A Novel Diagnostic Test for Invasive Fungal Infections

Michelle E. Matzko, MD, PhD[1,2]; Melanie Martinsen, BS[2]; Poppy Sephton-Clark, PhD[2]; Christina Cuomo, PhD[2]; Roby Bhattacharyya MD, PhD[1,2]

Mass General Brigham [1]

BROAD INSTITUTE [2]

@michellematzko mmatzkom@broadinstitute.org

Citations and Audio Script: Please scan QR code ^

Overview

Recognizing the critical need for better diagnostics for deadly invasive fungal infections, we have developed a fast and accurate rRNA-based test for identifying fungi from clinical samples within 4 hours.

Background

Developing new tests for invasive fungal infections is imperative for several key reasons (Fig 1):

- Rise in infections & new pathogens
- Slow, low-yield gold standard (culture)
- Limited treatments
- High mortality

We piloted an approach developed previously in our lab for bacteria which combines rapid identification (ID) + antimicrobial susceptibility testing (AST)

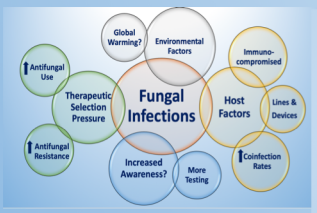
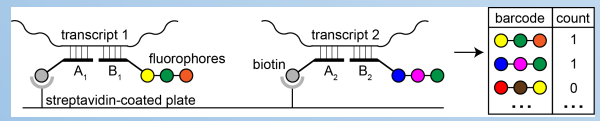


Figure 1 (above). *Numerous factors are responsible for more prevalent and resistant fungal infections.*

Figure 2 (left, top). *Two probes for each species hybridize to ribosomal rNA (albicans shown).*

Figure 3 (left, bottom). *Via Nanostring, probes bind target rRNA, a biotin molecule anchors the complex to a chip, unique fluorophores signal abundance of a target candida species.*



Methods

We employed NanoString, a multiplex RNA read technology that works on crude lysate within 4 hours to detect unique ribosomal RNA transcripts *(Figures 2 & 3)*. We sampled *candida* strains obtained from the CDC's AR bank + our clinical micro lab *via*:

- 19 rRNA probes to detect 10 unique *candida* species
- pan18S and 23S probes detect fungi nonspecifically

Results

Our probes detect the expected *candida* species with high accuracy and low cross-reactivity *(Figure 4)*. R^2 correlation matrix and heatmaps show that even closely called species (*tropicalis, parapsilosis*) can be distinguished *(Figures 5 and 6)*.

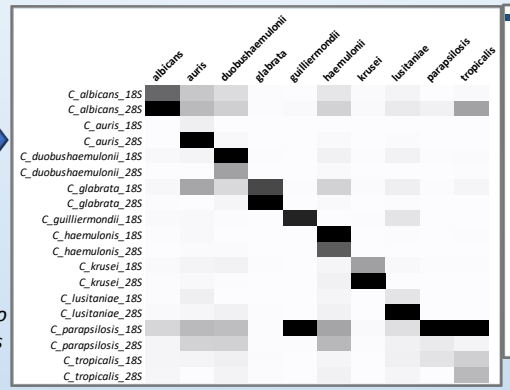
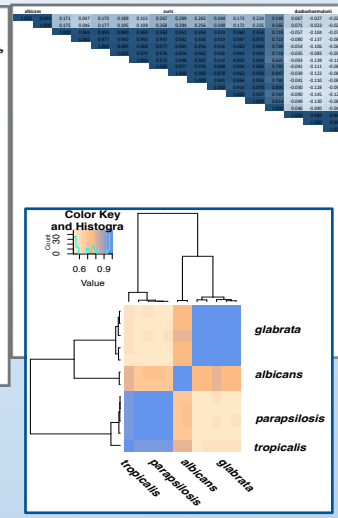


Figure 4 (above). *rRNA probes quantitatively detect their targeted candida species on Nanostring with excellent differentiation between closely-related species. Other than tropicalis, each species was best signaled by its own probes.*



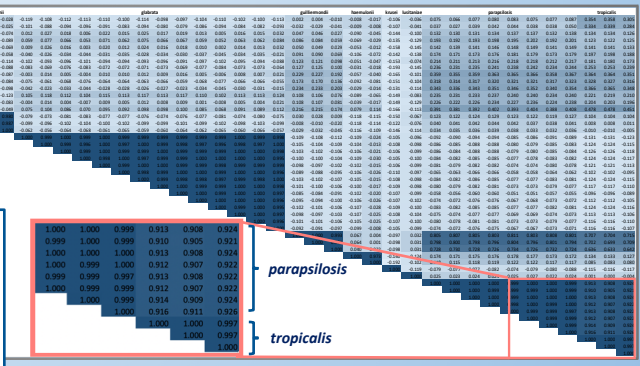


Figure 5 (above). *R2 correlation matrix demonstrates >99% correlation between yeast species. Because the overall pattern of uptake is unique, even tropicalis and parapsolisis can be distinguished by correlation (99% vs 92% shown in red inset).*
Figure 6 (left). *Heatmap demonstrates probable phylogenetic distances calculated based on pattern response to probe binding. Differences between tropicalis and parapsolisis are again seen.*

Results

Serial dilutions for three *candida* species show our test can detect accurately down to a single yeast cell (Fig. 7). *Tropicalis*, our weakest performing probe, can detect around 10 cells using a conservative limit of detection (LOD).

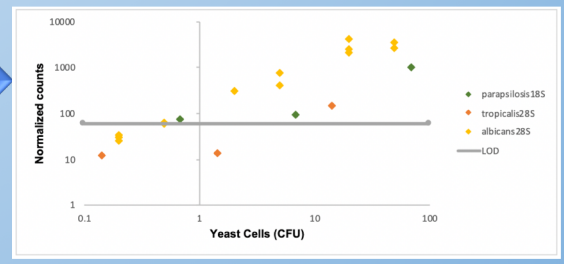


Figure 7. *Serial dilutions of candida species demonstrated Nanostring can detect RNA from a single yeast cell for all but tropicalis. LOD is estimated conservatively by sum of mean background signal from media + one standard deviation.*

Next Steps

Here we show rapid and accurate ID of *candida* species down to single yeast cells on Nanostring. We will next test for similar ID accuracy with other fungal classes, on primary clinical samples, and combine simultaneous antifungal susceptibility testing.