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ASPERGILLUS TECHNOLOGY CONSORTIUM

Diagnostics of Invasive Aspergillosis: From Experimental Models to Clinical Evaluation.

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INVASIVE ASPERGILLOSIS ANIMAL MODELS

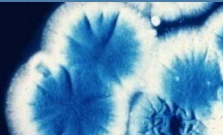
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DNA Extraction Methods

- Purpose to determine the optimal protocol for storing whole blood samples
- Freezing aliquots of whole blood versus storing whole blood in lysis buffer

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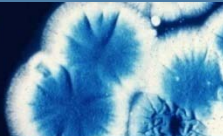
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Lessons Learned From Viral Load Tests

- Storage of whole blood by freezing
 - Defrost samples to use, cells are lysed releasing nucleases that can destroy nucleic acid
- Storage of whole blood in lysis buffer
 - Nucleases are inactivated, stabilizing NA
 - NA is stable at -80°C for years

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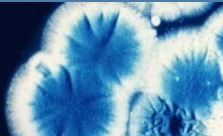
Lessons Learned From Viral Load Tests

- Lysis buffer may limit methods that can be used to extract NA from the blood specimens
 - Dilutes the specimen
- RNA Later
 - Stabilizes the nucleic acid
 - Remove the preservative prior to processing
 - Loose the plasma fraction, impact sensitivity

Lessons Learned From Viral Load Tests

- The large amount of human DNA in whole blood reduces the analytical sensitivity of molecular assays
- A target capture method would eliminate this problem, but not uniformly performed
 - Purify only target specific NA

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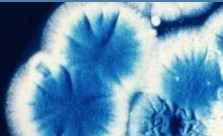
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Impact of Total Human DNA

- Different volumes of blood (1.0, 0.5, 0.25 mL) added to lysis buffer (4-5 ml)
- Spike with cultured *Candida albicans*
- Bead beat, then extraction of NA by Easy Mag (EM) and MagNa Pure Compact
- Conventional PCR for detection of *Candida sp*
 - Marshall Lyon's test

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Impact of Total Human DNA

- 3 mLs sample loaded on EasyMag
 - About 60% of total sample
- 1mL sample loaded on Compact
 - About 20% of total sample

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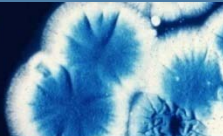
EZ MAG

MP COMPACT

1:30 CANDIDA				1:50 CANDIDA				1:30 CANDIDA				1:50 CANDIDA				PCR CONTROL	
1ML	0.5	0.25	NO BLD	1 ML	0.5	0.25	NO BLD	1 ML	0.5	0.25	NO BLD	1 ML	0.5	0.25	NO BLD	PC	NC
NO BAND	2+ POS	2+ POS	1+ POS	NO BAND	1+ POS	2+ POS	1+ POS	2+ POS	2+ POS	2+ POS	POS	2+ POS	2+ POS	2+ POS	POS	3+ POS	



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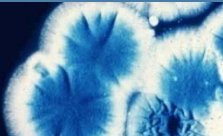


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Lessons Learned From Viral Load Tests

- Increasing input volume of extracted specimen can improve sensitivity but that will increase total human DNA
- Challenges of trying to detect very low concentrations of pathogen NA in a whole blood specimen

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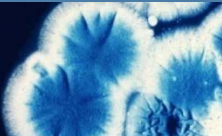
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Is storage of whole blood in lysis buffer feasible?

- Use animal models to determine if aspergillus DNA can be detected in whole blood samples stored in lysis buffer
- Once this is determined and optimal method to store blood is determined, move forward with stability studies



Experimental Approach

- Whole blood specimens collected from infected rats
- Samples into lysis buffer shipped to Emory, bead beating then two different automated NA extraction methods (EM, MP)
 - Purified asp NA in lysis buffer and blood added to lysis buffer and spiked with purified asp NA
- Extracted specimens shipped back to Denning
 - Compare extraction to Denning Lab method

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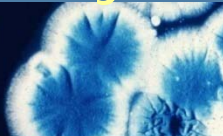
Results with Purified Aspergillus DNA

- DNA added to lysis buffer, EasyMag extraction

<u>ng DNA (AF 293)</u>	<u>Ct</u>
3840	22.0
384	25.6
38.4	28.8

- Compact consistently higher Cts (lower vol)
- Similar results when asp DNA spiked into blood/lysis buffer

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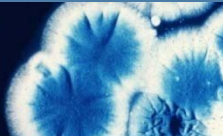
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Results with Blood Samples from Rats

- Unable to reliably detect aspergillus DNA with either extraction method
- Increase volume of blood extracted
- Increase volume of extracted NA that is added to PCR reaction

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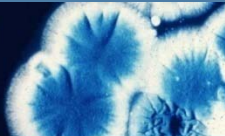
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Guinea Pig Model

- Able to detect aspergillus DNA in some specimens (blood and plasma)
 - Detection of DNA inconsistent
 - EM better than MP
- Detection lower than UTHSCSA method
 - Dilution that occurs with lysis buffer decreases sensitivity

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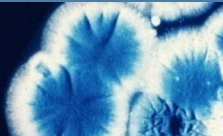
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Summary

- Evaluation of whether use of lysis buffer for storage of whole blood is still ongoing
- Once storage conditions determined stability studies will proceed

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