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The Clinical Validity of the Candida MycoDART conducted at MycoDART Inc.

And

The Clinical Indications for Testing of and Treating of Candida in Candidemia

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Why is clinical testing using MycoDART-PCR Candida so important to patients and the physicians who treat them?

Invasive fungal infections can cause disability and death. Patients can get fungal infections while receiving care for something else in a healthcare facility. For example, the fungus *Candida* is a leading cause of healthcare-associated bloodstream infection in US hospitals (1). These infections are also costly for patients and healthcare facilities. Each case of *Candida* bloodstream infection (also known as candidemia) is estimated to result in an additional 3 to 13 days of hospitalization and $6,000 to $29,000 in healthcare costs.(2) What's also concerning is when we find antifungal resistance in some types of *Candida*, which makes them harder to treat.

Yeasts of the genus *Candida* represent the most prevalent group of fungal pathogens in humans. Predominantly in patients with impaired immune response or upon trauma, *Candida* species can turn from endogenous colonizers to invasive pathogens. This is of particular significance in hematological or transplant patients. The most prominent members of the family are *Candida* (*C.*) *albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei,* accounting for >90% of invasive candidiasis or candidemia cases [3,4]. Some other important, yet infrequent, species are, for example, *C. guilliermondii*, *C. orthopsilosis*, *C. inconspicua, C. nivariensis*, and multi-resistant *C. auris* [5,6]. So far these species only constitute a small number of all invasive *Candida* infections [7], but these rare species might become increasingly important in the future, particularly with respect to resistance to antifungals [8]. Globally, the burden of invasive *Candida* infections, just like the burden of other invasive fungal infections, is rising [9–11]. Apart from the high level of morbidity or mortality associated with these infections, this is also reflected by the high healthcare costs attributed to fungal disease [12]. The highest risk of nosocomial infections is observed for patients above the age of 65 with prolonged hospital stays [13]. Candidemia hence poses a major threat to patients in intensive care units (ICUs).

For a long time, blood culture (BC) has been the gold standard of blood stream infection diagnostics, despite its drawbacks of low sensitivity (approx. 50%) and long turnaround times (3–5 days) [14]. However, experts have questioned the reliability of blood culture as the gold standard with respect to fungal infection and the evaluation of clinical test parameters (sensitivity and specificity), particularly in comparison to non-culture based assays such as real-time PCR [15]. As a consequence, quick and reliable diagnosis will be the key to optimized therapy and reduced morbidity and mortality in Candidemia [16,17].

The MycoDART-PCR-Candida system is a molecular assay which can test whole blood, plasma, or serum for the presence of DNA copies of different Candida species.

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The organisms that can be rapidly tested for are: *Candida* (*C.*) *albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei, and C. auris.*

Because these organism have and can develop a resistance to antifungals, it is extremely important that the clinician knows which organism is causing the trouble in the patient (Candemia or Candidiasis). Thus, upon evaluating the patient, the doctor can order a MycoDART-PCR- Candida test which can be evaluated in less than 24 hours. The results can change the morbidity and/or mortality of the patient as well as lower hospital stay costs.

What is the Clinical Validity and the clinical utility of the test?

Clinical validity refers to how well the the organism being analyzed for is related to the presence, absence, or risk of a specific disease. **Clinical utility** refers to whether the test can provide information about diagnosis, treatment, management, or prevention of the disease, which in this case is Candidemia (Candida in the blood) that will be helpful to the clinican.

In order for a test to be clinical valid, it must undergo very stringent testing in its validation procedures to determine sensitivity and specificity of the test. In regards to MycoDART-PCR- Candida, the sensitivity is 95% and specificity of 100% for the intended target organism. (See Attachment #1, Candida Test Specifications for MycoDART)

What type of doctor should see patients who are concerned about mold exposure?

Patients with Candidemia and/or Candidiasis are being treated and followed by surgeons, pulmonologists, gastroenterologists, internists, and infectious disease physicians. These physicians have been trained in their subspecialties to recognize and treat such diseases as Candidemia and Candidiasis.

What are the clinical indications for testing and possible treatment?

Candidemia is the most common fungal bloodstream infection and is the fourth most common all-type bloodstream infection seen in the ICU (18). The incidence of Candida in the bloodstream occurs with the elderly and the very young. These populations have the highest risk of any population suffering from Candidemia (18).

The most common risk factors include critical illness and prolonged ICU stays. The presence of a central venous catheter, antibiotic exposure, abdominal surgery, malignancy (solid organ or hematologic), organ transplant recipients and total parenteral nutrition. (19, 20).

What are the treatment modalities for fungal disease (Candidemia and/or Candidiasis).

Early antifungal therapy is considered in critically ill patients with the risk factors mentioned above. The antifungals of choice are listed in Attachment #2. The treatment of choice, once a diagnosis of Candidemia is identified is determined by the clinician. The identification of the Candida species is extremely useful to deter further complications in the patient as well as decrease hospital stay. Most physicians believe that the answer to decreased morbidity and mortality is to reverse the state of immunosuppression brought on by many factors.

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Attachment #1. MycoDART Panel Test Specifications

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MycoDART Panel Test Specifications

Clinical Background

Invasive fungal infections, such as Candidiasis remain leading causes of morbidity and mortality in adult and pediatric hospitalized patients, with resultant extended hospital stays, increased medical costs and poor prognosis. Hospitalized patients with the highest risk for contacting Invasive Candidiasis include those who: have a central venous catheter; are in the ICU; are immunocompromised; have neutropenia; have kidney failure or are on hemodialysis; have a recent surgery; or use broad-spectrum antibiotics to name a few .

Test Description:

The MycoDART-PCR™ Candida Panel utilizes a dual amplification real-time PCR procedure for the detection of six Candida species. The initial reaction utilizes specific oligonucleotides that are used to amplify the region of interest in the targeted genome. The amplicon generated from this process is then used as the template along with species-specific probes and primers for real-time PCR assay. The MYCODART-PCR™ process substantially improves assay analytical sensitivity and specificity compared to the traditional real-time PCR alone, resulting in an assay that is able to detect early and low amounts of target DNA in clinical blood samples.

References:

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Candida Panel

Assay Method: Dual Amplification Realtime PCR

Candida Targets: C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, C. auris, and Process Control Geotrichum candidum

PCR Amplification Efficiency

Amplification efficiency was evaluated by obtaining concentrated DNA for all assays from ZeptoMetrix and serially diluted ten-fold to produce dilutions of 1/10, 1/100, 1/1000, and 1/10000. The dilutions for each assay were run on each amplification of the duel amplification system generating two standard curves for each assay. All assays demonstrated a high amplification efficiency. Amplification Efficiency was calculated using the following equation:

$$\text{Efficiency} = -1+10^{\wedge(-1/\text{slope})} * 100\%$$

	CA	CG	CK	CP	CT	Cau	Geo
Initial PCR	93.8%	105.2%	98.7%	90.5%	84.3%	82.8%	n/a
Final PCR	98.9%	93.8%	104.6%	105.7%	98.2%	93.7%	99.6%

Limit of Detection (LOD) and Cutoff

Assay LOD and Cutoff was determined for all assays by obtaining concentrated DNA from ZeptoMetrix and diluting the DNA down to 1000 copies per mL in whole blood. DNA was further serially diluted ten-fold down to 1-copy per mL. LOD samples were processed in seven replicates with LOD determined at >95% detection and Assay Cutoff determined at >50% detection for each assay. See Results in table below:

	Copies/ml in Whole Blood						
	CA	CG	CK	CP	CT	Cau	Geo
LOD (>50% detection)	1	100	100	100	100	10	10
>95% detection	100	100	1000	2500	1000	10	10

Sensitivity and Reproducibility

Assay Sensitivity and reproducibility was determined by obtaining concentrated DNA from ZeptoMetrix and producing a ten-fold serial dilutions. All prepared samples were processed in 10 replicates. All assays demonstrate high sensitivity, with 100% detection of all replicates. Inter-run cycle threshold values after final amplification displayed high precision with less than 5% CV in all assays. Intra-run cycle threshold values also showed high precision with less than 16% CV in all assays.

Specificity

Assay Specificity was determined by obtaining concentrated DNA ZeptoMetrix and producing a ten-fold serial dilutions. All prepared sample were run with all assays in triplicate. All six assays shows 100% specificity for their intended target.

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Attachment #2
Antifungals used for systemic and local fungal infections. *

Amphotericin B - can be used iv for systemic infections or as a nasal spray (diluted) with EDTA for biofilm disruption and treatment of focal sinus infections

Caspofungin - iv only for candidemia and mucosal candidiasis

Fluconazole - iv or oral (used primarily for Coccidiomycosis and Cryptococcal meningitis)

Itraconazole - oral, can be used for thrush, esophageal candidiasis and prophylaxis for invasive aspergillosis and candidiasis

Posaconazole - oral candidiasis; used in oral candidiasis refractory to itraconazole

Voriconazole. - iv or oral. For Candidiasis or Fusariosis.

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