Fungal Contamination and Stability Testing

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Rates of contamination

- 8% of PCR assays were contaminated:
  - 5 DNA extractions (3.3%)
  - 7 PCR mixtures (4.7%)
- *A. fumigatus* or *S. cerevisiae* by sequencing
- Zymolase responsible for some

Loeffler et al, J Clin Microbiol 1999;37:1200

Contamination: *Aspergillus* PCR

Aspergillus PCR: 19% positivity among blood donors

Source	No pos/total	False pos. %
PCR reagents	24/992	2.4
Qiagen	17/82	12.5
Magnetic beats	6/104	5
Magnetic beads with blood		13

27 positive normal bloods ⇒ 12 different *Aspergillus* sequences

Palmer J. NIH & Roche. ICCAC 2001

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Sample tubes (1)

Type of vessel	Additive	Manufacturer	# of lots tested	# tested per lot
Blood collection tubes				
Whole blood collection tube 6mL	K_2EDTA (Spray Dried)	BD Vacutainer	3	25
Whole blood collection tube 2mL	K_2EDTA (Spray Dried)	BD Vacutainer	1	10
Whole blood Collection tube 6mL	K_3EDTA (Liquid)	BD Vacutainer	1	25
Serum blood collection tube 6mL	Clot Activator (Spray Dried)	BD Vacutainer	3	25
Serum blood collection tube 3mL	Clot Activator (Spray Dried)	BD Vacutainer	1	10
Serum blood collection tube (10 ml)	None	BD Vacutainer	1	25
Cell preparation tube (CPT)	Sodium citrate and Ficoll™ (a polysaccharide)	BD Vacutainer	3	6
PaxGeneRNA	Tetradecyltrimethylammonium oxalate solution	PreAnalytiX GmbH	2	6
RNA *later*®	RNA stabilization reagent	Ambion	2	10

Sample tubes (2)

Type of vessel	Additive	Manufacturer	# of lots tested	# tested per lot
Specimen collection and storage containers				
BAL collection container, 40cc	None	Busse	2	12
Urine collection container (Sterile cup only)	None	Medline	1	25
Urine collection container, (Sterile mid stream collection kit)	None	Medline	1	25
Cryovial container, 2mL	None	Simport	1	10
Cryovial container, 3mL	None	Simport	2	10
Pipette tips, 1000µL	None	Associates of Cape Cod/ Eppendorf	2	5

Harrison et al, ICAAC 2008 – Abstr. D1095

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Cultures

All tubes/containers tested for fungal sterility

1mL of sterile PDS/Tween vortexed in each

100uL plated on Sabouraud Dextrose agar

All (100%) negative

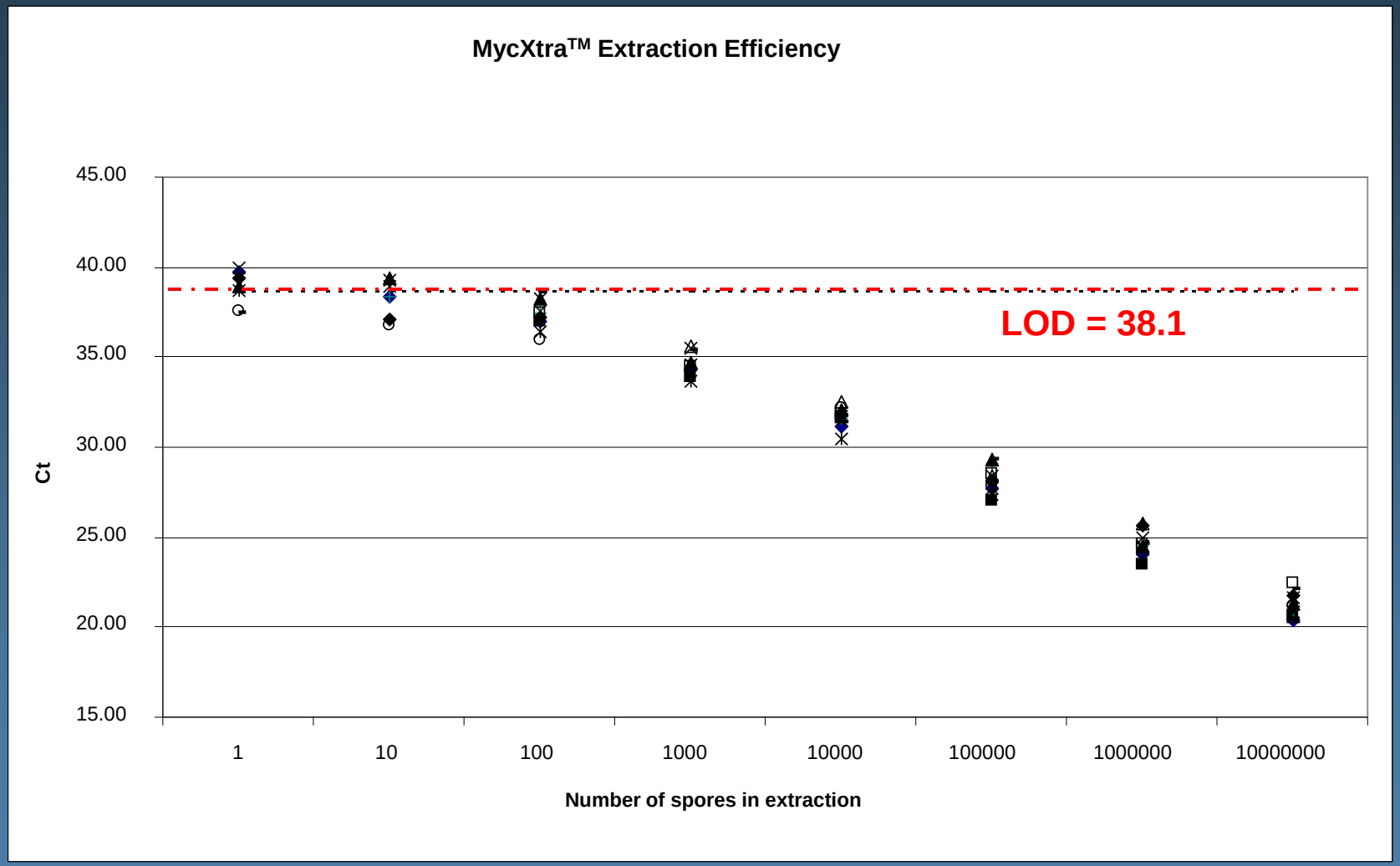
DNA Extraction

All tubes/containers tested for fungal DNA

1mL of sterile PDS/Tween vortexed in each

1mL used for DNA extraction using the MycXtra kit (Myconostica)

MycXtra™ Fungal DNA Extraction kit (Fungal DNA free)



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Real Time PCR

All tested with Aspergillus real-time PCR kit (Myconostica)

SmartCycler platform (Cepheid)

18S target for all Aspergilli and Penicillia

LoD ~50 target copies = ~1 genome

Real Time PCR results

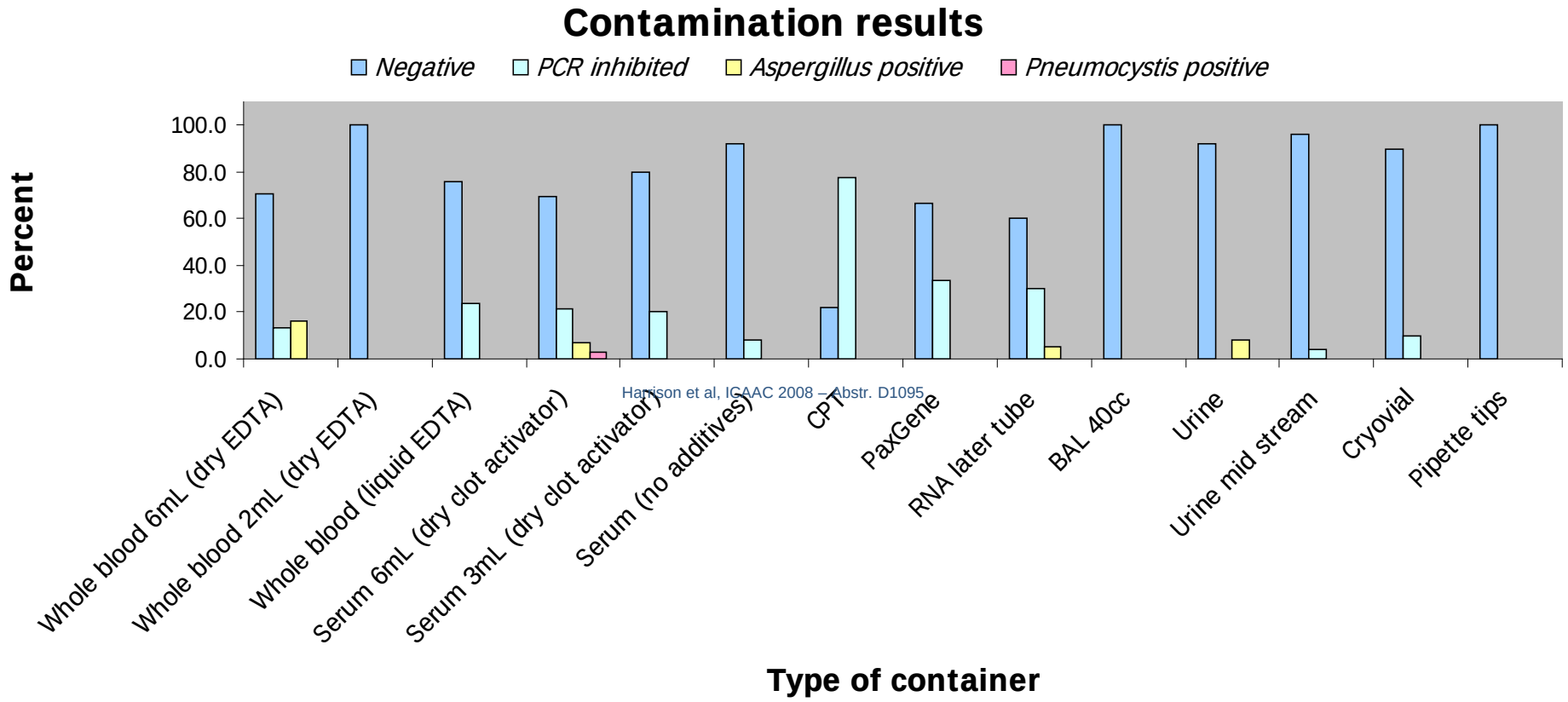

Figure 1: Results from each type of container shown as percentages

Real Time PCR results

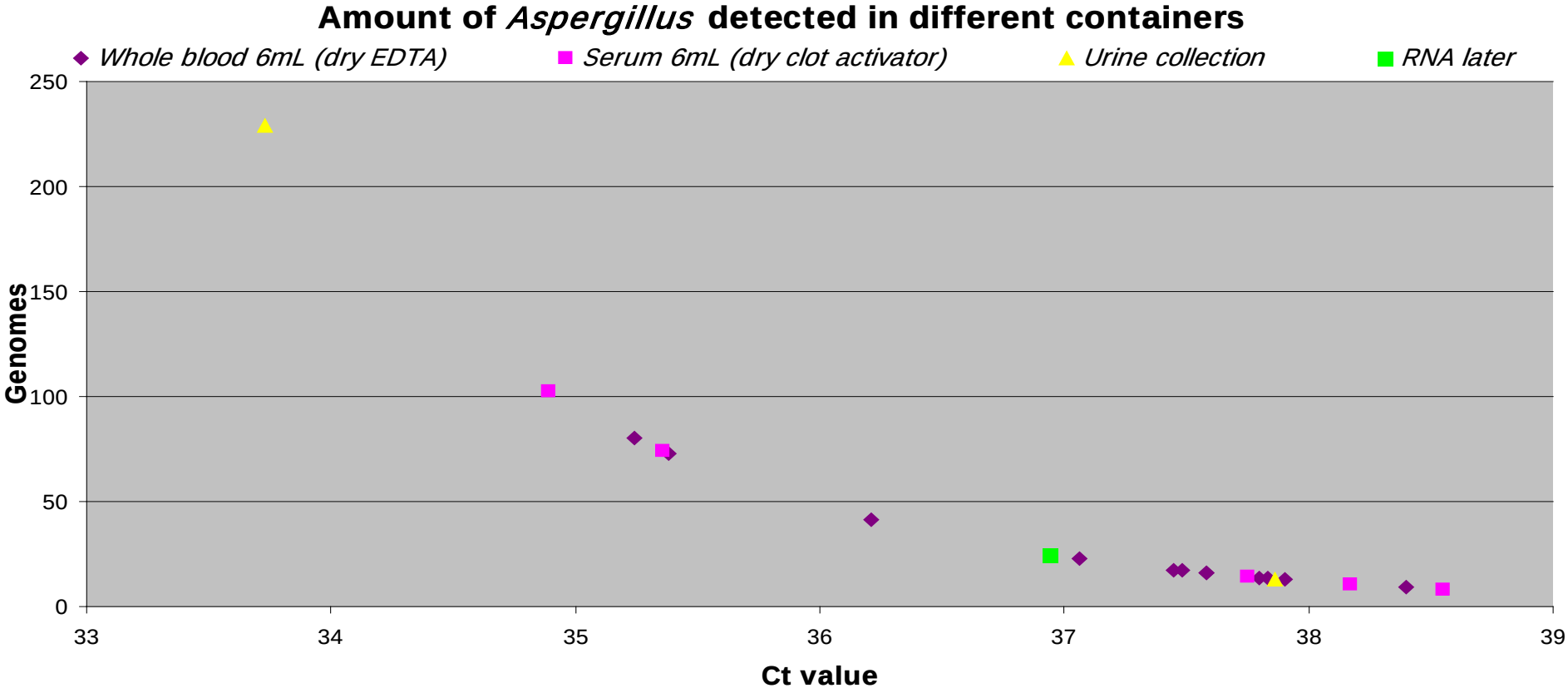

Figure 3: Real time PCR Ct values and corresponding contaminating genome copy number in different types of collection vessel

Additional testing in 2009

Collection container	LOT #	# tested	A. fumigatus positive	Inhibited	Positive Ct range
EDTA liquid 7ml	8219849	25	7	1	34.2 - 37.2
EDTA liquid 7ml	8339059	25	1	1	37.9
EDTA liquid 7ml	9007142	25	11	2	32.7 - 36.5
	TOTAL	**75**	**15%**		
Red top no additive 6ml	8331028	25	2	0	34.4 - 36.4
Red top no additive 6ml	9037525	25	9	0	35.4 - 37.9
	TOTAL	**50**	**18%**		

Confirmation of positives

Positives also tested with an *A. fumigatus* specific Taqman real-time assay

This showed 96% (48/50) agreement with samples that were MB positive

Suggests the results are real, and most contamination is *A. fumigatus*.

Challier S et al, J Clin Microbiol 2004;42:844

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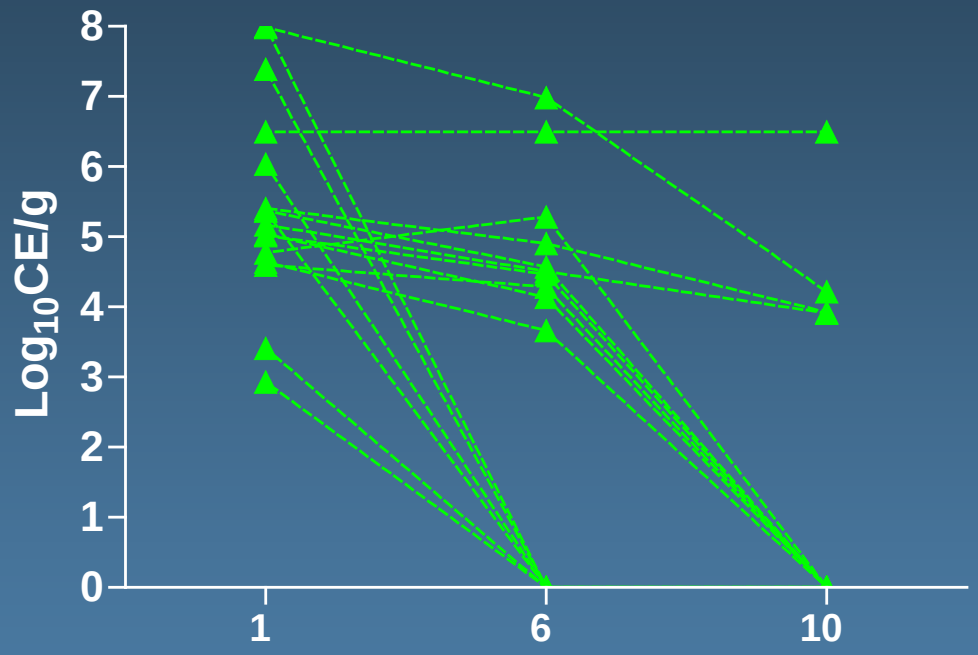
Conclusions

- Sample collection containers were investigated for fungal DNA contamination with two real-time PCR assays and culture
- All cultures were negative
- 17% of 185 whole blood collection tubes contaminated
- 10% of 160 serum blood collection tubes contaminated
- Other tubes and containers less frequently contaminated
- Most probably non-viable *A. fumigatus*

Diagnostics of Invasive Aspergillosis: From Experimental Models to Clinical Evaluation

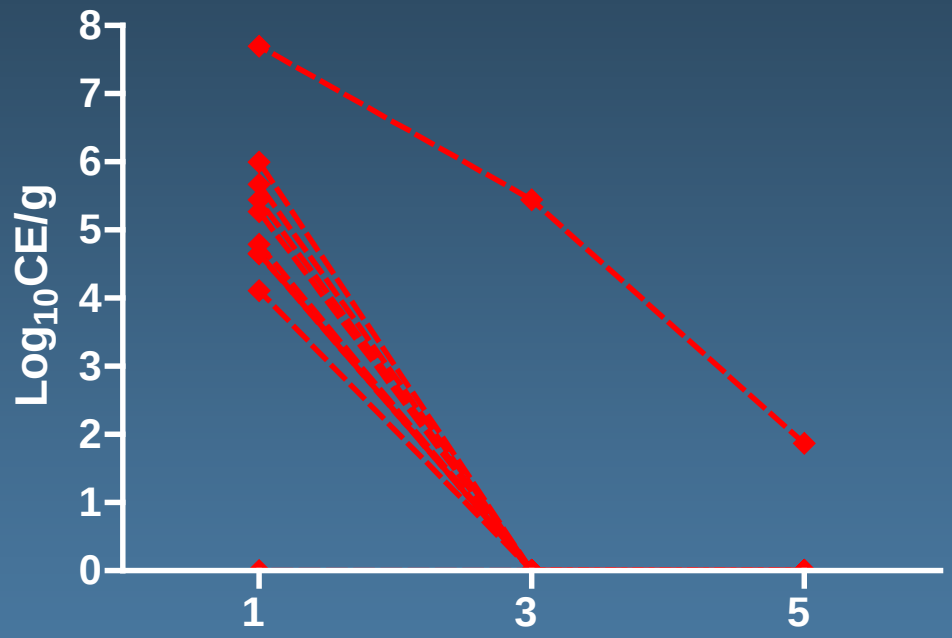
SAMPLE STABILITY

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



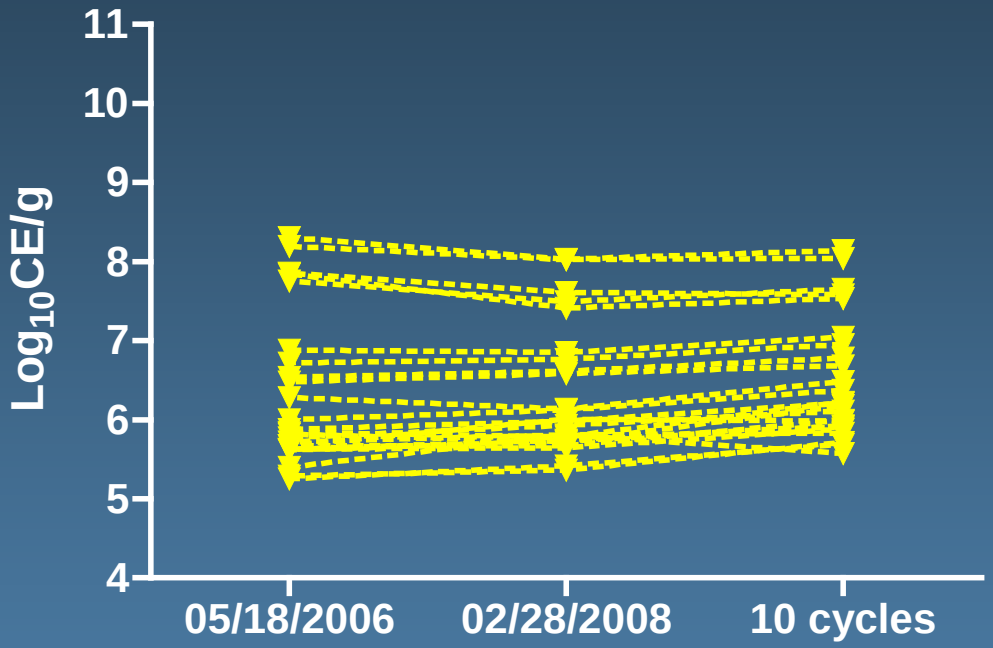
- n-=40
- 500µl aliquots of homogenate was immediately processed for DNA extraction and the rest was subsequently frozen in 1ml aliquots at -70°C.

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.

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



- N=24
- A small aliquot (15µl) 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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Future Studies

- Stability of DNA in fresh vs frozen whole blood
- Stability of DNA in fresh serum vs frozen sera
  - Intralaboratory
  - Interlaboratory (2-3)
- Stability of calibrator DNA in buffer, serum or blood, fresh vs frozen
  - Intralaboratory
  - Interlaboratory