

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

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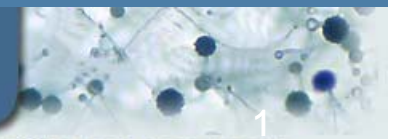
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INVASIVE ASPERGILLOSIS ANIMAL MODELS

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Why did we want to develop a rat model of IPA in Manchester

- ✓ We wanted to be able to collect sequential blood samples to monitor disease progression in a single animal
- ✓ We wanted to be able to collect at least 1ml of blood per day per animal
- ✓ We did not want to implant catheters
- ✓ We wanted to be able to work with large numbers of animals in a single experiment
- ✓ We wanted the cost to be reasonable

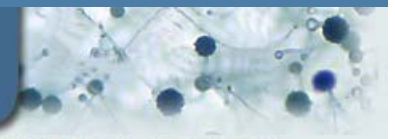
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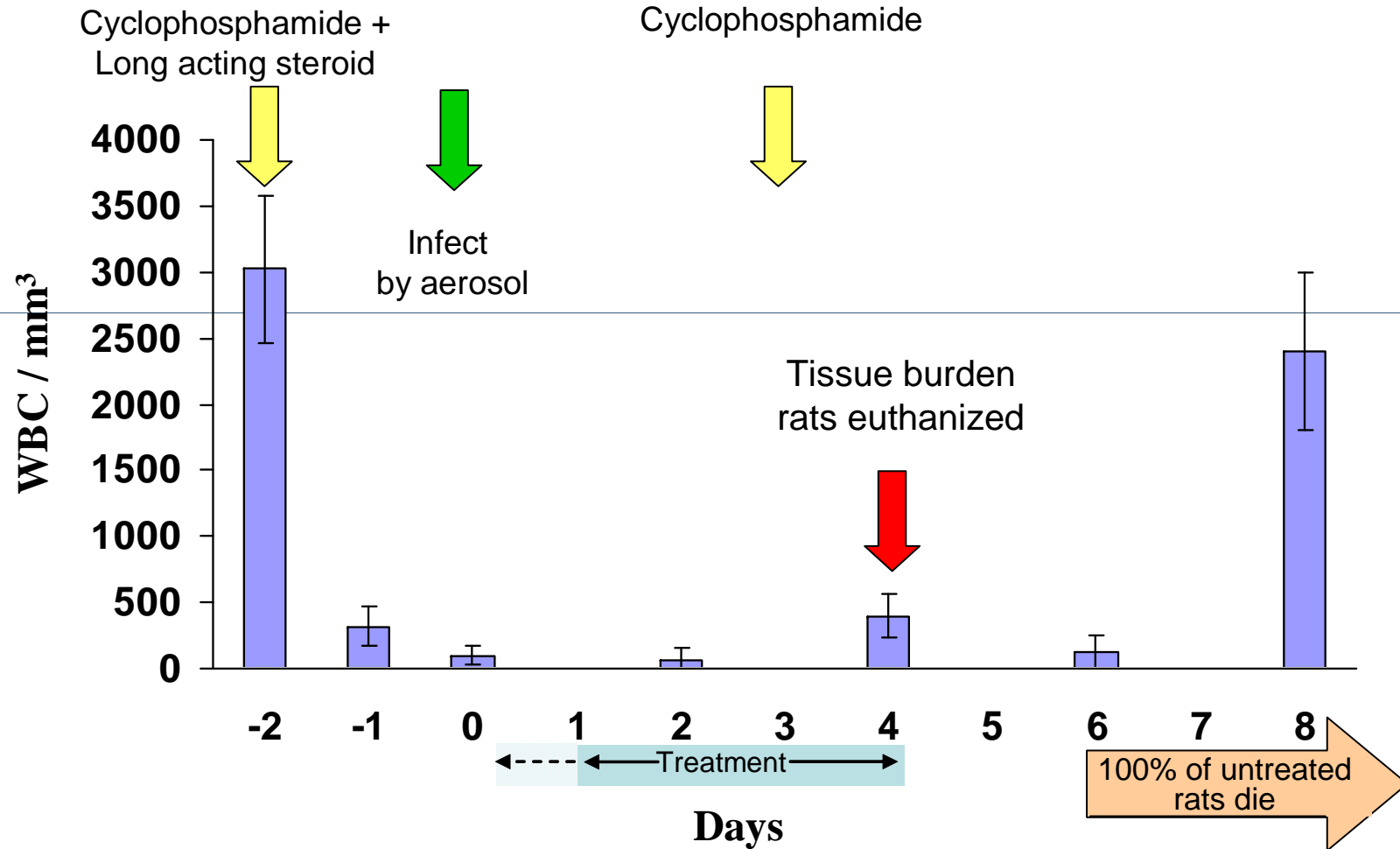
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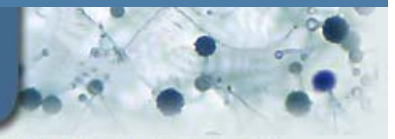


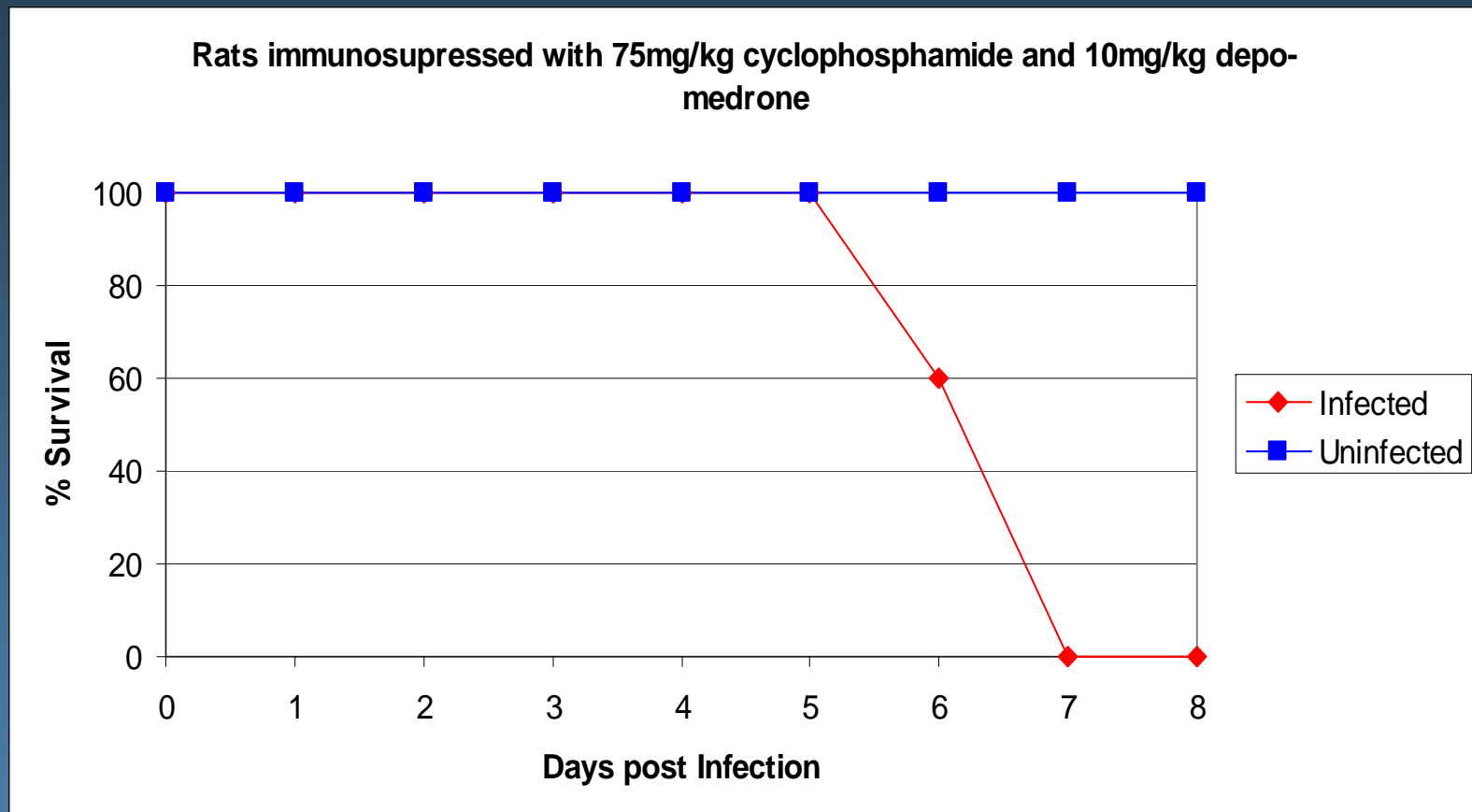
Time Course of IPA Models

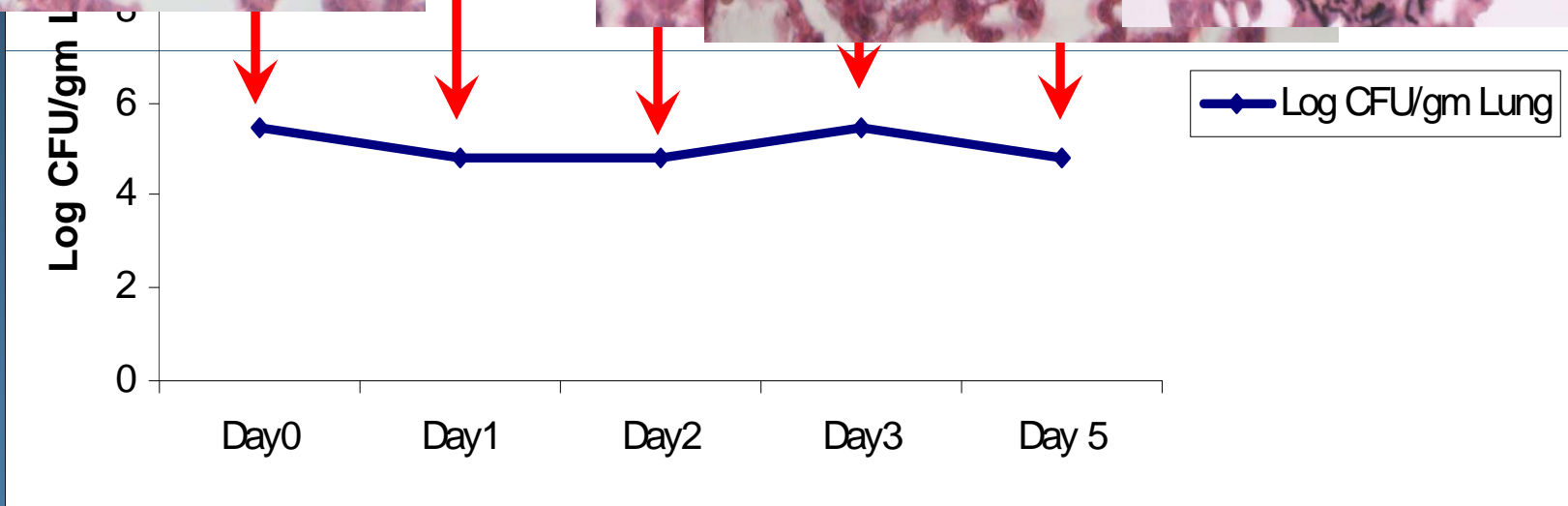
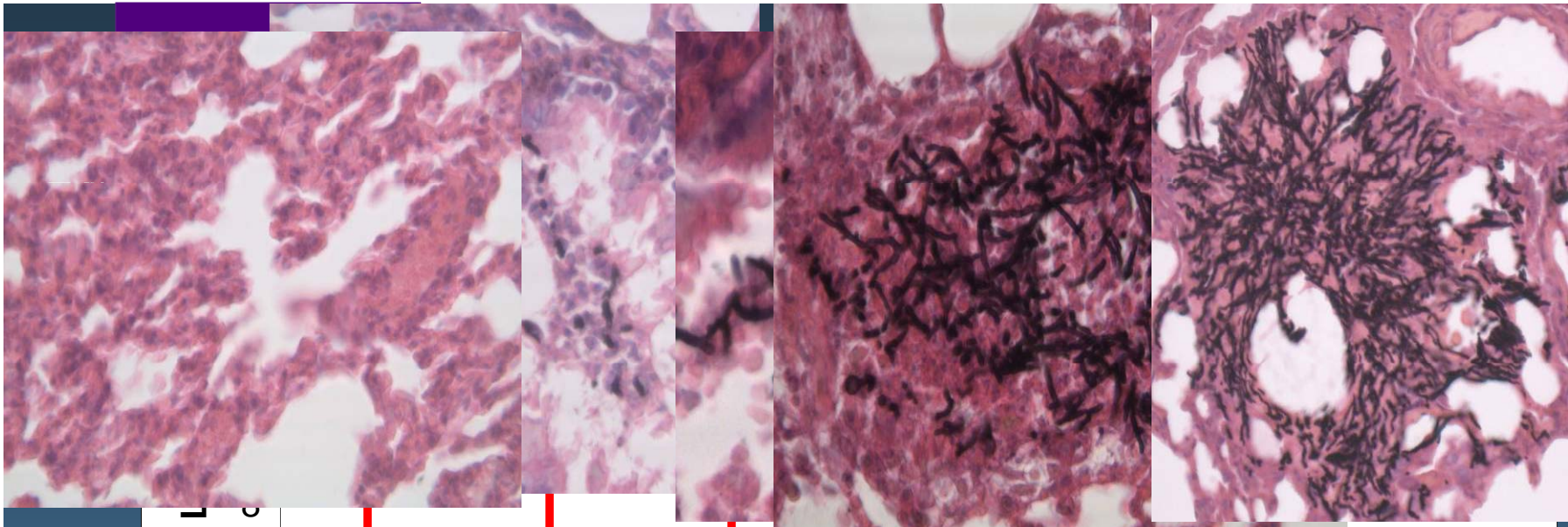


Experimental Variables

	Mice	Rats
Cytotoxics + cortisone	YES	YES
Cytotoxics + prednisolone	??	YES
Cortisone only	YES	??
Infectious challenge (delivered as 12ml aerosol)	$3 \times 10^7 - 1 \times 10^9 / \text{ml}$	$3 \times 10^7 - 1 \times 10^9 / \text{ml}$
BAL (plus urea)	YES	YES
Fungal strains	AF293 & A1163	A1163







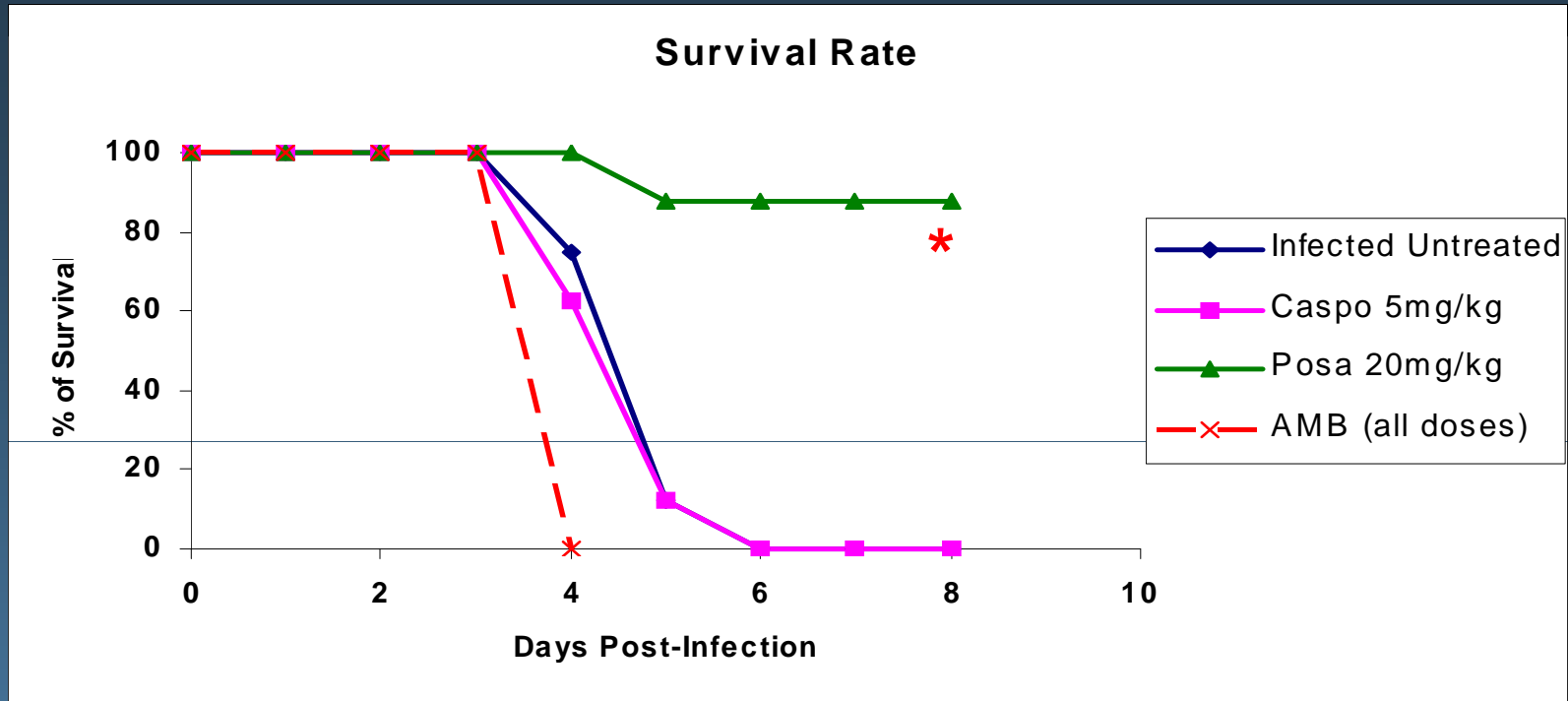
CFU of infected untreated rats showed little difference in the burden over time, but serial lung histopathology revealed; no hyphae invasion on day of infection, increase in *Afu* hyphae invading the lung parenchyma & destroying lung architecture on day-1, 2 ,3 & 5 post-infection with no difference between the 1st & 2nd days.

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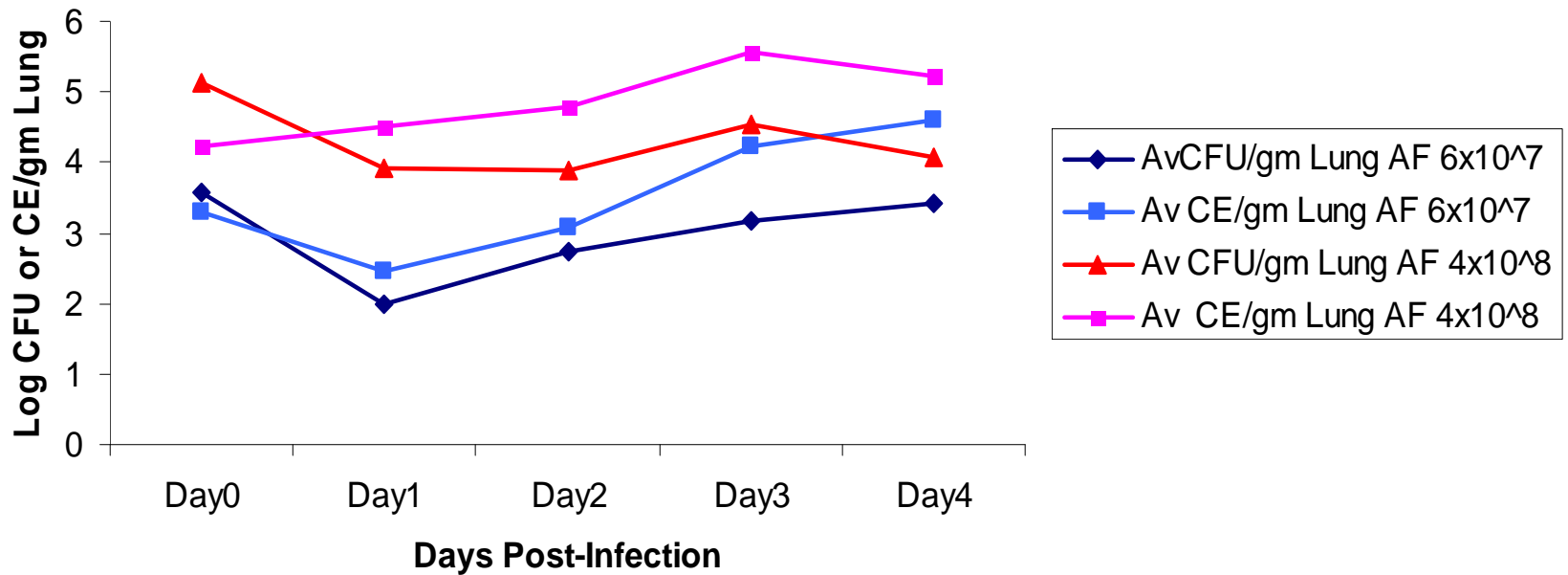


- Mortality of infected untreated rats was 100% 6 days post-infection with symptoms of severe pneumonia and weight loss.
- POS markedly prolonged the rats survival.
- AMB was nephrotoxic and increased the animals' mortality.

'Typical' Sequential sample model

- ✓ Blood samples collected daily from day -1 to day 5 (later samples are difficult to collect)
- ✓ Blood can be stored as whole blood, (EDTA) clotted blood, plasma or preservative (RNAlater Triazole etc)
- ✓ Paired rats can be serially euthanized for larger blood samples, terminal tissue samples and BAL collection
- ✓ All animals euthanized 5 days post infection due to severe disease development

AF Lung Burden progression over Time by CFU& QPCR



Sample	QPCT Ct
200µl Plasma AF1163 (Sample 1), LN, Qiagen	34.18
200µl Plasma AF1163 (Sample 2), LN, Qiagen	33.07
200µl Plasma AF1163 (Sample 3), LN, Qiagen	33.12
200µl Plasma AF293 (Sample 1), LN, Qiagen	34.54
200µl Plasma AF293 (Sample 2), LN, Qiagen	34.49
200µl Plasma AF293 (Sample 3), LN, Qiagen	33.55
100µl Water Blank (WB) LN, Qiagen	38.77
0.5 ml Blood AF1163, (Sample 1), Blood Cell Lysis, LN, Qiagen	33.00
0.5 ml Blood AF1163, (Sample 2), Blood Cell Lysis, LN, Qiagen	36.03
0.5 ml Blood AF1163, (Sample 3), Blood Cell Lysis, LN, Qiagen	35.61
0.5 ml WB, Blood Cell Lysis, LN, Qiagen	Undetermined
0.5 ml Blood AF1163, (Sample 1), Blood Cell Lysis Precellys 24, Qiagen	34.46
0.5 ml Blood AF1163, (Sample 2), Blood Cell Lysis Precellys 24, Qiagen	36.34
0.5 ml Blood AF1163, (Sample 3), Blood Cell Lysis Precellys 24, Qiagen	35.95
0.5 ml WB, Blood Cell Lysis, Precellys 24, Qiagen	Undetermined
PCR Positive Control (AF 50ng/reaction)	14.57
PCR Negative Control	Undetermined

Taqman primers and probes were designed targeting *Aspergillus fumigatus* 28s rDNA

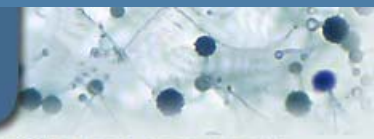
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Sample	QPCT Ct	CE/ 1ml Plasma/Blood
200µl Plasma AF1163 (Sample 1), LN, Qiagen	34.18	62.73
200µl Plasma AF1163 (Sample 2), LN, Qiagen	33.07	123.93
200µl Plasma AF1163 (Sample 3), LN, Qiagen	33.12	120.10
200µl Plasma AF293 (Sample 1), LN, Qiagen	34.54	50.26
200µl Plasma AF293 (Sample 2), LN, Qiagen	34.49	51.79
200µl Plasma AF293 (Sample 3), LN, Qiagen	33.55	92.23
100µl Water Blank (WB) LN, Qiagen	38.77	3.76
0.5 ml Blood AF1163, (Sample 1), Blood Cell Lysis, LN, Qiagen	33.00	51.74
0.5 ml Blood AF1163, (Sample 2), Blood Cell Lysis, LN, Qiagen	36.03	8.06
0.5 ml Blood AF1163, (Sample 3), Blood Cell Lysis, LN, Qiagen	35.61	10.44
0.5 ml WB, Blood Cell Lysis, LN, Qiagen	Undetermined	
0.5 ml Blood AF1163, (Sample 1), Blood Cell Lysis Precellys 24, Qiagen	34.46	21.10
0.5 ml Blood AF1163, (Sample 2), Blood Cell Lysis Precellys 24, Qiagen	36.34	6.68
0.5 ml Blood AF1163, (Sample 3), Blood Cell Lysis Precellys 24, Qiagen	35.95	8.49
0.5 ml WB, Blood Cell Lysis, Precellys 24, Qiagen	Undetermined	
PCR Positive Control (AF 50ng/reaction)	14.57	
PCR Negative Control	Undetermined	

Taqman primers and probes were designed targeting *Aspergillus fumigatus* 28s rDNA

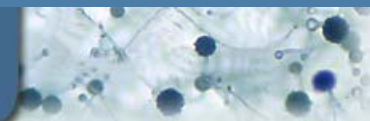
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Acknowledgements

Raghdaa Shrief

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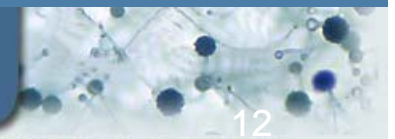
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