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Advances in *Candida* detection platforms for clinical and point-of-care applications

Mohammadali Safavieh^a, Chad Coarsey^{b,c}, Nwadiuto Esiobu^d, Adnan Memic^e, Jatin Mahesh Vyas^f, Hadi Shafiee^a, and Waseem Asghar^{b,c,d}

^aDivision of Biomedical Engineering, Division of Renal medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, USA

^bDepartment of Computer Engineering and Electrical Engineering and Computer Science, Florida Atlantic University, Boca Raton, FL, USA

^cCollege of Engineering and Computer Science, Asghar-Lab, Micro and Nanotechnologies for Medicine, Boca Raton, FL, USA

^dBiological Sciences Department, Florida Atlantic University, Davie, FL, USA

^eCenter of Nanotechnology, King Abdulaziz University, Jeddah, Saudi Arabia

^fDepartment of Medicine, Division of Infectious Disease, Massachusetts General Hospital, Boston, MA, USA

Abstract

Invasive candidiasis remains one of the most serious community and healthcare-acquired infections worldwide. Conventional *Candida* detection methods based on blood and plate culture are time-consuming and require at least 2–4 days to identify various *Candida* species. Despite considerable advances for candidiasis detection, the development of simple, compact and portable point-of-care diagnostics for rapid and precise testing that automatically performs cell lysis, nucleic acid extraction, purification and detection still remains a challenge. Here, we systematically review most prominent conventional and nonconventional techniques for the detection of various *Candida* species, including *Candida* staining, blood culture, serological testing and nucleic acid-based analysis. We also discuss the most advanced lab on a chip devices for candida detection.

Keywords

Blood culture; disease diagnostics; invasive Candidiasis; laboratory on a chip; loop-mediated isothermal amplification; microfluidics; nanotechnology

CONTACT: Waseem Asghar, wasghar@fau.edu, Department of Computer Engineering and Electrical Engineering and Computer Science, Florida Atlantic University, Boca Raton, Florida, 33431, USA; Hadi Shafiee, hshafiee@research.bwh.harvard.edu, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, 02139, USA.

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Introduction

The Fungal Kingdom consists of about one million species and it is estimated that only 400 of them can cause disease in humans.[1–3] Fungi reproduce mainly by the following two different ways: sexually by the fusion of haploid gametes/hyphae generated through meiosis or asexually by budding, fragmentation and spore formation. Fungi can also switch between these two forms.[4] Due to the highly dimorphic nature of fungi, its classification is difficult and continues to be revised. Fungi, including yeasts, molds and mushrooms, are involved in baking,[5] the production of alcoholic beverages, bio-remediation,[6] the production of antibiotics, vitamins, anticancer and cholesterol-lowering drugs,[7–9] and various types of chemical products.[10] Although fungi play a beneficial role in human life, some of them are hazardous to human health.[11] Yeast fungi, like *Candida albicans*, typically associate as a commensal symbiont with human hosts. *Candida* is also notoriously found as an opportunistic pathogen.[12]

The genus *Candida* contains about 150 species, but only few of them, such as *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. dubliniensis*, *C. krusei* and *C. parapsilosis*, are implicated in human pathogenesis. *Candida* species live as commensals on skin, the gastrointestinal and genitourinary tracts. Repeated and lengthy episodes of neutropenia provide favorable conditions for *Candida* growth especially in critically ill patients facing AIDS,[13] blood cancer, bone marrow or solid organ transplants.[14–16] Preterm neonates,[17] intensive care and immunosuppressed patients [18] are considered more prone to *Candida* infections. Increased use of immunosuppressive agents, broad spectrum antibiotics, anti-cancer therapies, the use of indwelling catheters and long-term corticosteroid therapy [19] are all predisposing factors for acquiring *Candida* infections.[20] Superficial candidiasis, including chronic stomatitis, vulvovaginitis and mucocutaneous candidiasis, can be treated easily with hygiene measures and application of local ointments. Common clinical features associated with different *Candida* spp. are described in Table 1.

Epidemiological data of recent decades place *Candida* as the fourth major cause of nosocomial infections,[21] with about 50% of these cases attributed to *C. albicans*. Typically, disease progression by *C. albicans* is controlled by the innate immune system that recognizes a component in *Candida*'s cell wall through toll-like receptors (TLRs) and carbohydrate lectin receptors (CLRs).[22–24] Key factors involved in the pathogenicity of *C. albicans* include the potential of hyphal switch and the ability to form biofilms with which *C. albicans* can adhere to surfaces. Biofilm formation enhances *C. albicans* resistance to drugs and the immune system.[25,26]

Data from United States National Nosocomial Infection Surveillance (NNIS) system between 1990 and 1999 [27–30] showed that *Candida* was the fourth leading nosocomial pathogen.[31] Recent reports [32] identify *Candida* at the top of the pathogen list causing healthcare-associated bloodstream infections in the United States. These *Candida*-related bloodstream infections require prolonged hospitalization (additional 3–13 days, average hospital days for patients with similar illness =7.3) and significantly increase healthcare costs (\$6000–\$29,000 – more in comparison with normal hospitalization cost).[33,34] With mortality rates as high as 30%, nosocomial candidemia remains a major threat in hospitals,

underscoring the need for rapid point-of-care (POC) detection to prevent wide-scale spread. *Candida* can also cause lethal infections leading to multiple organ failure and septic shock. [27,35–37] This situation could be exacerbated by drug resistance, which is relatively stable at 7% for fluconazole currently, is showing trends for multidrug resistance to other agents such as echinocandins. It is the emergence of multidrug resistance – a trend projected to worsen – that warrants developing a rapid and versatile POC detection platform that could be used for diagnosis and antimicrobial susceptibility screening. Sensitive and rapid detection of *Candida* can play a major role in the effective management of fungal infection and treatment monitoring.

The detection of *Candida* has become very important due to an increased incidence of *Candida* infection, especially in immunocompromised patients. Current laboratory settings and *Candida* detection methods are very time-consuming and require at least 2–4 days to identify *Candida spp.*[40] As *Candida* infects mostly the patients who are in intensive care units (ICUs) and require urgent treatment, the fast detection of *Candida* has become extremely important for these immunocompromised subjects and preterm neonates. There is an increasing need to identify other *Candida spp.* along with *C. albicans* because of differences in fluconazole susceptibility.[41] Although *C. albicans* shows low resistance to triazoles, other species such as *C. glabrata* and *C. krusei* are resistive to usual doses of triazoles.[42–46] These species are 32 times less susceptible to fluconazole than *C. albicans*. It has also been observed that more than one *Candida spp.* can be found in clinical samples, especially from neonates.[47] Rapid identification of *C. albicans* along with other species has led to the development of new commercial products and techniques. Previous reviews published on *Candida* detection are focused mainly on conventional techniques.[38,39] Here, we review conventional and nonconventional diagnostic assays in the market and in the development pipeline for *Candida* detection. We have discussed diagnostic assays for *Candida* detection at the POC. We also review the technological advancements and potential challenges faced by researchers, which has been schematized in Figure 1.

Detection of *Candida*

Methods available for the detection of *Candida* can be broadly classified into microscopic examination, conventional detection methods and nonconventional techniques. The microscopic examination consists of nonspecific staining with calcofluor white (CFW) and specific staining of *Candida* with fluorescent *in situ* hybridization (FISH)/antibodies. Conventional methods include carbohydrate assimilation and fermentation reactions, culture-based *Candida* detection, blood culture and nucleic acid detection techniques, while the nonconventional methods covered various lab chip (LOC) devices developed for *Candida* detection.

Microscopic examination

Nonspecific staining with calcofluor white (CFW)—The microscopic detection of *Candida* in clinical specimens can guide the laboratory selection of appropriate media and culture techniques to confirm the presence of *Candida*. The clinician and laboratory staff usually determine the detection technique based on the origin and form of the sample. For

specimens, such as nail clippings, skin scrapings, tissues, aspirates and respiratory specimens, microscopic observation and detection can be used, whereas for fluidic samples such as blood and urine, culture methods are preferred.[48,49] Calcofluor white (CFW) staining may be used for direct examination of most samples using fluorescent microscopy. CFW has been extensively used in textile and paper industry as “fluorescent brightner” as it prevents the yellowing of fabric or paper. It binds to β 1–3 and β 1–4 polysaccharides of the chitin ring found in the fungus cell wall.[50,51] It gives bright green to bluish color when excited by ultraviolet (UV) radiation with an absorbance peak maximum at 347 nm. The addition of potassium hydroxide (KOH) with CFW can enhance the visualization of fungal elements in specimens. Chitin, found in fungi cell wall, is resistant to KOH digestion as reported earlier.[52,53] KOH dissolves other cells faster than fungi because of chitinous cell walls of fungi.[54] CFW at 0.0025% concentration mixed into peptone and glucose medium can also be used to differentiate various *Candida* species.[55] *C. krusei* (elongated cells) and *C. torulopsis glabrata* (oval cells) gave only slight fluorescence at the end of the cells, whereas *C. albicans* showed characteristic pseudohyphae. Although the cell shape and size of *C. parapsilosis* were similar to those of *C. krusei*, the former showed fluorescence over the entire cell surface. *C. tropicalis* was also differentiated based on CFW fluorescence patterns as shown in Figure 2. A substantial amount of CFW nonspecific binding with human cellular material especially lung tissue, blood vessels and skin sections is expected, but microorganisms can be distinguished by experienced laboratory staff based on their characteristic morphology.[54] CFW staining of certain fungi, such as *Pneumocystis carinii* and *Coccidioides immitis*, have been reported to be problematic and fluorescence results have been found to be dependent on a CFW supplier.[56,57] Although CFW staining can serve as rapid fungi detection tool, due to the reported variability of results and nonspecific binding of CFW, the need for validating the results with other culture-based methods is always recommended. Additionally, CFW staining kills the organism, hence drug resistance testing becomes impossible.

Specific staining of *Candida* with FISH/antibodies—Specific fluorescent staining of tissue specimens based on immunochemistry and genetic markers is considered one of the most reliable methods for the diagnosis of *Candida* infection. FISH is a technique that uses fluorescent oligonucleotide probes that specifically bind to genes present in the cell’s genome. FISH technique was employed to differentiate *C. parapsilosis* from *C. albicans* using probes targeting 18S rRNA region of *Candida*.[58] *C. albicans* was successfully detected in tissue specimens of brain, kidney and spleen of infected mice. It was reported that FISH methods were prone to lengthy and dim fluorescent signals if DNA-based probes were used for *Candida* detection.[59] Efforts have also been made to overcome these challenges by using different DNA-based probes along with unlabeled helper probes. The helper probes significantly increased the fluorescent intensity of *C. albicans* when used with different FISH DNA-based probes (Calb-1249 or CalB-2208).[59] For enhanced FISH staining, peptide nucleic acid (PNA)-based probes targeting 26S rRNA region were also employed. PNA-based FISH-labeling technique improved signal intensity in shorter amounts of time.[60] In another report, *C. albicans* was identified after isolation from media using PNA FISH^{Flow} (AdvanDX, MA).[61] The PNA FISH^{Flow} Kit was also tested to analyze its efficiency to identify *Candida* from 150 clinical isolates collected from various sources.

Isolates were hybridized with PNA-based probes after 24-h culture on agar plates.[61] It successfully identified different *Candida spp.* including *C. glabrata*, *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. kefyr* and others. The specificity and sensitivity of PNA FISH^{Flow} probes for *Candida* identification were 100% (49 of 49) and 100% (101 of 101) respectively and the whole staining process completed in 1 h.[61] Gherna et al. also used the PNA FISH detection kit from AdvanDX and identified *C. albicans* and *C. glabrata* from 40 positive blood culture bottles in 1.25 h.[62] In another report, FISH oligonucleotide probes, labeled with fluorescein isothiocyanate (FITC; green signal) and fluorochrome CY3 (red signal), were synthesized by Microsynth (Switzerland, Table 2).[63,64] *Candida* was detected in 98% of positive blood samples (49 of 50) with PF2 all yeast probe. Different *Candida spp.* were also accurately identified with specific FISH probes. For example, in one specimen, FISH method detected *C. parapsilosis*, whereas the conventional method misidentified it as *C. glabrata*.[63,64] It was later confirmed with repeated culture methods that detected *Candida spp.* was *C. parapsilosis* as correctly identified by FISH method.[63,64] Overall, FISH detection methods offer a rapid platform for specific identification of *Candida spp.* from blood culture. Although it gives high sensitivity and specificity, FISH cannot be employed directly with patient blood samples without culture. Blood culture can take up to two weeks before giving positive results. There is a need to optimize FISH testing protocols for application in patient blood samples directly that may be achieved by incorporating efficient cell separation and centrifugation processes.

Conventional *Candida* detection methods

Carbohydrates assimilation and fermentation reactions—The most conventional method to identify *Candida* in a mycology laboratory is using carbohydrate assimilation and fermentation reactions. Traditionally, liquid media in test tubes are used for the assimilation of glucose, maltose, sucrose, lactose, rhamnose and trehalose, whereas glucose, maltose, sucrose, lactose are used for fermentation.[65] The test tubes are incubated and examined for growth after 7 and 14 days. These tests can now be performed easily on automated systems including the Auxacolor (Sanofic Diagnostcs Pasteur), API 20C AUX (bioMerieux-Vitek, Inc., Hazelwood, Missouri), the Uni-Yeast-Tek kit and Yeast Star (CLARC laboratories). The tests either utilize the production of color (Uni-Yeast-Tek and Auxacolor) or an increase in turbidity (API 20C AUX) in various wells having different substrates to produce a distinctive biochemical profile. A numerical code is generated for each isolate depending on the biochemical reaction profile. Identification of species is made by referring to a list of numerical profiles provided by the manufacturer. The API 20C AUX system is one of the first systems that is commercially available for the detection of *Candida*. It is faster than classical assimilation and fermentation methods but still requires up to 72-h incubation.[66–69] Auxacolor and API *Candida* are an improved version of API 20C AUX and can produce results after 24-h incubation. These two systems are intended to identify the most commonly encountered clinical pathogens; the Auxacolor system is capable of identifying 26 species, while API *Candida* systems can only identify 15 species.[66–69] These methods provide good identification results for more common species with an accuracy of 78.8% to 91.8% for API *Candida*, 85.7% to 91.2% for Auxacolor, 62.1% to 99.3% for API 20C, 89.7% for Vitek Yeast Biochemical Card (YBC), 99.8% for Uni-Yeast-Tek and 79.3% for Yeast Star. [67–71] On the other hand, there were many reports showing that all of these systems either

misidentified various fungal species or provided inconclusive results.[70,71] The API *Candida* system misidentified 7.5% of *Candida spp.* (12 of 159 isolates) and 14.5% of isolates (23 of 159) required further testing to validate their identification. About 27.2% isolates of *C. guilliermondii* were incorrectly identified as *S. cerevisiae*, whereas one of 11 was misidentified as either *C. famata* or *C. parapsilosis*. [67,70] API *Candida* systems also misidentified 10% of *C. tropicalis* isolates as *C. albicans* and 5% as *C. famata*. The Auxacolor system did not identify 8.1% (13 of 159) isolates; four isolates of *C. lusitaniae*, three of *S. cerevisiae*, two of *C. guilliermondii*, two of *Trichosporon beigelii*, one of *C. albicans* and one of *C. tropicalis*. [67,70] Morphological and culture-based *Candida* detection techniques are concurrently employed along with assimilation-/fertilization-based testing to confirm species identification that would further increase the identification time.

Culture-based *Candida* detection—Clinical specimens are routinely cultured on Sabouraud glucose agar (SGA) for fungal growth. The pathogen isolates are tested using assimilation/fermentation methods or are further cultured on Dalmau Plate, Corn meal-Tween 80 agar (CTA) for morphological identification on species level. [72,73] The development of a chromogenic medium improves the *Candida* differentiation method based on *albicans* and 100% of *C. krusei* the color/texture (Figure 2). The medium contains chromogenic substrates that react with enzymes produced by different pathogens and produce colonies of varying color and morphology. Chromogenic media culture kits are commercially available by various manufacturers including, but not limited to, BBL CHROMagar *Candida* (CHROMagar; Becton Dickinson Canada Inc.), Bio-Rad CandiSelect 4 (CandiSelect; Bio-Rad), Oxoid OCCA (OCCA; Oxoid Ltd.) and CHROMagar *Candida* (CMA, CHROMagar Company, Paris, France). [74] CHROMagar *Candida* has been used to successfully identify 95% of *C. albicans*, 94% of *C. glabrata*, 100% of *C. Tropicalis* and 100% of *C. krusei* isolates from other yeast *spp.* based on their color and morphology. [73,75] The CHROMagar has the ability to efficiently detect more than one yeast *spp.* from a clinical specimen; 43 out of 234 positive cultures contained a mixture of yeast *spp.* as detected by CHROMagar. On the other hand, 47% of these mixed cultures were not identified by SGA alone. [75] Adam et al. [74] compared the performance of three commercially available chromogenic media CHROMagar, CandiSelect and OCCA. [74] CHROMagar and CandiSelect have accurately identified 100% of *C. albicans* and *C. krusei* isolates, whereas OCCA identified 92% of *C. albicans* and 56% of *C. krusei* after 48-h incubation. Furthermore, CHROMagar, CandiSelect and OCCA correctly identified 50%, 60% and 40% of *C. Tropicalis* and 88%, 75% and 50% of *C. glabrata* respectively. There were few discrepancies found in all of the three media; CHROMagar falsely identified 1 *C. tropicalis* isolate as *C. glabrata*; CandiSelect falsely identified 1 *C. glabrata* as *C. tropicalis* and OCCA falsely identified 1 *C. albicans* as *C. tropicalis*, 1 *C. tropicalis* as *C. albicans* and 1 *C. tropicalis* as *C. glabrata*. [74] In another report, although CHROMagar identified 98.8% of *C. albicans* and 100% of *C. krusei*, only 66.7% of *C. tropicalis* (4 of 6) were correctly identified. It also identified *C. glabrata* with 98% sensitivity and 92.1% specificity, but some other stains of *Candida* (three stains of *C. parapsilosis*, two stains of *C. lusitaniae*, five out of nine stains of *C. cerevisiae*) also grew to pink colonies, similar to *C. glabrata*. Although the pink coloration of these species did not exactly match *C. glabrata*, there were high chances of a mistake without experienced laboratory staff. [73] Similar results were reported by

Sivakumar et al., [76] where CHROMagar misidentified three out of eight *C. tropicalis* as *C. albicans*, whereas *C. glabrata* could not be identified without experience. To overcome these shortcomings, a new scheme of using CTA (morphology) and CMA (color, morphology) at the same time was proposed and *C. tropicalis* was detected with >99% confidence.[72] *C. glabrata* was more efficiently identified by using combination of CTA and CMA as shown Figure 3.[72]

C. albicans and *C. dubliniensis* grow germ tubes (GT) that can be used as characteristic morphological feature for rapid identification of these two *Candida* species.[77–79] Yeast cells can be transformed into GTs in human blood, sera, plasma and cerebrospinal fluids (CSF). Corn meal agar medium and corn meal broth are usually used as GT culture media. *C. albicans* developed into GTs in all of 111 test stains in 1–2 h at optimal conditions (75% corn meal broth, 37 °C).[80] Although the GT test can be used as a rapid identification test, it cannot differentiate between *C. albicans* and *C. dubliniensis*. CHROMagar defines light green color for *C. albicans* and dark green color for *C. dubliniensis*. However, it was found that CHROMagar did not give light green color for *C. albicans* in 49 out of 98 oral swabs from HIV-infected patients and showed green to dark green coloration similar to *C. dubliniensis*. A novel chromogenic medium CHROM-Pal medium (CH-P) is proposed that is modified form of CHROMagar supplemented with Pal's agar. CH-P medium accurately differentiated *C. albicans* from *C. dubliniensis* with more than 95% detection efficiency.[81] CH-P medium has given very promising initial results, but it should be further analyzed with a wide range of rare *Candida* species. Notwithstanding the progress being made on chromogenic detection methods, there will probably be considerable limitations in specificity and sensitivity owing in part to differential gene expression of the targeted enzyme or substrate induced by changing environmental factors.

Overall, culture-based techniques are the most widely used methods for *Candida* detection in clinical applications. However, due to their time-consuming nature, it makes culture-based techniques unsuitable for rapid *Candida* testing.

Blood culture for *Candida* detection—Blood culture is one of the standard methods for fungal detection. The general technique of blood culture is based on taking 10 ml of blood injected into two or more blood bottles with specific media to culture either aerobic or anaerobic organisms.[33] If a vial test is positive, the Gram-stain can be determined in subsequent steps of the bacteria/fungal analysis.[33] Also, the blood can be subcultured onto agar plates to create distinctive colonies. A combination of blood culture and CHROMagar provides a better technique for the colony formation and detection of various *Candida* subtypes.[82] Horvath et al.[82] have investigated whether the colony size and shape if taken directly from blood culture were modified. In this regard, 50 clinically isolated samples from blood culture were isolated and cultured in CHROMagar.[82] Direct isolation yielded more rapid growth of colonies in the CHROMagar and consequently provided faster *Candida* detection analysis, despite the fact that it did not show significant changes in the colony color, shape and size (Figure 4).

One of the most frequently used blood culture media for *Candida* detection is BacT/ALERT®. With the leverage of liquid emulsion sensor and colorimetric analysis, BacT/

ALERT[®] provides color modification when the pH changes as a response to increasing CO₂ concentrations.[83] Other types of blood cultures that are commonly used for *Candida* detection, are BD Bactec[™] FX blood culture (BC) and VersaTREK[™] system (aerobic redox medium).[84] BC is a fully automated culturing machine integrated with fluorescent sensors, which provides high-throughput sample analysis. VersaTREK is a type of stir bar aerobic bottle, which has the magnet bar at the bottom of the bottle. By stirring, the oxygen will distribute around the media evenly yielding uninterrupted, continuous mixing, which provides consistent fungal growth in the bottle.[85] Despite significant advances in automation and throughput analysis, blood culturing is a very time-consuming method, thus, not suitable for rapid *Candida* detection. In addition, it is not easily amenable to miniaturized POC use by patients or care givers.

Serological detection of *Candida*

Antigenic detection of *Candida* infections has been developed over the past 30 to 40 years and provides a viable target for *Candida* detection. However, there is not a general consensus on a specific antibody detection convention.[86,87] Diagnosis by serological methods is resourceful due to the following: (1) the intrinsic nature of the humoral response to *Candida* infection, and the production of a multitude of antibodies to many different epitopes, (2) the high sensitivity of each antigen or antibody test allows for rapid detection of *Candida* infection [86] and (3) antibodies can be produced *in vitro* to meet the demand for novel tests for antigens with highly sensitive diagnostic application.[88] The current antigen and antibody diagnostic methods require serum samples from patient blood.[86,89] Since *Candida* is a common skin commensal organism, exposure confers development of antibodies regardless of infection.[90] This suggests that antigenic diagnosis of *Candida* is a better means of a quick diagnostic tool rather than the detection of anti-*Candida* antibodies in sera.

Some of the simple fungal antigen testing methods include the latex-agglutination tests.[91–93] This method utilizes latex or glass beads that are coated with an antigen-specific antibody within a buffer suspension. Though an older test, latex agglutination has made re-emergence with developments in antigen-specific monoclonal antibodies in serological testing.[92,94] Mitsutaki et al.[90] evaluated polyclonal anti-*Candida* antibody Cand-Tec, and monoclonal anti-*Candida* antibody Pastorex with latex agglutination systems. It was demonstrated that the Cand-Tec assay showed a 76.9% detection rate to the enolase antigen; whereas the Pastorex assay showed a lower (25.6%) detection rate. In the monoclonal antibody system, no superficial *Candida* infections were detected, unlike the polyclonal system. Another diagnostic tool, western blotting (immunoblotting),[90,95] is used to detect specific fungal antigens or host antibodies present in sera. The method separates the proteins by size with gel electrophoresis, and then, the gel is blotted onto absorbent paper. On the paper, patient serum is screened for *Candida* antigens by precipitation upon contact with specific antibodies. Similarly, antibodies against fungal antigens can be separated and used as assays for antigen detection in sera.[95] Other antigen–antibody paper tests include the immunoprecipitation and immunodiffusion assays.[91,96,97] These assays determine the presence of antibodies against fungal antigens on the basis of antibody–antigen precipitation.

Beginning in the early 1990s, enzyme-linked immunosorbent assays (ELISA) were developed to detect antibodies against *Candida* and showed high specificity and sensitivity. ELISA is a standard method based on antibody/antigen detection for a wide range of infectious and opportunistic diseases and can vary in protocol depending on which identification aspect is sought.[86,96–101] The assay uses a probe substrate that upon reaction with an enzyme conjugated to a specific antibody or antigen generates a fluorophore that can be spectrophotometrically analyzed as a result of substrate catalysis. [98–100] Laín et al.[99] found that an IgG ELISA had 81.0% sensitivity to an antienolase IgG antibody and showed 88.9% sensitivity to IgG antibody against an *N*-terminal hyphal wall protein.[98] The ease and practicality of these tests for anti-*Candida* IgG antibodies enables the development of a sensitive tool for early diagnosis of infection. Clancy et al.[86] recently used IgG ELISA to measure responses from a panel of antigens to accurately determine early infection markers of candidiasis. ELISA can also play a role for the evaluation of patient antibody responses as a quantification tool of antibody titer, and Fujita et al.[97] used this to show the various stages of diagnosis during the disease course, as well as mapped the increase in antibody titer against *Candida*. Although a useful tool, ELISA still must be further developed to a high level of consistency before becoming a standard for the antibody detection of *Candida* infection.

Several monoclonal antibodies have been produced against specific *Candida* antigens. [88,102,103] Reiss et al.[88] found that monoclonal antibodies directed against mannan components of the *Candida* cell wall were useful in antigen detection tests. Many of the antibodies produced confer the same reactivity as *in vivo* host antibodies.[102] A disadvantage to monoclonal antibody detection methods results from the high specificity of the monoclonal antibody epitope to the particular fungal antigen. This limited scope of the full antigenic epitope is prone to bias the ability of monoclonal antibody tests to detect antigens from the multiple strains of *Candida* as shown by Marot-Lebond et al.[92] Although minimal, monoclonal antibody testing has shown cross reactivity to other non-*Candida* antigens, which risks false-positive results.[104,105] Recently, in 2007, Rodriguez et al.[105] described a monoclonal antibody that cross-reacted with an overexpressed nuclear pore complex protein, *Nup88*, found in oral squamous cell carcinomas. This suggests that due to cross reactivity of the antibody, risk of generating false-positive results exists.

Polyclonal and monoclonal antibodies are also being used for immunofluorescence analysis of *Candida*. The production of specific antibodies for *Candida* has proved to be extremely difficult as antibodies often cross react with other fungi. Generally speaking, the antibodies specific for *Candida spp.* are useful in differentiating infections caused by various *Candida spp.* from other fungi.[106] Scientists have successfully produced a monoclonal antibody (Mab), 3H8, specific to *C. albicans*. [107] The 3H8 antibody recognizes mannoprotein, antigen present on the *C. albicans* cell wall, whereas this antigen is partially cryptic in cell walls of other *Candida spp.* (*C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*). [107–109] The 3H8 Mab was used in successful identification of *C. albicans* from various body tissues, including lung, kidney, thyroid, small bowel and esophagus tissue. [109] Bikandi et al. [110] have accurately differentiated *C. albicans* from *C. dubliniensis* based on their immunofluorescence using 3H8 Mab. Jarvensivu et al. [111] have also reported the specific

immunohistochemical identification of *C. albicans* using 3H8 Mab. These results are promising and antibodies can help in rapid *Candida* detection, but these antibodies should be tested with many clinical samples and blood cultures specimens before reaching any conclusion. Secondly, tremendous effort is needed to produce specific antibodies for other species of *Candida*. Overall, immunoassay-based techniques provide rapid tests. However, the main drawbacks are low specificity and cross-reactivity with other antibodies/antigens.

Nucleic acid detection of candida

Nucleic acid (NA)-based detection plays an important role in the identification of fungal analytes by providing sensitive and specific bioassays. There are major reasons that make this technique one of the most robust approaches [112]: (1) all living organisms have NAs in their cell body; (2) NAs regardless if they are single stranded or double stranded, they consist of only four different base pairs, which are relatively easy to detect; (3) NAs are easy to manipulate; and (4) there are very well established technologies existing that can replicate specific sequences of NAs at relatively low cost.[112] The use of molecular diagnostics for the detection of specific sequence of fungus has been reviewed.[113,114] NA-based detection or molecular diagnostics can be implemented based on hybridization techniques, microarrays and amplification. One of the most challenging steps when using these techniques is sample preparation. This consists of DNA extraction and purification steps to release the intracellular DNA from the fungus, concentration of the target DNA and removal of potential inhibitors, debris and contaminants.[115]

DNA microarray techniques have emerged as a viable platform for *Candida* detection. Thousands of gene-specific probes are attached to the surface of solid substrate, such as silicon wafer, glass or nylon. These probes are designed in a way to be complementary to target organism genome DNA sequences. Then, the sequences are queried by a label (fluorescent or radioactive labels). Once the sample is added to the microarray, either in bulk or with the help of some liquid handling device such as microfluidics, hybridization takes place. The array is then scanned to visualize the fluorescent pattern tiled on the surface of the substrate, and the pattern is determined using microarray reader. Various microarrays have been developed to detect *Candida*. [116–118] Leinberger et al. [118] have developed a microarray for the identification and detection of 12 different *spp.* of *Candida* and *Aspergillus*. Oligonucleotic probes were designed to detect the internal transcribed spacer (ITS) region of the rRNA gene. The whole process after DNA extraction took 4 h and showed a robust platform for clinical diagnostics by testing 21 unknown clinical patient samples on a micro-array. The microarray was simultaneously amplified with multiplex polymerase chain reaction (PCR) and labeled with a fluorescent dye for the sensitive detection of *Candida*. In a similar example, Spiess et al. [117] developed a microarray with multiplex PCR to detect 14 fungal *Candida spp.* from high-risk patient samples (22 patients) including blood, bronchoalveolar lavage and tissues. Although the assay is rapid, their sensitivity is often lower than amplification techniques.[119]

Since quantities of the NAs are relatively small, amplification techniques are routinely employed. Amplification techniques can be divided into two groups, namely (i) isothermal and (ii) nonisothermal [163] techniques.[112] Since its first publication in 1986,[120] PCR

is the most commonly known because of a nonisothermal amplification technique for the detection of bio-analytes and it has been exploited for *Candida* detection.[121–124] The process is based on three different reactions to amplify DNA by leveraging thermal cycling: (1) denaturation that makes double-stranded DNA into the single-stranded template at stringent temperature conditions, (2) annealing, which matches specifically designed primers to attach a single-stranded DNA template at stringent temperatures and (3) extension using polymerase enhances the polymerase reaction activity by generating a new strand, which is complementary to the single-strand sequence. Various PCR techniques have been developed based on different applications, such as multiplexed PCR (for the detection of various analytes in a sample), real-time PCR (for monitoring amplification in real time format), reverse-transcriptase PCR (RT-PCR) for the amplification of RNA templates, and nested PCR for increasing the specificity by using two pairs of primers to avoid unexpected primer binding and nonspecific amplification.

The requirement to use three different temperatures during PCR switched the attention into the development of isothermal amplification. There are various different isothermal amplification techniques that were used for NA amplification and detection such as (1) loop-mediated isothermal amplification (LAMP), (2) NA sequence-based amplification (NASBA), (3) rolling circle amplification (RCA) and (4) strand displacement amplification (SDA). Table 3 shows the different amplification methods used for *Candida* detection. For example, Inácio et al.[85] used the LAMP technique for amplification of the 26S rRNA gene in clinically relevant *Candida* yeast. The amplification occurred at 64 °C for 90 min with the sensitivity as low as 50 fg. In another example, Widjoatmodjo et al.[125] employed NASBA for the detection of 19 different *Candida* subtypes from 18S rRNA sequences at 41 °C with the detection limit of 0.01 CFU/ml. In the similar effort, Zhou et al.[126] detected 18 different *Candida* subtypes *Candida* based on RCA amplification in 2 h by synthesizing an RCA padlock probe. Overall, NA-based detection methods are the most accurate and precise techniques for screening *Candida* and various fungi. However, these methods are multistep and require sample purification, NA extraction prior to the detection step, a time-consuming process.

Nonconventional detection for *Candida*: lab chip (LOC) devices for *Candida* detection

With the development of micro total analysis systems (μTAS), various (LOC) devices have been developed for pathogen detection.[38,127–146, 164] Microfluidic LOC devices enhance early detection and identification of fungi in the blood stream. This technology allows for a device to be used in resource-constrained settings where there are no centralized laboratories available for implementing conventional culturing techniques.[132,133,147,148] However, the development of fungal detection based on NA requires various steps, including cell lysis, NA extraction and purification, together with the removal of possible inhibitors of PCR amplification.[149] For example, Cai et al. [150] have developed a microfluidic platform based on SlipChip® to simultaneously detect multiple fungi including *Candida spp.* such as *C. glabrata*, *C. albicans*, *C. krusei*, *C. parapsilosis* and other pathogens from blood samples. This was performed by combining multiplex PCR amplification and

dielectrophoresis (DEP) for the separation of 20 different pathogens and fungi from blood samples followed by fluorescent detection within 3 h (Figure 5A).

Digital microfluidics has also emerged as a robust platform for fungal detection. Boles et al. [151] have developed droplet-based microfluidic leveraging electrowetting concept to control and manipulate droplet samples for implication of various concepts such as merging liquid, mixing and moving to different regions of the chip. By combining the magnetic force and electro-wetting, the ability to merge, extend and move droplets to each direction allows for the implementation of various steps required for PCR, such as DNA extraction, purification and transfer to different thermal zones. Using this technique, Schell et al. [152] have employed an advanced liquid logic chip for the detection of *C. albicans* from blood samples collected from 16 candidemia patients. The DNA extraction was implemented off-chip and the purified DNA was applied to the biochip. Using this method, sensitivity of 56% (compared to 69% of conventional PCR real-time analysis) has been achieved within 45 min (Figure 5B). Other types of highly integrated LOC platforms have also been developed for *in vitro Candida* diagnosis. Schumacher et al. [153] have developed a Fraunhofer ® LOC platform for *in vitro* diagnosis of various fungi and pathogens using PCR by leveraging optical sensing and electrochemical sensing in a single platform (Figure 5C).

Microchip PCR has the robust ability to identify and quantify various species in biosamples. However, the main drawback is that these technologies are unable to determine the viability of the fungus or other pathogens. Another alternative is based on Raman spectroscopy that measures the distinct vibration spectrum of various molecules and structures such as DNA, cells and live/dead fungus. [24] This technique has been implemented for the detection of various *Candida spp.* which have been cultured on Sabouraud medium or blood culture. A modification of Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), employs metal nanoparticles (gold or silver) to enhance the intensity of Raman scattering. These metal nanoparticles are either attached chemically to the fungus/bacteria wall, or settle down in the proximity of pathogen. Combining the SERS technique for detection and DEP method for cell separation on a chip can provide rapid tools for multiplex *Candida* detection. Cheng et al. [24] have fabricated a microfluidic chip by combining planar electrodes into 3D DEP gates with latex beads to separate various pathogens such as *C. albicans*, *E. coli* and *Lactobacillus* (Figure 6).

A combination of field effect transistor (FET) and single wall carbon nanotube (SWCNT) has also been used for *Candida* detection. Villamizar et al. [32] have fabricated SWCNT-FET transistor based on chemical vapor deposition (CVD) on a silicon chip. The source and drain electrodes were screen-printed electrodes and have been fabricated using Ag ink. Monoclonal anti-*Candida* antibodies have been immobilized on the surface of SWCNT, and the selectivity of the assay has been analyzed with *Cryptococcus albidus* and *Saccharomyces cerevisiae*. A limit of detection (LOD) of 50 CFU/ml has been achieved within 1 h.

Nucleic magnetic resonance (NMR) is another useful technique for the diagnosis of biomolecules based on nuclei absorbance and the remittance of electromagnetic radiation in the presence of a magnetic field. NMR has the ability to detect the target with high sensitivity in highly turbid samples such as urine, blood, serum, without implementing any

filtration or purification step. Miniaturization of NMR can target and quantify multiplex bioanalytes such as various proteins and fungi. Lee et al.[154] have developed NMR-chip for multiplex target detection utilizing self-amplification of magnetic nanoparticles. Magnetic nanoparticles bind to the target through affinity ligand such as an antibody and form a magnetic cluster. The magnetic cluster corresponds to a decrease in bulk spin-spin relaxation time of surrounding molecules. The assay consists of NMR electronics, RF microcoils for NMR measurement and a microfluidic network for sample and permanent magnets. A 1.0 pM sensitivity has been achieved for multiple targets including *S. aureus* and others, as well as mammalian cells which was in agreement with benchmark detection using an NMR system. In a similar approach, a new device has been introduced by T2 Biosystem Inc. for the detection of the *Candida* amplicon by using a simple PCR tube for sample handling instead of a microfluidic chip and has a sensitivity of 10 CFU/ml.[23] Supernatant removal has been implemented off-chip by centrifugation followed by chemical lysis. Following the PCR, magnetic beads that have been attached to the PCR amplicon are incubated for 20–30 min before NMR T_2 measurement. Table 3 shows comparison of various commercially available platforms for *Candida* detection. These platforms are categorized based on their sensitivity, specificity and cost per test. One of the more successful commercial systems is the T2X platform, made by T2 Biosystem that utilizes T2 magnetic resonance (T2MR) approach. Particles coated with target-specific agent form clusters when bound to a specific pathogens. These clusters alter the microscopic environment of the water molecules around the target, which can be monitored in the presence of the magnetic field. As low as 1 CFU/ml of *Candida* is reported to be detected within 3–5 h without the need for sample purification steps.[137]

Summary and future perspective

Various technologies have been reviewed for *Candida* detection with significant development observed in rapid and more effective clinical diagnostics. Although cell culture-based methods are still the gold standard for *Candida* detection, NA-based diagnostic assays utilizing various amplification approaches provide a more sensitive and faster test analysis for fungal detection. One of the attractive amplification methods is LAMP, which can potentially reduce the test time, as it does not require thermal cycling and thermal ramping. Therefore, we envision that the integration of LAMP-based approaches with microfluidic-based platforms can potentially lead to the development of compact, self-contained and rapid POC assays for *Candida* detection.

Microfluidics is on the brink of decentralized testing.[155–158] These devices provide the opportunity for patients to monitor their own health condition without the need to seek consultation in the doctor's office. It is, however, important to consider the manufacturing ability to mass-produce low-cost microfluidic-based platforms as POC diagnostics that can meet the market demands and the guidelines made by public health agencies. For example, paper-based microchip technologies [115,132,147,159,160] integrated with optical/electrical sensing modalities may open a promising avenue in the development of POC diagnostics for *Candida* detection.

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References

1. De Hoog, G., Guarro, J., Gene, J., et al. Atlas of clinical fungi Centraalbureau voor Schimmelcultures. Universitat Rovira i Virgili, Amer Society for Microbiology; 2000. p. 164-174.
2. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev. 1996; 9:499–511. [PubMed: 8894349]
3. Guarro J, Gené J, Stchigel AM. Developments in fungal taxonomy. Clin Microbiol Rev. 1999; 12:454–500. [PubMed: 10398676]
4. Phaff, HJ. Yeasts eLS. 2001. p. 1-11.
5. Kulp, K. Handbook of Cereal Science and Technology, Revised and Expanded. CRC; 2000.
6. Christian V, Shrivastava R, Shukla D, et al. Degradation of xenobiotic compounds by lignin-degrading white-rot fungi: enzymology and mechanisms involved. Indian J Exp Biol. 2005; 43:301. [PubMed: 15875713]
7. Fincham J. Transformation in fungi. Microbiol Rev. 1989; 53:148–170. [PubMed: 2651864]
8. Hawkins KM, Smolke CD. Production of benzyloquinoline alkaloids in *Saccharomyces cerevisiae*. Nat Chem Biol. 2008; 4:564–573. [PubMed: 18690217]
9. Huang B, Guo J, Yi B, et al. Heterologous production of secondary metabolites as pharmaceuticals in *Saccharomyces cerevisiae*. Biotechnol Lett. 2008; 30:1121–1137. [PubMed: 18512022]
10. Joseph B, Ramteke PW, Thomas G. Cold active microbial lipases: some hot issues and recent developments. Biotechnol Adv. 2008; 26:457–470. [PubMed: 18571355]
11. Kendrick B. Fungi: ecological importance and impact on humans. eLS. 2001
12. Murray, PR., Rosenthal, KS., Pfaller, MA. Medical microbiology. 7. Vol. 44. Philadelphia, PA: Elsevier Saunders; 2013. Opportunistic mycoses/candidiasis; p. 6776-6783.
13. Pappas PG. Opportunistic fungi: a view to the future. Am J Med Sci. 2010; 340:253–257. [PubMed: 20823702]
14. Michallet M, Ito JI. Approaches to the management of invasive fungal infections in hematologic malignancy and hematopoietic cell transplantation. J Clin Oncol. 2009; 27:3398–3409. [PubMed: 19487382]
15. Baddley JW, Stroud TP, Salzman D, et al. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin Infect Dis. 2001; 32:1319–1324. [PubMed: 11303267]
16. Person AK, Kontoyiannis DP, Alexander BD. Fungal infections in transplant and oncology patients. Hematol Oncol Clin North Am. 2011; 25:193–213. [PubMed: 21236398]
17. Kaufman D. Fungal infections in neonates: update on prevention and treatment. Minerva Ginecol. 2007; 59:311–329. [PubMed: 17576407]
18. Smith JA, Kauffman CA. Recognition and prevention of nosocomial invasive fungal infections in the intensive care unit. Crit Care Med. 2010; 38:S380–S387. [PubMed: 20647796]
19. Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. The Lancet. 2003; 362:1828–1838.
20. Sullivan D, Coleman D. *Candida dubliniensis*: characteristics and identification. J Clin Microbiol. 1998; 36:329–334. [PubMed: 9466736]

21. Zilberberg MD, Shorr AF, Kollef MH. Secular trends in candidemia-related hospitalization in the United States, 2000–2005. *Infect Control Hosp Epidemiol*. 2008; 29:978–980. [PubMed: 18715153]
22. Ferwerda G, Meyer-Wentrup F, Kullberg BJ, et al. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. *Cell Microbiol*. 2008; 10:2058–2066. [PubMed: 18549457]
23. Neely, LA.Audeh, MJ.Blanco, M., et al., inventors. T2 Biosystems, Inc, assignee. NMR systems and methods for the rapid detection of analytes. United States patent US. 8409807 B2. 2012.
24. Cheng IF, Chang HC, Hou D, et al. An integrated dielectrophoretic chip for continuous bioparticle filtering, focusing, sorting, trapping, and detecting. *Biomicrofluidics*. 2007; 1:021503.
25. Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev*. 2003; 67:400–428. [PubMed: 12966142]
26. Whiteway M, Oberholzer U. *Candida* morphogenesis and host-pathogen interactions. *Curr Opin Microbiol*. 2004; 7:350–357. [PubMed: 15358253]
27. Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. *Clin Infect Dis*. 1995; 20:1526–1530. [PubMed: 7548503]
28. Jarvis WR, Martone WJ. Predominant pathogens in hospital infections. *J Antimicrob Chemother*. 1992; 29:19.
29. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in medical intensive care units in the United States. *Crit Care Med*. 1999; 27:887. [PubMed: 10362409]
30. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect Control Hosp Epidemiol*. 2000; 21:510–515. [PubMed: 10968716]
31. Edmond MB, Wallace SE, McClish DK, et al. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis*. 1999; 29:239–244. [PubMed: 10476719]
32. Villamizar RA, Maroto A, Riusa FX, et al. Fast detection of *Salmonella* *Infantis* with carbon nanotube field effect transistors. *Biosens Bioelectron*. 2008; 24:279–283. [PubMed: 18495470]
33. Hall KK, Lyman JA. Updated review of blood culture contamination. *Clin Microbiol Rev*. 2006; 19:788–802. [PubMed: 17041144]
34. Morgan J, Meltzer MI, Plikaytis BD, et al. Excess mortality, hospital stay, and cost due to candidemia: a case-control study using data from population-based candidemia surveillance. *Infect Control*. 2005; 26:540–547.
35. Wey SB, Mori M, Pfaller MA, et al. Hospital-acquired candidemia. The attributable mortality and excess length of stay. *Arch Inter Med*. 1988; 148:2642.
36. Fraser VJ, Jones M, Dunkel J, et al. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. *Clin Infect Dis*. 1992; 15:414–421. [PubMed: 1520786]
37. Leleu G, Aegerter P, Guidet B. Systemic candidiasis in intensive care units: a multicenter, matched-cohort study. *J Critical Care*. 2002; 17:168–175. [PubMed: 12297992]
38. Méan M, Marchetti O, Calandra T. Bench-to-bedside review: *Candida* infections in the intensive care unit. *Crit Care*. 2008; 12:204. [PubMed: 18279532]
39. Saghrouni F, Ben Abdeljelil J, Boukadida J, et al. Molecular methods for strain typing of *Candida albicans*: a review. *J Appl Microbiol*. 2013; 114:1559–1574. [PubMed: 23311504]
40. Jain N, Mathur P, Misra MC, et al. Rapid identification of yeast isolates from clinical specimens in critically ill trauma ICU patients. *J Lab Physicians*. 2012; 4:30. [PubMed: 22923919]
41. Zilberberg MD, Kollef MH, Arnold H, et al. Inappropriate empiric antifungal therapy for candidemia in the ICU and hospital resource utilization: a retrospective cohort study. *BMC Infect Dis*. 2010; 10:150. [PubMed: 20525301]
42. Rex JH, Pfaller MA, Barry AL, et al. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutropenic patients with candidemia. NIAID Mycoses study group and the Candidemia study group. *Antimicrob Agents Chemother*. 1995; 39:40–44. [PubMed: 7695326]

43. Wingard JR, Merz WG, Rinaldi MG, et al. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. *Antimicrob Agents Chemother.* 1993; 37:1847–1849. [PubMed: 8239594]
44. Canuto MM, Rodero FG. Antifungal drug resistance to azoles and polyenes. *Lancet Infect Dis.* 2002; 2:550–563. [PubMed: 12206971]
45. Rangel-Frausto MS, Wiblin T, Blumberg HM, et al. National epidemiology of mycoses survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. *Clin Infect Dis.* 1999; 29:253–258. [PubMed: 10476721]
46. Pfaller MA, Diekema DJ, Jones RN, et al. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *J Clin Microbiol.* 2001; 39:3254–3259. [PubMed: 11526159]
47. Agarwal S, Manchanda V, Verma N, et al. Yeast identification in routine clinical microbiology laboratory and its clinical relevance. *Indian J Med Microbiol.* 2012; 29:172.
48. Larone, DH. Medically important fungi: a guide to identification. 2. New York: Elsevier Science Publishing Co; 1987.
49. Koneman, EW., Roberts, GD. Practical laboratory mycology. Baltimore, MD: Williams & Wilkins; 1985.
50. Adams AEM, Stearns TIM. Fluorescence microscopy methods for yeast. *Methods in cell biology*, volume 31: Vesicular transport, Part A: Vesicular transport. 1989:357.
51. Hageage GJ, Harrington BJ. Use of calcofluor white in clinical mycology. *Lab Med.* 1984; 15:109–112.
52. Perry JL, Miller GR. Quality control slide for potassium hydroxide and cellufluor fungal preparations. *J Clin Microbiol.* 1989; 27:1411–1412. [PubMed: 2754012]
53. Ruiz-Herrera, J. Fungal cell wall: structure, synthesis, and assembly. Boca Raton, Florida: CRC press; 1991.
54. Harrington BJ, Hageage GJ. Calcofluor white: a review of its uses and applications in clinical mycology and parasitology. *Lab Med.* 2003; 34:361–367.
55. Harrington BJ, Williams DL. Rapid, presumptive identification of *Torulopsis (Candida) Glabrata* and *Candida Krusei* using *Calcofluor White*. *Lab Med.* 2007; 38:227–231.
56. Baselski VS, Robison MK, Pifer LW, et al. Rapid detection of *Pneumocystis carinii* in bronchoalveolar lavage samples by using *Cellufluor* staining. *J Clin Microbiol.* 1990; 28:393–394. [PubMed: 1690218]
57. Sautter RL, Kwee HG. Calcofluor white stain for fungi. *Am J Clin Pathol.* 1987; 87:295. [PubMed: 3812366]
58. Lischewski A, Kretschmar M, Hof H, et al. Detection and identification of *Candida* species in experimentally infected tissue and human blood by rRNA-specific fluorescent in situ hybridization. *J Clin Microbiol.* 1997; 35:2943–2948. [PubMed: 9350764]
59. Bisha B, Kim HJ, Brehm-Stecher BF. Improved DNA-FISH for cytometric detection of *Candida* spp. *J Appl Microbiol.* 2011; 110:881–892. [PubMed: 21205104]
60. Rigby S, Procop GW, Haase G, et al. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. *J Clin Microbiol.* 2002; 40:2182–2186. [PubMed: 12037084]
61. Trnovsky J, Merz W, Della-Latta P, et al. Rapid and accurate identification of *Candida albicans* isolates by use of PNA FISHFlow. *J Clin Microbiol.* 2008; 46:1537–1540. [PubMed: 18287325]
62. Gherna M, Merz WG. Identification of *Candida albicans* and *Candida glabrata* within 1.5 hours directly from positive blood culture bottles with a shortened peptide nucleic acid fluorescence in situ hybridization protocol. *J Clin Microbiol.* 2009; 47:247–248. [PubMed: 18987173]
63. Borekci G, Ersoz G, Otag F, et al. Identification of *Candida* species from blood cultures with fluorescent in situ hybridization (FISH), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and conventional methods. *Balkan Med J.* 2010; 27:183–191.

64. Kempf VAJ, Trebesius K, Autenrieth IB. Fluorescent in situ hybridization allows rapid identification of micro-organisms in blood cultures. *J Clin Microbiol.* 2000; 38:830–838. [PubMed: 10655393]
65. Rij, KV. A taxonomic study. New York, NY: Elsevier Science Publishers BV; 1984. The yeasts.
66. Espinel-Ingroff A, Stockman L, Roberts G, et al. Comparison of RapID yeast plus system with API 20C system for identification of common, new, and emerging yeast pathogens. *J Clin Microbiol.* 1998; 36:883–886. [PubMed: 9542903]
67. Davey KG, Chant PM, Downer CS, et al. Evaluation of the AUXACOLOR system, a new method of clinical yeast identification. *J Clin Pathol.* 1995; 48:807–809. [PubMed: 7490311]
68. El-Zaatari M, Pasarell L, McGinnis MR, et al. Evaluation of the updated Vitek yeast identification data base. *J Clin Microbiol.* 1990; 28:1938–1941. [PubMed: 2229376]
69. Fenn JP, Segal H, Barland B, et al. Comparison of updated Vitek yeast biochemical card and API 20C yeast identification systems. *J Clin Microbiol.* 1994; 32:1184–1187. [PubMed: 8051243]
70. Campbell CK, Davey KG, Holmes AD, et al. Comparison of the API *Candida* system with the AUXACOLOR system for identification of common yeast pathogens. *J Clin Microbiol.* 1999; 37:821–823. [PubMed: 9986865]
71. Verweij PE, Breuker IM, Rijs AJ, et al. Comparative study of seven commercial yeast identification systems. *J Clin Pathol.* 1999; 52:271–273. [PubMed: 10474518]
72. Koehler AP, Chu KC, Houang ETS, et al. Simple, reliable, and cost-effective yeast identification scheme for the clinical laboratory. *J Clin Microbiol.* 1999; 37:422–426. [PubMed: 9889232]
73. Willinger B, Manafi M. Evaluation of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida* species. *Mycoses.* 2002; 42:61–65.
74. Adam HJ, Richardson SE, Roscoe M, et al. An implementation strategy for the use of chromogenic media in the rapid, presumptive identification of *Candida* species. *Open Mycol J.* 2010; 4:33–38.
75. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J Clin Microbiol.* 1996; 34:58–61. [PubMed: 8748273]
76. Sivakumar VG, Shankar P, Nalina K, et al. Use of CHROMagar in the differentiation of common species of *Candida*. *Mycopathologia.* 2009; 167:47–49. [PubMed: 18716895]
77. Sudbery P, Gow N, Berman J. The distinct morphogenic states of *Candida albicans*. *Trends Microbiol.* 2004; 12:317–324. [PubMed: 15223059]
78. Whiteway M, Bachewich C. Morphogenesis in *Candida albicans**. *Annu Rev Microbiol.* 2007; 61:529–553. [PubMed: 17506678]
79. Kadosh D, Johnson AD. Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Mol Biol Cell.* 2005; 16:2903–2912. [PubMed: 15814840]
80. Nakamoto S. Germ tube formation of *Candida albicans* in corn meal broth using the non-slip slide glass incubation method. *Yonago Acta Medica.* 1998; 41:65–72.
81. Sahand IH, Maza JL, Eraso E, et al. Evaluation of CHROM-Pal medium for the isolation and direct identification of *Candida dubliniensis* in primary cultures from the oral cavity. *J Med Microbiol.* 2009; 58:1437–1442. [PubMed: 19608690]
82. Horvath LL, Hospenthal DR, Murray CK, et al. Direct isolation of *Candida spp.* from blood cultures on the chromogenic medium CHROMagar *Candida*. *J Clin Microbiol.* 2003; 41:2629–2632. [PubMed: 12791890]
83. BacT/ALERT® MB, BioMerieux. [cited 2015 Dec 26]. Available from: <http://www.biomérieux-usa.com/clinical/bact-alert-3d-healthcare>
84. TREK Diagnostic System. [cited 2015 Feb 04]. Available from: <http://www.trekds.com/products/ver-satrek/media.asp>
85. João Inácio, OFaIS-M. Efficient identification of clinically relevant *Candida* yeast species by use of an assay combining Panfungal loop-mediated isothermal DNA amplification with hybridization to species-specific oligonucleotide probes. *J Clin Microbiol.* 2008; 46:713–720. [PubMed: 18077626]

86. Clancy CJ, Nguyen ML, Cheng S, et al. Immunoglobulin G responses to a panel of *Candida albicans* antigens as accurate and early markers for the presence of systemic candidiasis. *J Clin Microbiol.* 2008; 46:1647–1654. [PubMed: 18322056]
87. Medical microbiology. 7. Philadelphia, PA: Elsevier Saunders; 2013.
88. Reiss E, De Repentigny L, Kuykendall R, et al. Monoclonal antibodies against *Candida tropicalis* mannan: antigen detection by enzyme immunoassay and immunofluorescence. *J Clin Microbiol.* 1986; 24:796–802. [PubMed: 2429989]
89. Philip A, Odabasi Z, Matiuizzi G, et al. Syscan3, a kit for detection of anti-candida antibodies for diagnosis of invasive candidiasis. *J Clin Microbiol.* 2005; 43:4834–4835. [PubMed: 16145152]
90. Mitsutake K, Miyazaki T, Tashiro T, et al. Enolase antigen, mannan antigen, Cand-Tec antigen, and beta-glucan in patients with candidemia. *J Clin Microbiol.* 1996; 34:1918–1921. [PubMed: 8818881]
91. Stickle D, Kaufman L, Blumer SO, et al. Comparison of a newly developed latex agglutination test and an immunodiffusion test in the diagnosis of systemic candidiasis. *Appl Microbiol.* 1972; 23:490–499. [PubMed: 4623278]
92. Marot-Leblond A, Beucher B, David S, et al. Development and evaluation of a rapid latex agglutination test using a monoclonal antibody to identify *Candida dubliniensis* colonies. *J Clin Microbiol.* 2006; 44:138–142. [PubMed: 16390961]
93. Fujita S, Hashimoto T. Detection of serum *Candida* antigens by enzyme-linked immunosorbent assay and a latex agglutination test with anti-*Candida albicans* and anti-*Candida krusei* antibodies. *J Clin Microbiol.* 1992; 30:3132–3137. [PubMed: 1452696]
94. Matthews R, Burnie J. Diagnosis of systemic candidiasis by an enzyme-linked dot immunobinding assay for a circulating immunodominant 47-kilodalton antigen. *J Clin Microbiol.* 1988; 26:459–463. [PubMed: 3281974]
95. Manning-Zweierink M, Maloney C, Mitchell T, et al. Immunoblot analyses of *Candida albicans*-associated antigens and antibodies in human sera. *J Clin Microbiol.* 1986; 23:46–52. [PubMed: 2422198]
96. Kostiala I, Kostiala A, Larinkari U, et al. Antibodies against antigens of *Candida albicans* in patients with fungaemia and bacteraemia, studied by ELISA, precipitation, passive haemagglutination and immunofluorescence techniques. *J Med Microbiol.* 1981; 14:483–492. [PubMed: 6796694]
97. Fujita S, Matsubara F, Matsuda T. Enzyme-linked immunosorbent assay measurement of fluctuations in antibody titer and antigenemia in cancer patients with and without candidiasis. *J Clin Microbiol.* 1986; 23:568–575. [PubMed: 3082925]
98. Laín A, Elguezaal N, Brena S, et al. Diagnosis of invasive candidiasis by enzyme-linked immunosorbent assay using the N-terminal fragment of *Candida albicans* hyphal wall protein 1. *BMC Microbiol.* 2007; 7:35. [PubMed: 17448251]
99. Laín A, Moragues MD, Ruiz JCG, et al. Evaluation of a novel enzyme-linked immunosorbent assay to detect immunoglobulin G antibody to enolase for serodiagnosis of invasive candidiasis. *Clin Vaccine Immunol.* 2007; 14:318–319. [PubMed: 17229884]
100. Van Deventer A, Van Vliet H, Hop W, et al. Diagnostic value of anti-*Candida enolase* antibodies. *J Clin Microbiol.* 1994; 32:17–23. [PubMed: 8126174]
101. Pisa D, Ramos M, Molina S, et al. Evolution of antibody response and fungal antigens in the serum of a patient infected with *Candida famata*. *J Med Microbiol.* 2007; 56:571–578. [PubMed: 17446276]
102. Strockbine NA, Largen MT, Buckley HR. Production and characterization of three monoclonal antibodies to *Candida albicans* proteins. *Infect Immun.* 1984; 43:1012–1018. [PubMed: 6365778]
103. Ponton J, Marot-Leblond A, Ezkurra P, et al. Characterization of *Candida albicans* cell wall antigens with monoclonal antibodies. *Infect Immun.* 1993; 61:4842–4847. [PubMed: 8406886]
104. Schneider J, Moragues D, Martáñez N, et al. Cross-reactivity between *Candida albicans* and human ovarian carcinoma as revealed by monoclonal antibodies PA10F and C6. *Br J Cancer.* 1998; 77:1015. [PubMed: 9528850]

105. Rodriguez MJ, Schneider J, Moragues MD, et al. Cross-reactivity between *Candida albicans* and oral squamous cell carcinoma revealed by monoclonal antibody C7. *Anticancer Res.* 2007; 27:3639–3643. [PubMed: 17972529]
106. Kaufman, L., Kovacs, JA., Reiss, E. Manual of clinical laboratory immunology. Washington, DC: American Society for Microbiology; 1997. Clinical immunomycology; p. 575-583.
107. Marcilla A, Monteagudo C, Mormeneo S, et al. Monoclonal antibody 3H8: a useful tool in the diagnosis of candidiasis. *Microbiology.* 1999; 145:695–701. [PubMed: 10217504]
108. Yeo SF, Wong B. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin Microbiol Rev.* 2002; 15:465–484. [PubMed: 12097252]
109. Jarvensivu A, Hietanen J, Rautemaa R, et al. *Candida* yeasts in chronic periodontitis tissues and subgingival microbial biofilms in vivo. *Oral Dis.* 2004; 10:106–112. [PubMed: 14996281]
110. Bikandi J, San Millán R, Moragues MD, et al. Rapid identification of *Candida dubliniensis* by indirect immunofluorescence based on differential localization of antigens on *C. dubliniensis* blastospores and *Candida albicans* germ tubes. *J Clin Microbiol.* 1998; 36:2428–2433. [PubMed: 9705368]
111. Jarvensivu A, Rautemaa R, Sorsa T, et al. Specificity of the monoclonal antibody 3H8 in the immunohistochemical identification of *Candida* species. *Oral Dis.* 2006; 12:428–433. [PubMed: 16792731]
112. Chang CM, Chang WH, Wang CH, et al. Nucleic acid amplification using microfluidic systems. *Lab Chip.* 2013; 13:1225–1242. [PubMed: 23407669]
113. Simon D, Atkins IMC. Fungal molecular diagnostics: a mini review. *J Appl Genet.* 2004; 45:3–15. [PubMed: 14960763]
114. Arvanitis M, Anagnostou T, Fuchs BB, et al. Molecular and nonmolecular diagnostic methods for invasive fungal infections. *Clin Microbiol Rev.* 2014; 27:490–526. [PubMed: 24982319]
115. Zourob, M., Elwary, S., Turner, AP. Principles of bacterial detection: biosensors, recognition receptors and microsystems. New York (NY): Springer Science & Business Media; 2008.
116. Campa D, Tavanti A, Gemignani F, et al. DNA micro-array based on arrayed-primer extension technique for identification of pathogenic fungi responsible for invasive and superficial mycoses. *J Clin Microbiol.* 2008; 46:909–915. [PubMed: 18160452]
117. Spiess B, Seifarth W, Hummel M, et al. DNA micro-array-based detection and identification of fungal pathogens in clinical samples from neutropenic patients. *J Clin Microbiol.* 2007; 45:3743–3753. [PubMed: 17715373]
118. Leinberger DM, Schumacher U, Autenrieth IB, et al. Development of a DNA microarray for detection and identification of fungal pathogens involved in invasive mycoses. *J Clin Microbiol.* 2005; 43:4943–4953. [PubMed: 16207946]
119. Reiss E, Tanaka K, Bruker G, et al. Molecular diagnosis and epidemiology of fungal infections. *Med Mycol.* 1997; 36:249–257.
120. Mullis K, Faloona F, Scharf S, et al. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symp Quant Biol.* 1986; 51:263–273. [PubMed: 3472723]
121. Kan VL. Polymerase chain reaction for the diagnosis of candidemia. *J Infect Dis.* 1993; 168:779–783. [PubMed: 8354923]
122. Jordan JA. PCR identification of four medically important *Candida* species by using a single primer pair. *J Clin Microbiol.* 1994; 32:2962–2967. [PubMed: 7883883]
123. Einsele H, Hebart H, Roller G, et al. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin Microbiol.* 1997; 35:1353–1360. [PubMed: 9163443]
124. Burnie JP, Golband N, Matthews RC. Semiquantitative polymerase chain reaction enzyme immunoassay for diagnosis of disseminated candidiasis. *Eur J Clin Microbiol Dis.* 1997; 16:346–350.
125. Widjoatmodjo MN, Borst A, Schukkink RAF, et al. Nucleic acid sequence-based amplification (NASBA) detection of medically important *Candida* species. *J Microbiol Meth.* 1999; 38:81–90.
126. Zhou X, Kong F, Sorrell TC, et al. Practical method for detection and identification of *Candida*, *Aspergillus*, and *Scedosporium* spp. by use of rolling-circle amplification. *J Clin Microbiol.* 2008; 46:2423–2427. [PubMed: 18495860]

127. Prasad JK, Feller I, Thomson PD. A ten-year review of *Candida sepsis* and mortality in burn patients. *Surgery*. 1987; 101:213–216. [PubMed: 3810491]
128. Kurita H, Kamata T, Zhao C, et al. Usefulness of a commercial enzyme-linked immunosorbent assay kit for *Candida mannan* antigen for detecting *Candida* in oral rinse solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009; 107:531–534. [PubMed: 19327638]
129. Lehmann LE, Hunfeld K-P, Emrich T, et al. A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. *Med Microbiol Immunol*. 2008; 197:313–324. [PubMed: 18008085]
130. Ilyas A, Asghar W, Allen PB, et al. Electrical detection of cancer biomarker using aptamers with nanogap break-junctions. *Nanotechnology*. 2012; 23:275502. [PubMed: 22706642]
131. Wan Y, Tan J, Asghar W, et al. Velocity effect on aptamer-based circulating tumor cell isolation in microfluidic devices. *J Phys Chem B*. 2011; 115:13891–13896. [PubMed: 22029250]
132. Pulcrano G, Iula DV, Vollaro A, et al. Rapid and reliable MALDI-TOF mass spectrometry identification of *Candida non-albicans* isolates from bloodstream infections. *J Microbiol Methods*. 2013; 94:262–266. [PubMed: 23845229]
133. Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. *Nature*. 2014; 507:181–189. [PubMed: 24622198]
134. Asghar W, El Assal R, Shafiee H, et al. Preserving human cells for regenerative, reproductive, and transfusion medicine. *Biotechnol J*. 2014; 9:895–903. [PubMed: 24995723]
135. Asghar W, El Assal R, Shafiee H, et al. Engineering cancer microenvironments for in vitro 3-D tumor models. *Mater Today*. 2015; 18:539–553.
136. Asghar W, Ramachandran PP, Adewumi A, et al. Rapid nanomanufacturing of metallic break junctions using focused ion beam scratching and electromigration. *J Manufact Sci Eng*. 2010; 132:030911.
137. Asghar W, Velasco V, Kingsley JL, et al. Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. *Adv Healthc Mater*. 2014; 3:1671–1679. [PubMed: 24753434]
138. Asghar W, Wan Y, Ilyas A, et al. Electrical fingerprinting, 3D profiling and detection of tumor cells with solid-state micropores. *Lab on a Chip*. 2012; 12:2345–2352. [PubMed: 22549275]
139. Ilyas A, Asghar W, Ahmed S, et al. Electrophysiological analysis of biopsy samples using elasticity as an inherent cell marker for cancer detection. *Anal Methods*. 2014; 6:7166–7174.
140. Ilyas A, Asghar W, Kim YT, et al. Parallel recognition of cancer cells using an addressable array of solid-state micropores. *Biosens Bioelectron*. 2014; 62:343–349. [PubMed: 25038540]
141. Wang L, Asghar W, Demirci U, et al. Nanostructured substrates for isolation of circulating tumor cells. *Nano today*. 2013; 8:374–387.
142. Asghar W, Islam M, Wadajkar AS, et al. PLGA micro-and nanoparticles loaded into gelatin scaffold for controlled drug release. *IEEE Trans Nanotechnol*. 2012; 11:546–553.
143. Ramachandran A, Liu Y, Asghar W, et al. Characterization of DNA-nanopore interactions by molecular dynamics. *Am J Biomed Sci*. 2009; 1:344–351.
144. Vidyala SD, Asghar W, Iqbal SM. Porous organic nano-layers for coating of solid-state devices. *J Nanobiotechnol*. 2011; 9:18.
145. Ilyas A, Islam M, Asghar W, et al. Salt-leaching synthesis of porous PLGA nanoparticles. *IEEE Trans Nanotechnol*. 2013; 12:1082–1088.
146. Ilyas, A., Asghar, W., Billo, J., et al. From molecular electronics to proteonics: break junctions for biomarker detection. *Life Science Systems and Applications Workshop (LiSSA)*, 2011 IEEE/NIH; 2011; Bethesda, MD: IEEE;
147. Sachse S, Straube E, Lehmann M, et al. Truncated human cytidylate-phosphatedeoxyguanylate-binding protein for improved nucleic acid amplification technique-based detection of bacterial species in human samples. *J Clin Microbiol*. 2009; 47:1050–1057. [PubMed: 19193840]
148. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. *Clin Infect Dis*. 2015; 60:892–899. [PubMed: 25586686]
149. Baeumner SJ. Microfluidic isolation of nucleic acids. *Angew Chem Int Ed*. 2014; 53:2–16.

150. Cai D, Xiao M, Xu P, et al. An integrated microfluidic device utilizing dielectrophoresis and multiplex array PCR for point-of-care detection of pathogens. *Lab Chip*. 2014; 14:3917–3924. [PubMed: 25082458]
151. Boles DJ, Benton JL, Siew GJ, et al. Droplet-based pyrosequencing using digital microfluidics. *Anal Chem*. 2011; 83:8439–8447. [PubMed: 21932784]
152. Schell WA, Benton JL, Smith PB, et al. Evaluation of a digital microfluidic real-time PCR platform to detect DNA of *Candida albicans* in blood. *Eur J Microbiol Infect Dis*. 2012; 31:2237–2245.
153. Schumacher S, Nestler J, Otto T, et al. Highly-integrated lab-on-chip system for point-of-care multiparameter analysis. *Lab Chip*. 2012; 12:464–473. [PubMed: 22038328]
154. Lee H, Sun E, Ham D, et al. Chip-NMR biosensor for detection and molecular analysis of cells. *Nat Med*. 2008; 14:869–874. [PubMed: 18607350]
155. Gervais L, de Rooij N, Delamarche E. Microfluidic chips for point-of-care immunodiagnostics. *Adv Mater Weinheim*. 2011; 23:H151–HH76. [PubMed: 21567479]
156. Shafiee H, Sano MB, Henslee EA, et al. Selective isolation of live/dead cells using contactless dielectrophoresis (cDEP). *Lab on a Chip*. 2010; 10:438–445. [PubMed: 20126683]
157. Fujita SI, Takamura T, Nagahara M, et al. Evaluation of a newly developed down-flow immunoassay for detection of serum mannan antigens in patients with candidaemia. *J Med Microbiol*. 2006; 55:537–543. [PubMed: 16585640]
158. Sogawa K, Watanabe M, Sato K, et al. Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms. *Anal Bioanal Chem*. 2011; 400:1905–1911. [PubMed: 21442367]
159. Yetisen AK, Akram MS, Lowe CR. Paper-based micro-fluidic point-of-care diagnostic devices. *Lab Chip*. 2013; 13:2210. [PubMed: 23652632]
160. Shafiee H, Asghar W, Inci F, et al. Paper and flexible substrates as materials for biosensing platforms to detect multiple biotargets. *Scientific Rep*. 2015; 5:8719.
161. Sista R, Hua Z, Thwar P, et al. Development of a digital microfluidic platform for point of care testing. *Lab Chip*. 2008; 8:2091–2104. [PubMed: 19023472]
162. Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis*. 2003; 3:685–702. [PubMed: 14592598]
163. Safavieh M, Kanakasabapathy MK, Tarlan F, et al. Emerging loop-mediated isothermal amplification-based microchip and microdevice technologies for nucleic acid detection. *ACS Biomater Sci Eng*. 2016; 3:278–294.
164. Asghar W, Yuksekkaya M, Shafiee H, et al. Engineering long shelf life multi-layer biologically active surfaces on microfluidic devices for point of care applications. *Sci Rep*. 2016; 6:21163. [PubMed: 26883474]

Figure 1.
Schematic of conventional and nonconventional methods for *Candida* detection.

Figure 2.

CFW staining of *Candida spp.* (A) Fluorescent image of *C. albicans* after germ tube production. The long tubes are pseudohyphae, the characteristic morphology of *C. albicans*. (B) UV fluorescent image of *C. tropicalis* having oval shape and bright CFW staining over whole cell. (C) Fluorescent image of *C. torulopsis glabrata*, very slight CFW staining at the ends of oval cells. (D) Fluorescent image of *C. krusei*, slight CFW staining at the end of elongated cells. (E) Fluorescent image of *C. parapsilosis*, dull fluorescence over whole elongated cells. Reproduced with the permission from.[54]

Figure 3.

Appearance of colonies on CHROMagar (Left: 1× magnification) and Corn meal-Tween 80 agar (Right: 400× magnification) after 48 h of incubation. (A) *C. albicans*. (B) *C. dubliniensis*. (C) *C. tropicalis*. (D) *T. beigeli*. (E) *C. krusei*. (F) *C. glabrata*. (G) *C. parapsilosis*. (H) *C. neoformans*. Reproduced with the permission from.[72]

Figure 4.

Detection and comparison of colonies of *Candida* on blood agar and CHROMagar. Growth of *C. albicans* (A and B), *C. krusei* (C and D), *C. tropicalis* (E and F), and *C. glabrata* (G and H) on CHROMagar, with direct isolation from blood culture bottled medium (A, C, E and G) compared to that seen with inoculation from culture on standard solid medium (B, D, F and H). Reproduced with the permission from.[82]

Figure 5.

Microchips for *Candida* detection. (A) Integration of SlipChip and Dielectrophoresis (DEP) for fungal separation and PCR amplification. (a) Schematic workflow process of fungal and pathogen detections from blood sample taken from patient using multiplex PCR. (b) Photograph of the chip loaded with food dye. (c–f) Water diluted sample, containing blood were mixed properly with the blood cell ghost (BCGs), fungal and pathogens and was injected into the channels. (g–k) The top plate consists of PCR mixture and the bottom plate has the pathogens and fungal *spp.* that are separated by the DEP and the contaminant is washed away. The bottom plate is slip back to be mixed with the captured pathogens and then initiates PCR reaction followed by fluorescent detection. (B) (a–c) Image of the advanced liquid logic chip, its reader and fully assembled microwell cartridge showing location of sample, reagent wells, and other solutions. (d) Schematic of the on/off magnet to merge the droplets, extend the droplet into the column, and split two droplets. (e) The droplet logic has been used for extraction, washing, purification and elution of DNA from beads over the magnet. (C) Fraunhofer iVD platform. (a–b) Schematic and image of the Fraunhofer iVD cartridge consists of the total internal reflectance fluorescence (TRIF) optical sensor as well as electrochemical sensor. The cartridge was fabricated based on half microtiter plate (credit card size) and has various micropumps and eight reservoirs for sample and reagent storage. The amplified product is pumped to the TRIF sensor or electrochemical sensor for further analysis. (c) Images of TRIF sensor for various pathogen; *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albican*. Reproduced with the permission from [150,153,161].

Figure 6.

Integrated DEP and SERS chip for *Candida* detection. (A) Microimage of integrated DEP chip consists of several sections of filtering, focusing, sorting and separation. (B) Schematic representation of 3D electrode configuration for the separation of different particles including *C. albicans* which is detected by SERS. Reproduced with the permission from [24].

Table 1

Candida infections in humans: Species related clinical features [162].

Species	Related clinical features
<i>C. albicans</i>	Mucocutaneous infections: oropharyngeal, esophagitis, vaginitis; Deep-seated infections: pyelonephritis, peritonitis; Hematogenous infections: candidemia, meningitis, hepatosplenic
<i>C. parapsilosis</i>	Candidemia, deep infections associated with implanted devices, infections related to contaminated solutions responsible for most candidemia among neonates
<i>C. tropicalis</i>	Candidemia and systemic candidiasis in immunosuppressed patients Candidemia may be associated with severe myalgia and myositis
<i>C. glabrata</i>	Systemic candidiasis, candidemia, urinary tract infections
<i>C. krusei</i>	Candidemia, endophthalmitis, diarrhea in newborns

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Table 2PNA FISH probes for specific *Candida* detection.[63,64]

FISH probe name	Oligonucleotide sequence (5'-3')	Specificity	rRNA position^a
PF2	CTC TGG CTT CAC CCT ATT C	All yeast	18/618
Caal	GCC AAG GCT TAT ACT CGC T	<i>C. albicans</i>	18/1249
Cagl	CCG CCA AGC CAC AAG GAC T	<i>C. glabrata</i>	18/651
Cpara	CCT GGT TCG CCA AAA AGG C	<i>C. parapsilosis</i>	18/651
Ckrus	GAT TCT CGG CCC CAT GGG	<i>C. Krusei</i>	18/1433
Non Eub	ACT CCT ACG GGA GGC AGC	None	None

^aFirst number represents type of rRNA (18S); second number represents the position of first base of target sequence.

All probes were labeled with FITC or CY3.

Table 3

Commercially available platforms for *Candida* detection.

Product name	Cost/test	Sensitivity	Specificity	References
SeptiFast	100–180\$	75%	92%	[130]
SepsiTest	83.5 \$	87.0%	85.8%	[131]
VYOO	TBD	Range of 38.0–60.0%	77%	[147]
Magicplex sepsis real-time test	TBD	65.0%	92.0%,	[133]
T2Dx	TBD	91.1%	99.4%	[156]
T2MR	TBD	91.1%	99.4%	[148]
Unimedi <i>Candida</i>	TBD	82%	96%	[128,157]
Bruker Autoflex III MALDI-TOF	TBD	91.7%	97%	[132,158]