

ASTECC

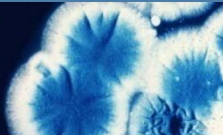
ASPERGILLUS TECHNOLOGY CONSORTIUM

Sample Stability

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IAAM

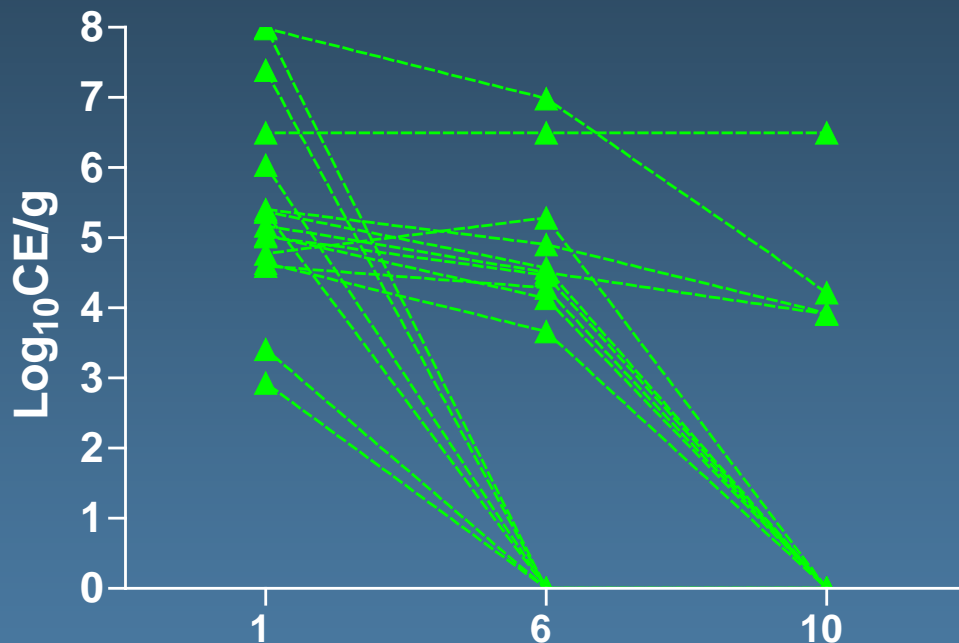


INVASIVE ASPERGILLOSIS ANIMAL MODELS

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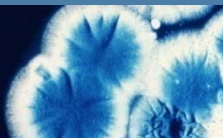
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The Effect of Repeated Freeze-Thaw Conditions on DNA Yield in Infected Lungs Tissue



- n=40
- 500µl aliquots of homogenate was immediately processed for DNA extraction and the rest was subsequently frozen in 1ml aliquots at -70°C.

IAAM

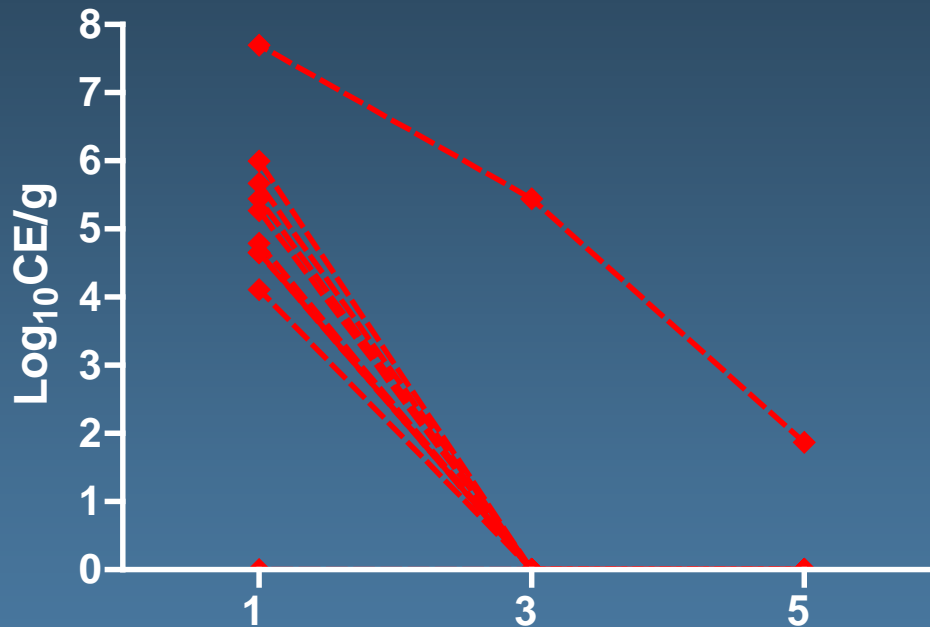


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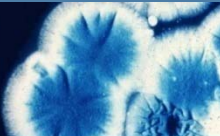
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The Effect of Repeated Freeze-Thaw Conditions on DNA Yield In Serum



- n= 10
- DNA extraction from a 500µl sample of serum was immediately performed. The rest of the serum was subsequently frozen in 1.8 ml cryovials in 1ml aliquots at -70°C.

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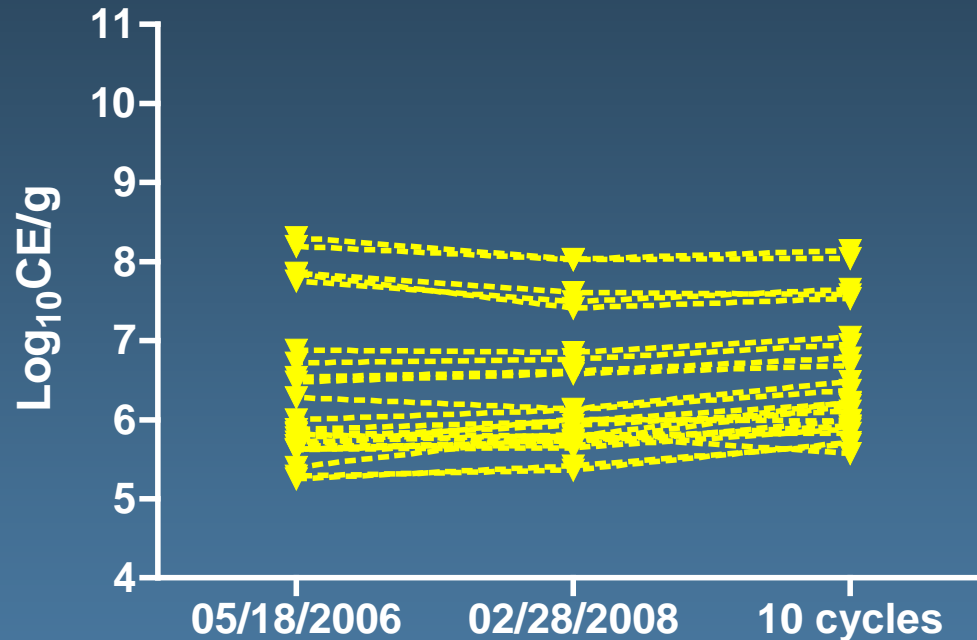


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The Effect of Time (21 months) and Freeze-Thaw Cycling on DNA quantitation



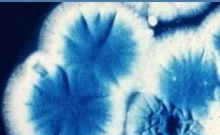
■ N=24

■ A small aliquot (15 μ l) of the DNA sample was used for quantitative PCR analysis. All samples were then placed into the -70°C freezer to be frozen again for the next cycle.

Result and Conclusions

- There was a statistically significant decrease in the mean quantity of CE/ml of AF 293 detected from lung samples after repeated freeze thaw cycles (initial: mean log₁₀: 5.4 ± 0.4 vs. cycle 10: mean log₁₀ of 1.16 ± 0.5 ; $p < 0.0001$).
- Similar results were obtained when assessing the CE/ml in serum samples (initial: mean log₁₀ 4.8 ± 0.62 vs. cycle 5: mean log₁₀ of 0.49 ± 0.49 ; $p < 0.0003$).
- In comparison, no significant change was detected in the quantity of AF 293 from DNA samples stored over 21 months or after 10 repeated freeze thaw cycles.
- Assessment of AF293 DNA extracted from frozen samples was stable and consistent after prolonged storage at -70°C and repeated freeze thaw cycles.
- Repeated freeze - thaw cycles of lung and serum samples prior to extraction of AF293 DNA led to a steady decrease in DNA yield over time.

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Tissue samples and Aspergillus PCR

- 43 tissue biopsies from 43 patients in Innsbruck
- 30 were lung (1 pleura), 4 skin, 1 ethmoid sinus, 1 lymph node, 1 liver and 1 unspecified
- 21 were culture positive for Aspergillus, 8 for *A. fumigatus*, 4 for *A. flavus* and 8 for *A. terreus*.
- 2 were also culture positive for *Candida* spp. One sample grew *Mucor* sp.
- Microscopy was positive in 34 of 42 (81%) cases, and narrow septate hyphae were found in 28 cases, broad rarely septate hyphae in 1 and non-septate hyphae in 5.
- 12 recent samples (Q2+3, 2008) were processed only for culture, microscopy and PCR (Myconostica), after freezing.
- 32 samples were collected over some years, also processed for in house PCR and GM, and stored frozen.

Lass-Flörl et al. ICAAC 2008 M697



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The logo for ASTEC (Aspergillus Technology Consortium) is displayed in a white serif font on a dark blue background. To the right of the text is a microscopic image showing blue-stained, branching, septate hyphae characteristic of Aspergillus.

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12 new tissue samples

	FXG : RESP (Asp +) positive	FXG : RESP (Asp +) negative	Totals
Microscopy			
Septate hyphae seen	4	2	6
Non-septate/broad hyphae seen	0	2	2
No hyphae seen	0	4	4
Totals	6	6	12
Culture			
<i>Aspergillus</i> positive	4	0	4
<i>Aspergillus</i> negative	0	8	8
Totals	4	8	12

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The logo for ASTEC (Aspergillus Technology Consortium) features the acronym 'ASTEC' in a large, white, serif font on a dark blue background. To the right of the text is a circular inset image showing a microscopic view of blue, branching, non-septate hyphae.

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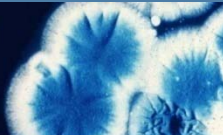
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All 43 tissue samples

Microscopy	FXG : RESP (Asp +) positive	FXG : RESP (Asp +) negative	Totals
Septate hyphae seen	18	11	29
Non-septate hyphae seen	0	5	5
No hyphae seen	0	8	8
Totals	14	16	42
Culture			
Aspergillus positive	12	9	21
Aspergillus negative	2	20	22
Totals	14	29	43

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