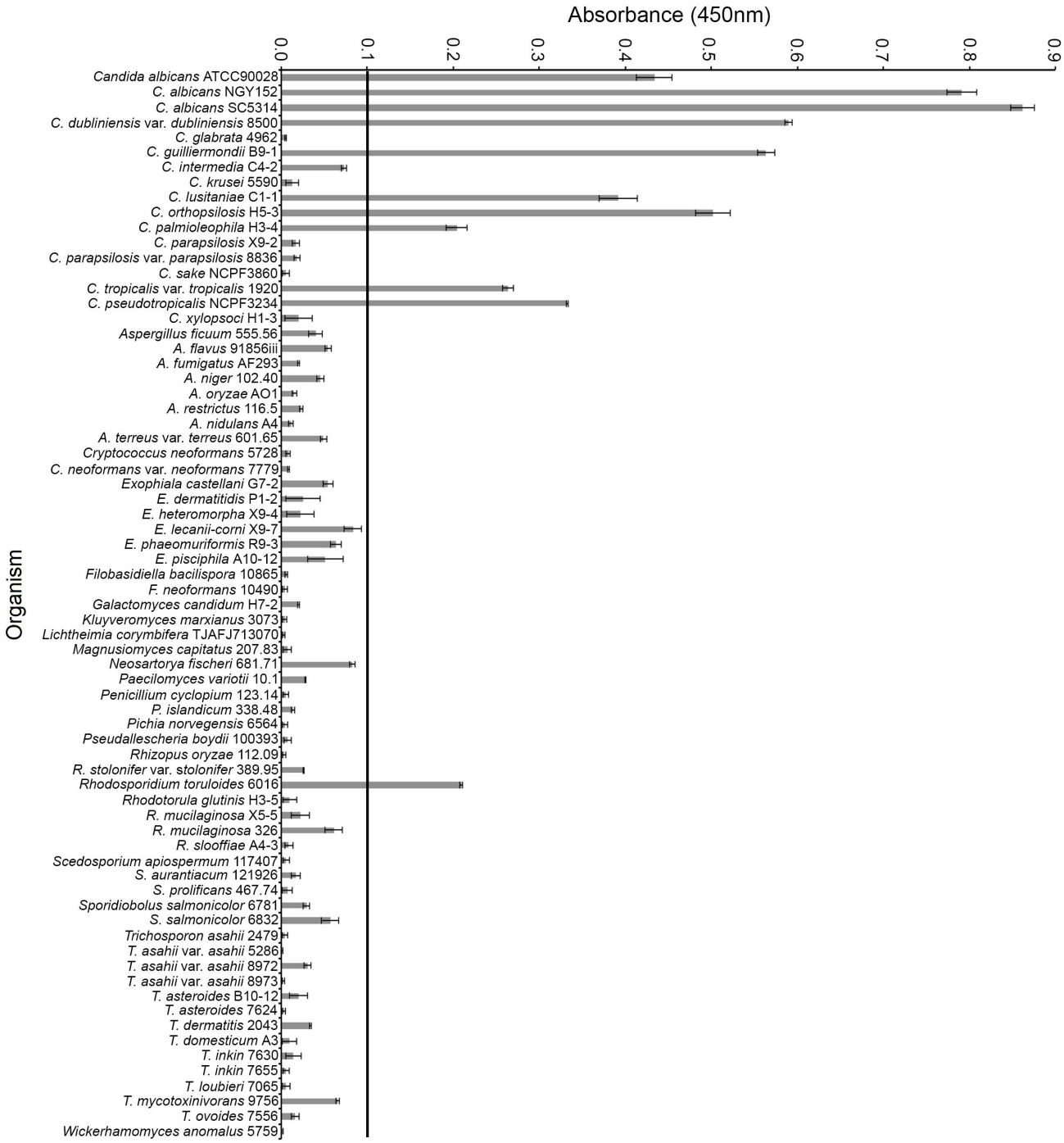


*Figure 1 ELISA absorbance values from specificity screenings using MAb MC3*. Bars represent the means of three biological replicates ± standard error. The threshold absorbance value for the detection of antigen was >0.1 (see methods), indicated by the line. Full details of the fungal cultures tested are shown in Table 1.

## Recognition of Purified *Candida albicans* Cell Wall Mannans

Mannan, the major carbohydrate of the *Candida* cell wall, consisting of α-1-6, α-1-2. α-1-3 and β-1-2 structures (Hall and Gow, 2013; Netea et al., 2008), was investigated as the putative antigen bound by MAb MC3 (Figures 2A & 2B). MAb MC3 was shown to recognise purified *C. albicans* cell wall mannans at concentrations as low as ≥1.3 µg/mL in ELISA (absorbance threshold for positivity >0.1) (Figure 2A). MAb MC3 also recognised purified mannan in Western blotting studies, where it reacted with mannan structures >200 kDa (Figure 2B). This pattern is consistent with MAbs shown to bind to β-1,2-mannan structures (Jouault et al., 1998; Poulain et al., 2002).

## Western Blotting of the *Candida albicans* Wild-Type

Western blot binding patterns with wild-type *C. albicans* SC5314 showed strong reaction of MAb MC3 with high molecular weight glycosylated antigens, between ~100 kDa to >200 kDa and less strong reaction with those of lower molecular weight, between ~50 kDa and ~100 kDa (Figure 2B). This staining pattern is consistent with MAbs shown to bind to *C. albicans* mannan-containing antigens (Torosantucci et al., 1991). MAb MC3 also reacted with an antigen with a molecular weight of ~10 kDa, which is consistent with MAb recognition of the cell wall glycolipid antigen phospholipomannan (PLM) (Figure 2B) (Jouault et al., 1998; Mille et al., 2012; Poulain et al., 2002; Trinel et al., 1993). PLM contains exclusively β-1,2-mannans, which are also found to decorate mannoprotein structures in the *C. albicans* cell wall (Fradin et al., 2008; Mille et al., 2004; Trinel et al., 1999). Thus, together, these data suggest β-1,2-mannan structures form the epitope for MAb MC3.

## *Candida albicans* Mannan Mutants

Extensive literature on the properties of *C. albicans* mannan mutants has been published elsewhere (Bates et al., 2005; Hall and Gow, 2013; Munro et al., 2005; Shahana et al., 2013). In summary, the mutant Δ*och1* has no branched outer chain N-mannan, mutant Δ*mnn4* lacks phosphomannan on the N-branched chain and mutant Δ*mnt1,2* has truncated O-mannan. Δ*pmr1* differs in that disruption of the *PMR1* gene reduces the activity of all the mannosyltransferases mentioned above, along with many others, resulting in truncation of all branched N-mannan, O-mannan and PLM structures (Bates et al., 2005; Hall and Gow, 2013; Munro et al., 2005). MAb MC3 reacted with all four mutant strains of *C. albicans* in ELISA tests and no significant differences in absorbance values were noted despite the

differing mannan structures (Figure 2C). Western blots conducted with the same samples similarly showed strong reaction of MAb MC3 with high molecular weight mannan antigens and weaker reaction with those of lower molecular weight, down to ~50 kDa (Figure 2D). This staining pattern was similar for all the strains tested (Figure 2D). MC3 also reacted with the ~10 kDa antigen, assumed to be PLM, in all strains except Δ*pmr1* (Figure 2D). Δ*pmr1* was the only strain in which the low molecular weight band was absent (Figure 2D). However, recognition of PLM does not appear to affect the overall strength of binding of MAb MC3 (Figure 2C), and thus the dominant epitope is assumed to be β-1,2-mannan residues on *C. albicans* mannan-containing antigens.

## Extracellular Antigen Detection

Samples of extracellular fluids from wild-type and mutant *C. albicans* strains were tested, after 5 days of growth, by ELISA (Figure 2E). MAb MC3 detected antigen in all strains, revealing that the antigens recognised by the MAb are released extracellularly (Figure 2E). MC3 reacted significantly less with the Δ*pmr1* mutant than with the other strains (Figure 2E). A Western blot conducted with the same samples showed that MAb MC3 reacted strongly with extracellular mannan antigens (Figure 2F). The staining pattern with Δ*pmr1* signified severely truncated mannosylation of the extracellular antigen (Figure 2F). With the exception of mutant Δ*mnt1,2*, the ~10 kDa antigen was absent in extracellular fluids (Figure 2F). This supports the hypothesis that the dominant epitope for MAb MC3 is located in the mannan residues on *C. albicans* antigens.

## Heat, Chemical and Enzymatic Characterisation of Antigens

*Candida albicans* SC5314 antigens were subjected to different heat (Figure 2G), chemical (Figure 2H) and enzymatic (Table 2) modifications in order to further characterise the epitope bound by MAb MC3. Reductions in MAb binding in ELISA following heat treatment would show that an epitope is heat labile. There was no significant reduction in MAb MC3 binding over 60 mins of heating, revealing that its epitope is heat-stable (Figure 2G). Reductions in MAb binding following treatment with pronase would show that the epitope consists of protein, while reductions with trypsin would indicate a protein epitope that contains positively charged lysine and arginine side chains. Consequently, the lack of reduction in MAb MC3 binding following digestion of immobilized antigen with pronase or trypsin confirmed that the antibody does not bind to a protein epitope (Table 2). Reductions in MAb binding

following chemical digestion of an antigen with periodate would show that the epitope is carbohydrate with vicinal hydroxyl groups. Significant reduction in MC3 binding was observed after 1 h of periodate treatment, depicting that its epitope consists of carbohydrate residues (Figure 2H). Reductions in MAb binding following β-elimination (treatment with NaOH) would show that the epitope is present on O-linked glycan. Consequently, the lack of reduction with MAb MC3 (data not shown), suggest the epitope is present on N-linked glycan

*Table 2 Absorbance values from ELISA tests of protease-treated antigen with MAb MC3.*

| | **Absorbance (450 nm[a])** | | | |
|---|---|---|---|---|
| **Temp ($^{o}$C)** | **Trypsin** | **$H_2O$** | **Pronase** | **PBS** |
| **4** | 1.366 ± 0.018 | 1.457 ± 0.010 | 1.313 ± 0.014 | 1.438 ± 0.013 |
| **37** | 1.347 ± 0.009 | 1.474 ± 0.014 | 1.313 ± 0.011 | 1.402 ± 0.028 |

[a] Each value represents the mean of three biological replicates ± standard error.

## Immunofluorescence

Immunofluorescence studies showed the MAb MC3 antigen is present on the cell walls of *Candida albicans* yeast cells, pseudohyphae and hyphae (Figure 2I-N). This is consistent with the observation that the epitope likely consists of β-1,2-linked mannan residues on PLM and N-linked mannoprotein antigens located in the cell wall of *C. albicans*.

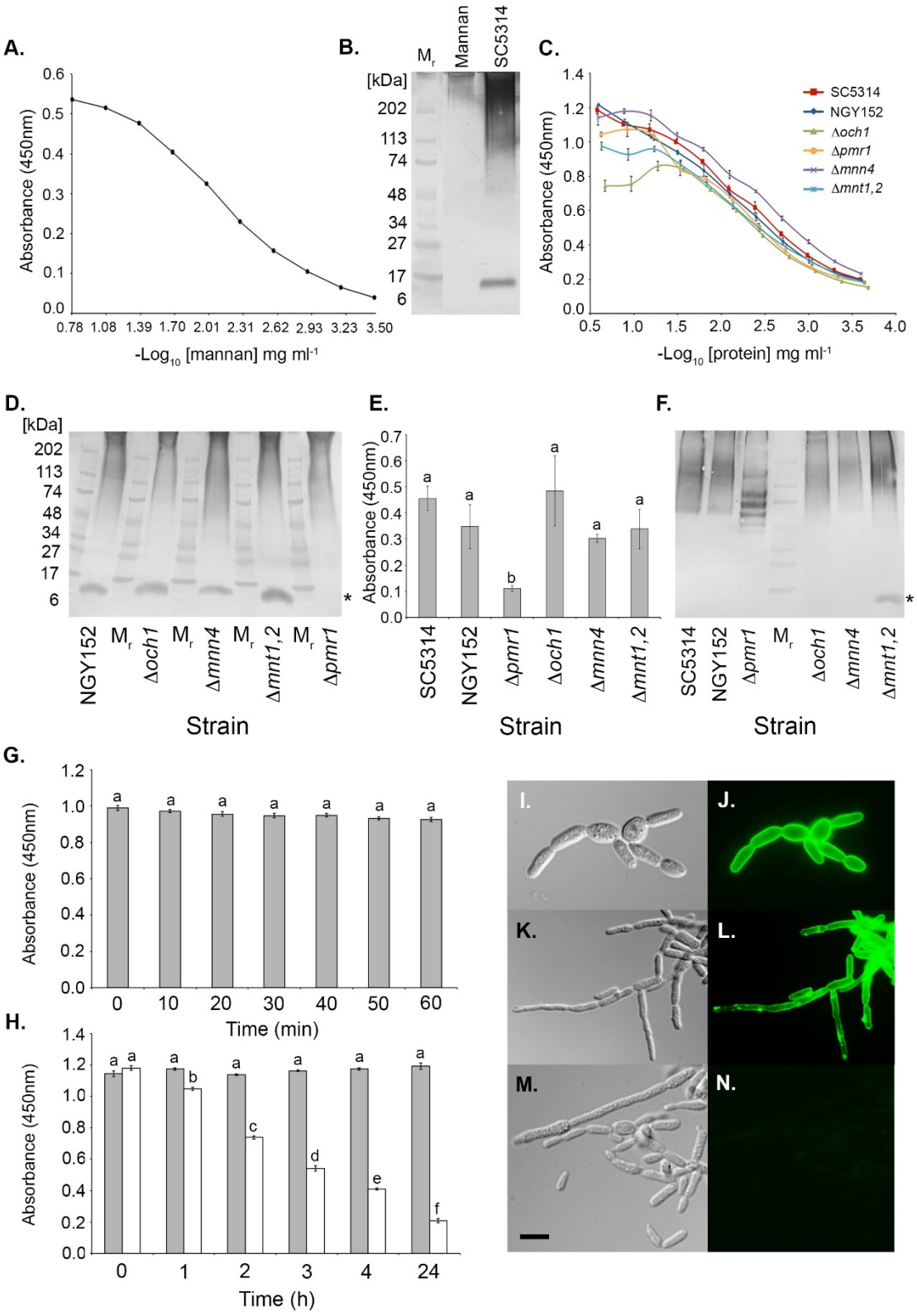


*Figure 2 Characterisation of antigens bound by MAb MC3.* *(A) ELISA absorbance values for MAb MC3 recognition of purified C. albicans cell wall mannans. (B) Western immunoblot with MAb MC3 of purified C. albicans cell wall mannan (lane 2) and C. albicans SC5314 antigens (lane 3). $M_r$ (lane 1) is broad range molecular weight markers. (C) ELISA absorbance values for MAb MC3 recognition of the C. albicans wild-type and mutant strains. (D) Western immunoblot comparing MAb MC3 recognition of antigens from the C. albicans wild-type strain NGY152 and the mutant stains. * indicates*

*the low molecular weight antigen, assumed to be PLM, which is not detected in the Δpmr1 mutant. (E) ELISA absorbance values for MAb MC3 recognition of extracellular antigens from the C. albicans wild-type and mutant strains. (F) Western immunoblot comparing MAb MC3 recognition of extracellular antigens from the wild-type and mutant C. albicans strains. * indicates that the same low molecular weight antigen in (D) is only produced extracellularly in mutant Δmnt1,2. (G) ELISA absorbance values for heat treated C. albicans antigen. (H) ELISA absorbance values for periodate treated C. albicans antigen (white bars) and controls (grey bars). (I-N) Photomicrographs of C. albicans SC5314 cells immunostained with MAb MC3 (I-L) or TCM (M,N) and anti-mouse polyvalent Ig fluorescein isothiocyanate. (I,K) Bright field images of budding yeast cells and of hyphal growth, respectively. (J,L) Same field of view as (I,K), but examined under epifluorescence. (M) Bright field view of yeast and hyphae, (N) Same field of view but examined under epifluorescence. Scale bar = 4 µm. (A,C,E,G,H) Each point/bar is the mean of three biological repeats ± standard errors. (E,G,H) Bars with the same letter are not significantly different at p<0.001 (ANOVA and Tukey-Kramer test).*

## Differentiation of *Candida albicans* from *Candida glabrata* and Other Yeasts Grown in Mixed Species Cultures

Yeasts were cultured on SDA plates for 24 h (Figure 3A) and antigen solutions tested by ELISA using MAb MC3 (Figure 3B). MC3 was highly accurate at detecting *C. albicans* both when grown in single species culture and when grown on plates containing mixed populations of *C. glabrata*, *T. asahii* or *C. neoformans* (Figure 3B). MC3 did not cross-react with any yeast, other than *C. albicans*, when grown either as single species or mixed species populations (Figure 3B).

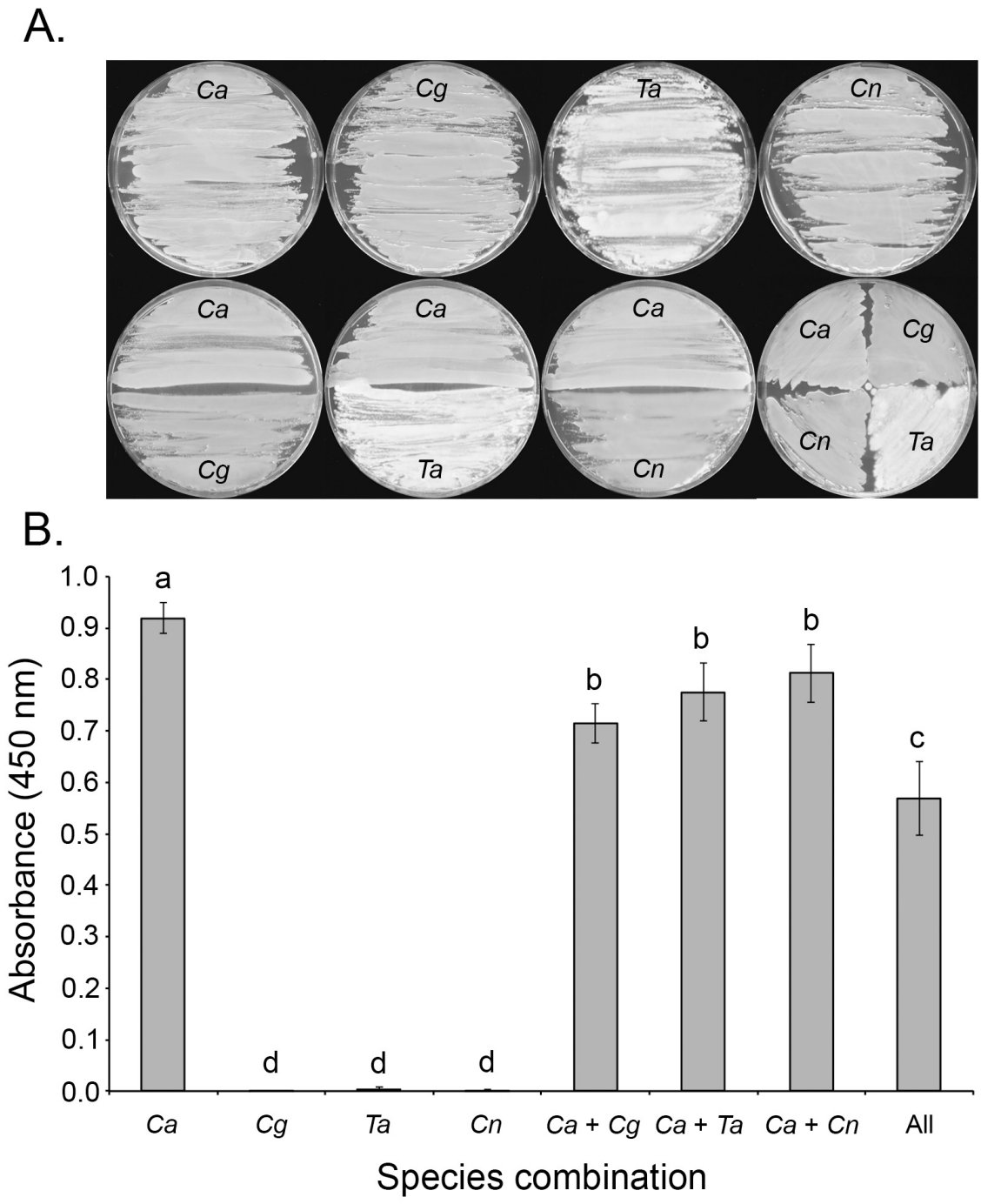


*Figure 3 Growth of yeasts for 24 h as single or mixed species cultures and ELISA tests of soluble antigens using MAb MC3.* *(A) SDA plates inoculated with different combinations of species; C. albicans SC5314 (Ca), C. glabrata CBS4962 (Cg), T. ashaii var asahii CBS5286 (Ta) and C. neoformans var neoformans CBS7779 (Cn). (B) ELISA absorbance values for antigen solutions tested with MAb MC3. Each bar is the mean of three biological replicates ± standard errors. Bars with the same letter are not significantly different at p<0.001 (ANOVA and Tukey-Kramer test).*

## Discussion

This paper describes the development of a murine IgG3 monoclonal antibody, MAb MC3, raised against a heat-stable carbohydrate epitope on an extracellular mannan antigen located in the cell wall of *Candida albicans* yeast and hyphae. Specificity tests showed that MAb MC3 reacted with antigens from several *Candida* species known to cause IC (Cantón et al., 2011; Jensen and Arendrup, 2011; Morgan et al., 1984; Pfaller et al., 2014), but not with a wide range of related and unrelated clinically-relevant yeasts and moulds, including the invasive human pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus* (Dagenais and Keller, 2009; Eisenman et al., 2008).

The *Candida* species recognised by MAb MC3 all reside within a phylogenetic subgroup known as the CTG clade (Butler et al., 2009; Santos et al., 2011). Members of this clade, which include most medically relevant *Candida* species, are very closely related and have a high conservation of protein-coding genes, with all members translating the CTG codon as serine instead of leucine (Santos and Tuite, 1995; Santos et al., 2011). The high similarity between members of the clade has led to several previously unknown species being unmasked. *Candida palmioleophila*, for example, was discovered within the phenotypically indistinguishable *Candida guilliermondii* grouping (Jensen and Arendrup, 2011), while *Candida orthopsilosis* and *Candida metapsilosis* have replaced *Candida parapsilosis* groups II and III, respectively (Tavanti et al., 2005).

Antigen characterisation studies showed MAb MC3 to be specific to cell wall mannans of *Candida albicans* yeast, pseudohyphal and hyphal morphotypes. This is important from a diagnostic perspective since *C. albicans* is a pleomorphic yeast, which regularly switches morphologies during infection (Calderone and Fonzi, 2001; Staniszewska et al., 2012). The apparent recognition of PLM (demonstrated by absence of the ~10 kDa immunoreactive antigen in the PLM mutant Δ*pmr1*) suggests that the MC3 epitope consists of β-1,2-mannan residues (Fradin et al., 2008; Mille et al., 2004; Trinel et al., 1999). This is consistent with immunodominant (1→2)-β-mannan structures in *C. albicans* PLM bound by the protective β-mannan-specific IgG3 MAb, C3.1 (Han et al., 2000; Johnson et al., 2012). The staining pattern in Western blots suggested this β-1,2-linked epitope is present in cell wall mannans of *C. albicans* (Fradin et al., 2008; Mille et al., 2004; Torosantucci et al., 1991; Trinel et al., 1999). The MC3 mannan antigen was also shown to be released extracellularly, which is similarly

advantageous since detection of extracellular markers of IC circulating in the bloodstream have been shown to indicate disseminated infection up to 7 days prior to culture positivity (Mikulska et al., 2010). Moreover, deep-seated infections not associated with candidemia are rarely detected by cultures, but detection of released, circulating antigen in the serum might indicate cryptic infections (Clancy and Nguyen, 2013).

All of the *Candida* species recognised by MAb MC3 are reported to cause IC (Cantón et al., 2011; Jensen and Arendrup, 2011; Morgan et al., 1984; Pfaller et al., 2014). *Candida albicans* is the most prevalent cause worldwide and thus of the highest clinical significance (Diekema et al., 2012; Guinea, 2014), but several of the other MC3-reactive species are emerging as significant pathogens (Chi et al., 2011; Pfaller et al., 2014). *Candida tropicalis*, for example, has an unusually high incidence among patients with haematological malignancies (Leung et al., 2002), *Candida lusitaniae* infection has become more prevalent with the use of aggressive chemotherapies (Krcmery and Barnes, 2002; Wingard, 1995), and *Candida orthopsilosis* is commonly associated with nosocomial infections (Barbedo et al., 2014). *Candida guilliermondii* and *Candida dubliniensis*, while less widespread, are becoming more common as causes of IC (Brandt et al.; Krcmery and Barnes, 2002; Pfaller et al., 2006). MAb MC3 also displayed cross-reactivity with *Rhodosporidium toruloides*, but this yeast has not been reported as a cause of invasive fungal infection in humans.

MAb MC3 is not able to detect *Candida glabrata*, *C. krusei* and *C. parapsilosis*, which are emerging as more frequent agents of IC (Chi et al., 2011; Pfaller et al., 2014). Unlike *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* are found outside the CTG clade (Butler et al., 2009; Santos and Tuite, 1995), with *C. glabrata* more closely related to *Saccharomyces cerevisiae* than *Candida albicans* (Butler et al., 2009). The ability of MAb MC3 to discriminate against these three species offers a diagnostic advantage, due to their antifungal resistance profiles. The azole drug fluconazole is the most widely used *Candida*-active antifungal in clinical practice (Pappas et al., 2009), and *C. parapsilosis* (Sarvikivi et al., 2005), *C. glabrata* (Panackal et al., 2006; Sanguinetti et al., 2005),and *C. krusei* (Espinel-Ingroff et al., 2014) all display differing levels of fluconazole resistance. Over recent years, the echinocandin drug caspofungin has become widely adopted (Pappas et al., 2009), and *C. parapsilosis* (Forrest et al., 2008; Moudgal et al., 2005), *C. glabrata* (Alexander et al., 2013; Singh-Babak et al., 2012) and *C. krusei* (Hakki et al., 2006; Kahn et al., 2007) have also all exhibited resistance to this

drug. In contrast, the species recognised by MAb MC3 do not commonly show resistance to the drugs most widely used in clinical practice (Pappas et al., 2009; Pfaller et al., 2010). Detection of infection by MC3-reactive species, for example through use in an ELISA or LFD, would therefore allow effective antifungal treatment to be delivered immediately, with confidence of drug efficacy. Test negativity would indicate infection by alternative species, requiring blood culture and drug resistance profiling (Jones, 1990; Pfaller and Diekema, 2007).

Non-invasive, non-culture based serodiagnostic tests are already used in clinical practice. Combined determination of Mn and A-Mn using the Platelia *Candida* ELISA formats, for example, has become a useful aid in monitoring patients at high risk for IC (Mikulska et al., 2010). The previously discussed limitations of the assay, however, hamper its effectiveness and few prospective evaluating studies have been conducted thus far (Ellis et al., 2009; Mikulska et al., 2010). Moreover, the genus specific test does not give an indication of which *Candida* species may be causing the IC, which could present difficulties in choosing an appropriate treatment strategy due to the potential for antifungal resistance (Pappas et al., 2009). Furthermore, another limitation lies in the fact that two separate ELISAs must be conducted in order to obtain meaningful results, which is time consuming when the speed of diagnosis is crucial to patient outcome (Garey et al., 2006; Morrell et al., 2005). The issue of time, along with the need for expensive equipment, also applies to promising nucleic-acid based detection methods (Avni et al., 2011; Lucignano et al., 2011; McMullan et al., 2008).

The "pan-fungal" (1→3)-β-D-glucan test is recommended as an indicator of invasive fungal infection (Lamoth et al., 2012). However, the specificity and sensitivity of the assay vary, from 38% - 100% and 45% - 99%, respectively, hindering its usefulness (Theel and Doern, 2013). Moreover, the test lacks specificity to *Candida*, cross-reacting with a number of yeasts and moulds, including *Trichosporon* and *Aspergillus* species, so its use in detecting IC is limited (Karageorgopoulos et al., 2011; Pickering et al., 2005). Nonetheless, the test does serve as broad marker of invasive fungal infection, which may be caused by a single species, or a combination of different species (Yang et al., 2014).

Combinations of different fungal species causing mixed yeast infections in high risk patient groups is increasing, and accurate identification of individual pathogens in polymicrobial infections is of paramount importance for appropriate treatment (Badiye et al., 2012; Yang et

al., 2014). Diagnosis in these cases regularly relies on selective media, such as CHROMagar Candida, which identifies yeasts by producing different coloured colonies (Otero Silva et al., 2004; Pfaller et al., 1996). However, CHROMagar medium testing is often not definitive and must be combined with other culture media, as well as requiring expert mycological analysis to identify the specific species present (Otero Silva et al., 2004). In order to address these issues, antigen solutions from mixed cultures of *Candida albicans*, *Candida glabrata*, *Trichosporon asahii* and *Cryptococcus neoformans*, grown on SDA plates, were tested in ELISA using MAb MC3. The antibody allowed unambiguous identification of *Candida albicans* when grown individually or in combination with the other pathogenic yeasts, thus offering a substantial improvement to *in vitro* detection methods using mycological culture.

## Conclusion

Hybridoma technology was used to generate a murine IgG3 monoclonal antibody, MAb MC3. The MAb reacted with antigens from several *Candida* species known to cause IC, but was able to discriminate against those which exhibit multi-drug resistance and did not react with a wide range of related and unrelated clinically-relevant yeasts and moulds. The MAb has been shown to improve the diagnostic accuracy of mycological cultures employing a standardised growth medium, through use in a highly specific ELISA. The MAb was specific to a mannan antigen released extracellularly by *C. albicans*, which might translate to the detection of released circulating antigens in the bloodstream of IC patients (Clancy and Nguyen, 2013). However, patient sera must be tested to confirm that MC3 is able to detect these *Candida* antigens without the need for prior *in vitro* culture. Confirmation would allow rapid immunoassay testing, yielding results in hours with an ELISA, or just minutes with the MAb incorporated into an LFD platform (Thornton, 2008). The use of MAb MC3 in these diagnostic tests would allow high risk patient groups, such as those with haematological malignancies, chemotherapy induced neutropenia, solid-organ transplant recipients, stem cell transplant patients and ICU patients to be routinely monitored for IC.

## Future Work

MAb MC3 is currently being incorporated into an LFD diagnostic format. The specificity and sensitivity of the LFD should be configured by testing human serum spiked with a range of yeasts and moulds, compared to control serum. Following this, the sera of patients with IC,

suspected IC and healthy controls should be tested using the LFD and the results compared to those using the Mn/A-Mn and (1→3)-β-D-glucan tests. An LFD format would provide a rapid point-of-care diagnostic platform that is inexpensive, non-invasive and requires minimal training for use.

## Acknowledgements

The author would like to thank Christopher. R. Thornton for guidance in compiling this report and Christopher. R. Thornton and Genna E. Davies for laboratory support.

## References

Alexander, B.D., Johnson, M.D., Pfeiffer, C.D., Jiménez-Ortigosa, C., Catania, J., Booker, R., Castanheira, M., Messer, S.A., Perlin, D.S., Pfaller, M.A., 2013. Increasing echinocandin resistance in Candida glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin. Infect. Dis. 56, 1724–32.

Alexander, B.D., Smith, P.B., Davis, R.D., Perfect, J.R., Reller, L.B., 2010. The (1,3){beta}-D-glucan test as an aid to early diagnosis of invasive fungal infections following lung transplantation. J. Clin. Microbiol. 48, 4083–8.

Ang, B.S., Telenti, A., King, B., Steckelberg, J.M., Wilson, W.R., 1993. Candidemia from a urinary tract source: microbiological aspects and clinical significance. Clin. Infect. Dis. 17, 662–6.

Arendrup, M.C., Fuursted, K., Gahrn-Hansen, B., Jensen, I.M., Knudsen, J.D., Lundgren, B., Schønheyder, H.C., Tvede, M., 2005. Seminational surveillance of fungemia in Denmark: notably high rates of fungemia and numbers of isolates with reduced azole susceptibility. J. Clin. Microbiol. 43, 4434–40.

Ascioglu, S., Rex, J.H., de Pauw, B., Bennett, J.E., Bille, J., Crokaert, F., Denning, D.W., Donnelly, J.P., Edwards, J.E., Erjavec, Z., Fiere, D., Lortholary, O., Maertens, J., Meis, J.F., Patterson, T.F., Ritter, J., Selleslag, D., Shah, P.M., Stevens, D.A., Walsh, T.J., 2002. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin. Infect. Dis. 34, 7–14.

Avni, T., Leibovici, L., Paul, M., 2011. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J. Clin. Microbiol. 49, 665–70.

Badiye, A., Patnaik, M., Deshpande, A., Rajendran, C., Chandrashekara, K. V, 2012. Think fungus NOT just a crypto-meningitis in AIDS! J. Assoc. Physicians India 60, 21–4.

Bailey, J.W., Sada, E., Brass, C., Bennett, J.E., 1985. Diagnosis of systemic candidiasis by latex agglutination for serum antigen. J. Clin. Microbiol. 21, 749–752.

Barbedo, L.S., Vaz, C., Pais, C., Figueiredo-Carvalho, M.H.G., Muniz, M. d. M., Zancope-Oliveira, R.M., Sampaio, P., 2014. Different scenarios for Candida parapsilosis fungaemia reveal high numbers of mixed C. parapsilosis and Candida orthopsilosis infections. J. Med. Microbiol. 64, 7–17.

Bassetti, M., Righi, E., Ansaldi, F., Merelli, M., Trucchi, C., Cecilia, T., De Pascale, G., Diaz-Martin, A., Luzzati, R., Rosin, C., Lagunes, L., Trecarichi, E.M., Sanguinetti, M., Posteraro, B., Garnacho-Montero, J., Sartor, A., Rello, J., Rocca, G. Della, Antonelli, M., Tumbarello, M., 2014. A multicenter study of septic shock due to candidemia: outcomes and predictors of mortality. Intensive Care Med. 40, 839–45.

Bates, S., MacCallum, D.M., Bertram, G., Munro, C.A., Hughes, H.B., Buurman, E.T., Brown, A.J.P., Odds, F.C., Gow, N.A.R., 2005. Candida albicans Pmr1p, a secretory pathway P-type Ca2+/Mn2+-ATPase, is required for glycosylation and virulence. J. Biol. Chem. 280, 23408–15.

Bodey, G.P., Mardani, M., Hanna, H.A., Boktour, M., Abbas, J., Girgawy, E., Hachem, R.Y., Kontoyiannis, D.P., Raad, I.I., 2002. The epidemiology of Candida glabrata and Candida albicans fungemia in immunocompromised patients with cancer. Am. J. Med. 112, 380–385.

Brandt, M.E., Harrison, L.H., Pass, M., Sofair, A.N., Huie, S., Li, R.K., Morrison, C.J., Warnock, D.W., Hajjeh, R.A.,. Candida dubliniensis fungemia: the first four cases in North America. Emerg. Infect. Dis. 6, 46–9.

Butler, G., Rasmussen, M.D., Lin, M.F., Santos, M.A.S., Sakthikumar, S., Munro, C.A., Rheinbay, E., Grabherr, M., Forche, A., Reedy, J.L., Agrafioti, I., Arnaud, M.B., Bates, S., Brown, A.J.P., Brunke, S., Costanzo, M.C., Fitzpatrick, D.A., de Groot, P.W.J., Harris, D., Hoyer, L.L., Hube, B., Klis, F.M., Kodira, C., Lennard, N., Logue, M.E., Martin, R., Neiman,

A.M., Nikolaou, E., Quail, M.A., Quinn, J., Santos, M.C., Schmitzberger, F.F., Sherlock, G., Shah, P., Silverstein, K.A.T., Skrzypek, M.S., Soll, D., Staggs, R., Stansfield, I., Stumpf, M.P.H., Sudbery, P.E., Srikantha, T., Zeng, Q., Berman, J., Berriman, M., Heitman, J., Gow, N.A.R., Lorenz, M.C., Birren, B.W., Kellis, M., Cuomo, C.A., 2009. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature 459, 657–62.

Calderone & Clancy, 2011. Candida and Candidiasis. American Society for Microbiology Press.

Calderone, R.A., Fonzi, W.A., 2001. Virulence factors of Candida albicans. Trends Microbiol. 9, 327–335.

Cantón, E., Pemán, J., Quindós, G., Eraso, E., Miranda-Zapico, I., Álvarez, M., Merino, P., Campos-Herrero, I., Marco, F., de la Pedrosa, E.G.G., Yagüe, G., Guna, R., Rubio, C., Miranda, C., Pazos, C., Velasco, D., 2011. Prospective multicenter study of the epidemiology, molecular identification, and antifungal susceptibility of Candida parapsilosis, Candida orthopsilosis, and Candida metapsilosis isolated from patients with candidemia. Antimicrob. Agents Chemother. 55, 5590–6.

Chi, H.-W., Yang, Y.-S., Shang, S.-T., Chen, K.-H., Yeh, K.-M., Chang, F.-Y., Lin, J.-C., 2011. Candida albicans versus non-albicans bloodstream infections: the comparison of risk factors and outcome. J. Microbiol. Immunol. Infect. 44, 369–75.

Clancy, C.J., Nguyen, M.H., 2013. Finding the “missing 50%” of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin. Infect. Dis. 56, 1284–92.

Cole, G.T., Halawa, A.A., Anaissie, E.J., 1996. The role of the gastrointestinal tract in hematogenous candidiasis: from the laboratory to the bedside. Clin. Infect. Dis. 22 Suppl 2, S73–88.

Dagenais, T.R.T., Keller, N.P., 2009. Pathogenesis of Aspergillus fumigatus in Invasive Aspergillosis. Clin. Microbiol. Rev. 22, 447–465.

de Repentigny, L., Lewandowski, D., Jolicoeur, P., 2004. Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. Clin. Microbiol. Rev. 17, 729–59.

Diekema, D., Arbefeville, S., Boyken, L., Kroeger, J., Pfaller, M., 2012. The changing

epidemiology of healthcare-associated candidemia over three decades. Diagn. Microbiol. Infect. Dis. 73, 45–8.

Eisenman, H.C., Casadevall, A., McClelland, E.E., 2008. New insights on the pathogenesis of invasive Cryptococcus neoformans infection. Curr. Infect. Dis. Rep. 9, 457–464.

Ellis, M., Al-Ramadi, B., Bernsen, R., Kristensen, J., Alizadeh, H., Hedstrom, U., 2009. Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive Candida infections in patients with neutropenic fever. J. Med. Microbiol. 58, 606–15.

Espinel-Ingroff, A., Pfaller, M.A., Bustamante, B., Canton, E., Fothergill, A., Fuller, J., Gonzalez, G.M., Lass-Flörl, C., Lockhart, S.R., Martin-Mazuelos, E., Meis, J.F., Melhem, M.S.C., Ostrosky-Zeichner, L., Pelaez, T., Szeszs, M.W., St-Germain, G., Bonfietti, L.X., Guarro, J., Turnidge, J., 2014. Multilaboratory study of epidemiological cutoff values for detection of resistance in eight Candida species to fluconazole, posaconazole, and voriconazole. Antimicrob. Agents Chemother. 58, 2006–12.

Fidel, P.L., 2006. Candida-Host Interactions in HIV Disease: Relationships in Oropharyngeal Candidiasis. Adv. Dent. Res. 19, 80–84.

Forrest, G.N., Weekes, E., Johnson, J.K., 2008. Increasing incidence of Candida parapsilosis candidemia with caspofungin usage. J. Infect. 56, 126–9.

Fradin, C., Slomianny, M.C., Mille, C., Masset, A., Robert, R., Sendid, B., Ernst, J.F., Michalski, J.C., Poulain, D., 2008. Beta-1,2 oligomannose adhesin epitopes are widely distributed over the different families of Candida albicans cell wall mannoproteins and are associated through both N- and O-glycosylation processes. Infect. Immun. 76, 4509–17.

Garey, K.W., Rege, M., Pai, M.P., Mingo, D.E., Suda, K.J., Turpin, R.S., Bearden, D.T., 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. 43, 25–31.

Gow, N.A.R., van de Veerdonk, F.L., Brown, A.J.P., Netea, M.G., 2012. Candida albicans morphogenesis and host defence: discriminating invasion from colonization. Nat. Rev. Microbiol. 10, 112–22.

Gudlaugsson, O., Gillespie, S., Lee, K., Vande Berg, J., Hu, J., Messer, S., Herwaldt, L., Pfaller, M., Diekema, D., 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. 37, 1172–7.

Guinea, J., 2014. Global trends in the distribution of Candida species causing candidemia. Clin. Microbiol. Infect. 20 Suppl 6, 5–10.

Hakki, M., Staab, J.F., Marr, K.A., 2006. Emergence of a Candida krusei isolate with reduced susceptibility to caspofungin during therapy. Antimicrob. Agents Chemother. 50, 2522–4.

Hall, R.A., Gow, N.A.R., 2013. Mannosylation in Candida albicans: role in cell wall function and immune recognition. Mol. Microbiol. 90, 1147–61.

Han, Y., Riesselman, M.H., Cutler, J.E., 2000. Protection against candidiasis by an immunoglobulin G3 (IgG3) monoclonal antibody specific for the same mannotriose as an IgM protective antibody. Infect. Immun. 68, 1649–54.

Held, J., Schmidt, T., Thornton, C.R., Kotter, E., Bertz, H., 2013. Comparison of a novel Aspergillus lateral-flow device and the Platelia® galactomannan assay for the diagnosis of invasive aspergillosis following haematopoietic stem cell transplantation. Infection 41, 1163–9.

Jacquinot, P.M., Plancke, Y., Sendid, B., Strecker, G., Poulain, D., 1998. Nature of Candida albicans-derived carbohydrate antigen recognized by a monoclonal antibody in patient sera and distribution over Candida species. FEMS Microbiol. Lett. 169, 131–8.

Jaijakul, S., Vazquez, J.A., Swanson, R.N., Ostrosky-Zeichner, L., 2012. (1,3)-β-D-glucan as a prognostic marker of treatment response in invasive candidiasis. Clin. Infect. Dis. 55, 521–6.

Jensen, R.H., Arendrup, M.C., 2011. Candida palmioleophila: characterization of a previously overlooked pathogen and its unique susceptibility profile in comparison with five related species. J. Clin. Microbiol. 49, 549–56.

Johnson, M., Cartmell, J., Weisser, N., 2012. Molecular recognition of Candida albicans (1→2)-β-mannan oligosaccharides by a protective monoclonal antibody reveals the immunodominance of internal. J. Biol. Chem. 287, 18078–90.

Jones, J.M., 1990. Laboratory diagnosis of invasive candidiasis. Clin. Microbiol. Rev. 3, 32–45.

Jouault, T., Fradin, C., Trinel, P. a, Bernigaud, a, Poulain, D., 1998. Early signal transduction induced by Candida albicans in macrophages through shedding of a glycolipid. J. Infect.

Dis. 178, 792–802.

Kahn, J.N., Garcia-Effron, G., Hsu, M.-J., Park, S., Marr, K.A., Perlin, D.S., 2007. Acquired echinocandin resistance in a Candida krusei isolate due to modification of glucan synthase. Antimicrob. Agents Chemother. 51, 1876–8.

Karageorgopoulos, D.E., Vouloumanou, E.K., Ntziora, F., Michalopoulos, A., Rafailidis, P.I., Falagas, M.E., 2011. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin. Infect. Dis. 52, 750–70.

Kedzierska, A., Kochan, P., Pietrzyk, A., Kedzierska, J., 2007. Current status of fungal cell wall components in the immunodiagnostics of invasive fungal infections in humans: galactomannan, mannan and (1-->3)-beta-D-glucan antigens. Eur. J. Clin. Microbiol. Infect. Dis. 26, 755–66.

Klis, F.M., 1994. Review: cell wall assembly in yeast. Yeast 10, 851–69.

Kojic, E.M., Darouiche, R.O., 2004. Candida Infections of Medical Devices. Clin. Microbiol. Rev. 17, 255–267.

Krcmery, V., Barnes, A.J., 2002. Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J. Hosp. Infect. 50, 243–60.

Lamoth, F., Cruciani, M., Mengoli, C., Castagnola, E., Lortholary, O., Richardson, M., Marchetti, O., 2012. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin. Infect. Dis. 54, 633–43.

Leung, A.Y.H., Chim, C.S., Ho, P.L., Cheng, V.C.C., Yuen, K.Y., Lie, A.K.W., Au, W.Y., Liang, R., Kwong, Y.L., 2002. Candida tropicalis fungaemia in adult patients with haematological malignancies: clinical features and risk factors. J. Hosp. Infect. 50, 316–9.

Lewis, R.E., 2009. Overview of the changing epidemiology of candidemia. Curr. Med. Res. Opin. 25, 1732–40.

Lucignano, B., Ranno, S., Liesenfeld, O., Pizzorno, B., Putignani, L., Bernaschi, P., Menichella, D., 2011. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. J. Clin. Microbiol. 49, 2252–8.

Marchetti, O., Bille, J., Fluckiger, U., Eggimann, P., Ruef, C., Garbino, J., Calandra, T., Glauser, M.-P., Täuber, M.G., Pittet, D., 2004. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clin. Infect. Dis. 38, 311–20.

Martin, R., Wächtler, B., Schaller, M., Wilson, D., Hube, B., 2011. Host-pathogen interactions and virulence-associated genes during Candida albicans oral infections. Int. J. Med. Microbiol. 301, 417–22.

McMullan, R., Metwally, L., Coyle, P. V, Hedderwick, S., McCloskey, B., O'Neill, H.J., Patterson, C.C., Thompson, G., Webb, C.H., Hay, R.J., 2008. A prospective clinical trial of a real-time polymerase chain reaction assay for the diagnosis of candidemia in nonneutropenic, critically ill adults. Clin. Infect. Dis. 46, 890–6.

Mikulska, M., Calandra, T., Sanguinetti, M., Poulain, D., Viscoli, C., 2010. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit. care 14, R222.

Mille, C., Fradin, C., Delplace, F., Trinel, P.-A., Masset, A., François, N., Coddeville, B., Bobrowicz, P., Jouault, T., Guerardel, Y., Wildt, S., Janbon, G., Poulain, D., 2012. Members 5 and 6 of the Candida albicans BMT family encode enzymes acting specifically on β-mannosylation of the phospholipomannan cell-wall glycosphingolipid. Glycobiology 22, 1332–42.

Mille, C., Janbon, G., Delplace, F., Ibata-Ombetta, S., Gaillardin, C., Strecker, G., Jouault, T., Trinel, P.-A., Poulain, D., 2004. Inactivation of CaMIT1 inhibits Candida albicans phospholipomannan beta-mannosylation, reduces virulence, and alters cell wall protein beta-mannosylation. J. Biol. Chem. 279, 47952–60.

Morgan, J., Meltzer, M.I., Plikaytis, B.D., Sofair, A.N., Huie-White, S., Wilcox, S., Harrison, L.H., Seaberg, E.C., Hajjeh, R.A., Teutsch, S.M., 2005. Excess mortality, hospital stay, and cost due to candidemia: a case-control study using data from population-based candidemia surveillance. Infect. Control Hosp. Epidemiol. 26, 540–7.

Morgan, M.A., Wilkowske, C.J., Roberts, G.D., 1984. Candida pseudotropicalis fungemia and invasive disease in an immunocompromised patient. J. Clin. Microbiol. 20, 1006–7.

Morrell, M., Fraser, V.J., Kollef, M.H., 2005. Delaying the empiric treatment of candida

bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob. Agents Chemother. 49, 3640–5.

Moudgal, V., Little, T., Boikov, D., Vazquez, J.A., 2005. Multiechinocandin- and multiazole-resistant Candida parapsilosis isolates serially obtained during therapy for prosthetic valve endocarditis. Antimicrob. Agents Chemother. 49, 767–9.

Munro, C.A., Bates, S., Buurman, E.T., Hughes, H.B., Maccallum, D.M., Bertram, G., Atrih, A., Ferguson, M.A.J., Bain, J.M., Brand, A., Hamilton, S., Westwater, C., Thomson, L.M., Brown, A.J.P., Odds, F.C., Gow, N.A.R., 2005. Mnt1p and Mnt2p of Candida albicans are partially redundant alpha-1,2-mannosyltransferases that participate in O-linked mannosylation and are required for adhesion and virulence. J. Biol. Chem. 280, 1051–60.

Ness, M.J., Vaughan, W.P., Woods, G.L., 1989. Candida antigen latex test for detection of invasive candidiasis in immunocompromised patients. J. Infect. Dis. 159, 495–502.

Netea, M.G., Brown, G.D., Kullberg, B.J., Gow, N.A.R., 2008. An integrated model of the recognition of Candida albicans by the innate immune system. Nat. Rev. Microbiol. 6, 67–78.

Obayashi, T., Negishi, K., Suzuki, T., Funata, N., 2008. Reappraisal of the serum (1-->3)-beta-D-glucan assay for the diagnosis of invasive fungal infections--a study based on autopsy cases from 6 years. Clin. Infect. Dis. 46, 1864–70.

Otero Silva, J., Franceschini, S.A., Lavrador, M.A.S., Candido, R.C., 2004. Performance of Selective and Differential Media in the Primary Isolation of Yeasts from Different Biological Samples. Mycopathologia 157, 29–36.

Panackal, A.A., Gribskov, J.L., Staab, J.F., Kirby, K.A., Rinaldi, M., Marr, K.A., 2006. Clinical significance of azole antifungal drug cross-resistance in Candida glabrata. J. Clin. Microbiol. 44, 1740–3.

Pappas, P.G., Kauffman, C.A., Andes, D., Benjamin, D.K., Calandra, T.F., Edwards, J.E., Filler, S.G., Fisher, J.F., Kullberg, B.-J., Ostrosky-Zeichner, L., Reboli, A.C., Rex, J.H., Walsh, T.J., Sobel, J.D., 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. 48, 503–35.

Pfaller, M., Houston, A., Coffmann, S., 1996. Application of CHROMagar Candida for rapid

screening of clinical specimens for Candida albicans, Candida tropicalis, Candida krusei, and Candida (Torulopsis) glabrata. J. Clin. Microbiol. 34, 58–61.

Pfaller, M.A., Andes, D.R., Diekema, D.J., Horn, D.L., Reboli, A.C., Rotstein, C., Franks, B., Azie, N.E., 2014. Epidemiology and outcomes of invasive candidiasis due to non-albicans species of Candida in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004-2008. PLoS One 9, e101510.

Pfaller, M.A., Diekema, D.J., 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. 20, 133–63.

Pfaller, M.A., Diekema, D.J., Gibbs, D.L., Newell, V.A., Ellis, D., Tullio, V., Rodloff, A., Fu, W., Ling, T.A., 2010. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J. Clin. Microbiol. 48, 1366–77.

Pfaller, M.A., Diekema, D.J., Mendez, M., Kibbler, C., Erzsebet, P., Chang, S.-C., Gibbs, D.L., Newell, V.A., 2006. Candida guilliermondii, an opportunistic fungal pathogen with decreased susceptibility to fluconazole: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance program. J. Clin. Microbiol. 44, 3551–6.

Pfeiffer, C.D., Samsa, G.P., Schell, W.A., Reller, L.B., Perfect, J.R., Alexander, B.D., 2011. Quantitation of Candida CFU in initial positive blood cultures. J. Clin. Microbiol. 49, 2879–83.

Pickering, J.W., Sant, H.W., Bowles, C.A.P., Roberts, W.L., Woods, G.L., 2005. Evaluation of a (1->3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J. Clin. Microbiol. 43, 5957–62.

Poulain, D., Slomianny, C., Jouault, T., Gomez, J.M., Trinel, P.A., 2002. Contribution of phospholipomannan to the surface expression of beta-1,2-oligomannosides in Candida albicans and its presence in cell wall extracts. Infect. Immun. 70, 4323–8.

Richet, H., Roux, P., Des Champs, C., Esnault, Y., Andremont, A., 2002. Candidemia in French hospitals: incidence rates and characteristics. Clin. Microbiol. Infect. 8, 405–12.

Ringdahl, E.N., 2000. Treatment of recurrent vulvovaginal candidiasis. Am. Fam. Physician 61, 3306–12.

Safdar, A., Armstrong, D., 2011. Infections in patients with hematologic neoplasms and hematopoietic stem cell transplantation: neutropenia, humoral, and splenic defects. Clin. Infect. Dis. 53, 798–806.

Sanguinetti, M., Posteraro, B., Fiori, B., Ranno, S., Torelli, R., Fadda, G., 2005. Mechanisms of azole resistance in clinical isolates of Candida glabrata collected during a hospital survey of antifungal resistance. Antimicrob. Agents Chemother. 49, 668–79.

Santos, M.A., Tuite, M.F., 1995. The CUG codon is decoded in vivo as serine and not leucine in Candida albicans. Nucleic Acids Res. 23, 1481–6.

Santos, M.A.S., Gomes, A.C., Santos, M.C., Carreto, L.C., Moura, G.R., 2011. The genetic code of the fungal CTG clade. C. R. Biol. 334, 607–11.

Sarvikivi, E., Lyytikäinen, O., Soll, D.R., Pujol, C., Pfaller, M.A., Richardson, M., Koukila-Kähkölä, P., Luukkainen, P., Saxén, H., 2005. Emergence of fluconazole resistance in a Candida parapsilosis strain that caused infections in a neonatal intensive care unit. J. Clin. Microbiol. 43, 2729–35.

Schell, W.A., Benton, J.L., Smith, P.B., Poore, M., Rouse, J.L., Boles, D.J., Johnson, M.D., Alexander, B.D., Pamula, V.K., Eckhardt, A.E., Pollack, M.G., Benjamin, D.K., Perfect, J.R., Mitchell, T.G., 2012. Evaluation of a digital microfluidic real-time PCR platform to detect DNA of Candida albicans in blood. Eur. J. Clin. Microbiol. Infect. Dis. 31, 2237–45.

Sendid, B., Poirot, J.L., Tabouret, M., Bonnin, A., Caillot, D., Camus, D., Poulain, D., 2002. Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic Candida species. J. Med. Microbiol. 51, 433–42.

Sendid, B., Tabouret, M., Poirot, J.L., Mathieu, D., Fruit, J., Poulain, D., 1999. New enzyme immunoassays for sensitive detection of circulating Candida albicans mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. J. Clin. Microbiol. 37, 1510–7.

Shahana, S., Mora-Montes, H.M., Castillo, L., Bohovych, I., Sheth, C.C., Odds, F.C., Gow, N.A.R., Brown, A.J.P., 2013. Reporters for the analysis of N-glycosylation in Candida albicans. Fungal Genet. Biol. 56, 107–15.

Singh-Babak, S.D., Babak, T., Diezmann, S., Hill, J.A., Xie, J.L., Chen, Y.-L., Poutanen, S.M.,

Rennie, R.P., Heitman, J., Cowen, L.E., 2012. Global analysis of the evolution and mechanism of echinocandin resistance in Candida glabrata. PLoS Pathog. 8, e1002718.

Staniszewska, M., Bondaryk, M., Siennicka, K., Kurzatkowski, W., 2012. Ultrastructure of Candida albicans pleomorphic forms: phase-contrast microscopy, scanning and transmission electron microscopy. Pol. J. Microbiol. 61, 129–35.

Tavanti, A., Davidson, A.D., Gow, N.A.R., Maiden, M.C.J., Odds, F.C., 2005. Candida orthopsilosis and Candida metapsilosis spp. nov. to replace Candida parapsilosis groups II and III. J. Clin. Microbiol. 43, 284–92.

Theel, E.S., Doern, C.D., 2013. β-D-glucan testing is important for diagnosis of invasive fungal infections. J. Clin. Microbiol. 51, 3478–83.

Thornton, C., Johnson, G., Agrawal, S., 2012. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. J. Vis. Exp.

Thornton, C.R., 2001. Molecular and Cellular Biology of Filamentous Fungi: A Practical Approach. Oxford University Press.

Thornton, C.R., 2008. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. Clin. Vaccine Immunol. 15, 1095–105.

Thornton, C.R., 2009. Tracking the emerging human pathogen Pseudallescheria boydii by using highly specific monoclonal antibodies. Clin. Vaccine Immunol. 16, 756–64.

Torosantucci, A., Gomez, M.J., Bromuro, C., Casalinuovo, I., Cassone, A., 1991. Biochemical and antigenic characterization of mannoprotein constituents released from yeast and mycelial forms of Candida albicans. Med. Mycol. 29, 361–372.

Trinel, P.A., Borg-von-Zepelin, M., Lepage, G., Jouault, T., Mackenzie, D., Poulain, D., 1993. Isolation and preliminary characterization of the 14- to 18-kilodalton Candida albicans antigen as a phospholipomannan containing beta-1,2-linked oligomannosides. Infect. Immun. 61, 4398–4405.

Trinel, P.-A., Plancke, Y., Gerold, P., Jouault, T., Delplace, F., Schwarz, R.T., Strecker, G., Poulain, D., 1999. The Candida albicans Phospholipomannan Is a Family of Glycolipids Presenting Phosphoinositolmannosides with Long Linear Chains of  -1,2-Linked Mannose Residues. J. Biol. Chem. 274, 30520–30526.

Verduyn Lunel, F.M., Donnelly, J.P., van der Lee, H.A.L., Blijlevens, N.M.A., Verweij, P.E., 2009. Circulating Candida-specific anti-mannan antibodies precede invasive candidiasis in patients undergoing myelo-ablative chemotherapy. Clin. Microbiol. Infect. 15, 380–6.

Vincent, J.L., Anaissie, E., Bruining, H., Demajo, W., El-Ebiary, M., Haber, J., Hiramatsu, Y., Nitenberg, G., Nyström, P.O., Pittet, D., Rogers, T., Sandven, P., Sganga, G., Schaller, M.D., Solomkin, J., 2014. Epidemiology, diagnosis and treatment of systemic Candida infection in surgical patients under intensive care. Intensive Care Med. 24, 206–216.

White, P.L., Parr, C., Thornton, C., Barnes, R.A., 2013. Evaluation of real-time PCR, galactomannan enzyme-linked immunosorbent assay (ELISA), and a novel lateral-flow device for diagnosis of invasive aspergillosis. J. Clin. Microbiol. 51, 1510–6.

Wingard, J.R., 1995. Importance of Candida species other than C. albicans as pathogens in oncology patients. Clin. Infect. Dis. 20, 115–25.

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P., Edmond, M.B., 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39, 309–17.

Yang, Y.-L., Chu, W.-L., Lin, C.-C., Tsai, S.-H., Chang, T.-P., Lo, H.-J., 2014. An emerging issue of mixed yeast cultures. J. Microbiol. Immunol. Infect. 47, 339–44.

Yera, H., Sendid, B., Francois, N., Camus, D., Poulain, D., 2001. Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. Eur. J. Clin. Microbiol. Infect. Dis. 20, 864–70.

Ylipalosaari, P., Ala-Kokko, T.I., Karhu, J., Koskela, M., Laurila, J., Ohtonen, P., Syrjälä, H., 2012. Comparison of the epidemiology, risk factors, outcome and degree of organ failures of patients with candidemia acquired before or during ICU treatment. Crit. care 16, 62.