Efficacious drug testing for fungal infections using Fountain Flow Cytometry

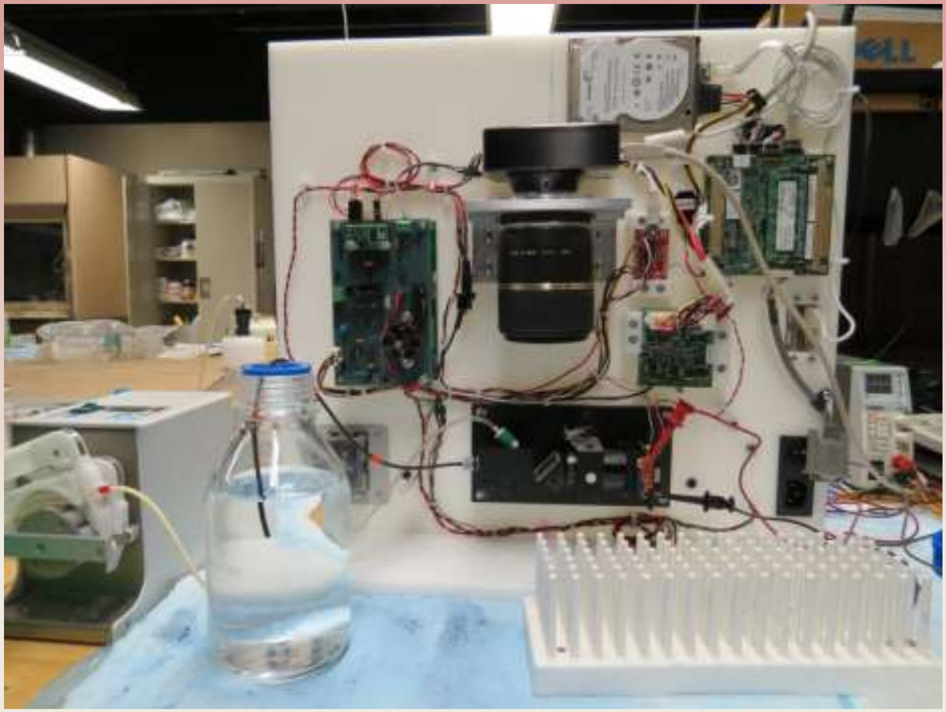
Andy Jones
Dr. Paul Johnson

Abstract

- Every year, billions of people around the world contract fungal diseases, and millions of these infections end in death. The most common fungal infection is from *Candida albicans*, and causes high mortality rates in immunocompromised patients. There are multiple commonly accepted methods for detecting fungal drug resistance, and all of these have been used for Candida spp, but they fall short at rapidly determining the choice of antifungal for septic patients. Dr. Paul Johnson at the University of Wyoming has developed an instrument, the Fountain Flow Cytometer (FFC), which is used for rapid detection and counting of microorganisms in an aqueous environment. I have developed a nutrient broth to grow large concentrations (>10,000) of yeast over an 4 hour time period. This has proven that we can get a measurable increase in yeast to prove as a control group. The final goal of this project is to develop a procedure for antibiotic resistance testing of blood samples.

Background

- Fungal infections are common
- *Candida albicans*
- Current antibiotic resistance methods are lacking
- Fountain Flow Cytometry (FFC)



Etest

http://www.biomerieux-diagnostics.com/upload/Etest2530.jpg

Purpose

- The purpose of this project is to develop a rapid method for determining whether a fungal infection in the blood is resistant to an antifungal agent.

Project Plan

- Stain blood samples inoculated with *C. albicans* with a chitin stain
- Enumerate with FFC
- Add growth media and an antibiotic to blood, incubate for 8 hours
- Stain again and enumerate with FFC

Results

- Developed the media to add for rapid *C. albicans* growth

Media composition	Initial count	8 hour count
YM agar	45	21
5g/L Bacto Peptone, 5g/L D-glucose	68	36
10g/L Bacto Peptone, 10g/L D-glucose	73	42
10g/L Bacto Peptone, 10g/L D-glucose, .1M NaCL	53	223

Next steps

- Repeat with the effective media, including thiabendazole, an antibiotic
- Transform yeast to be thiabendazole resistant
- Grow these resistant yeast with and without thiabendazole
- Move to smaller concentrations with FFC

Advantages and Drawbacks

- Advantages
  - Simple process
  - Much shorter than other methods
  - Can provide Minimum Inhibitory Concentration
  - Starting concentrations can be 10-100 cells/ml (estimated)
- Drawbacks
  - Requires a Fountain Flow Cytometer

Questions?