## **Supplementary Information**

## Early and non-invasive diagnosis of aspergillosis revealed by infection kinetics monitored in a rat model

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**Figure S2:** Fe-TAFC concentration detected in rat urine in the HD and LD infection models. Fe-TAFC concentration detected in rat urine is plotted in a logarithmic scale. A hash (#) denotes the Fe-TAFC concentration below the limit of detection, while an asterisk (\*) denotes the Fe-TAFC concentration below limit of quantification. Missing bars at 48h – D6 post-infection indicate animal death.

**Figure S3:** Whole body three-dimensional volume-rendered PET/CT images of  ${}^{68}$ Ga-TAFC in infected (yellow arrow indicates the infection) and non-infected rats. (A) PET/CT images of high-dose infected animals on the day of infection (on the left) and three days after inoculation (on the right). (B) PET/CT images of low-dose-infected animals on the day of infection (on the left) and ten days after inoculation (on the right). (C) PET/CT images of non-infected animals at the beginning (on the left) and at the end (on the right) of the experiment. S = site of injection, K = kidneys, G = gastrointestinal tract, U = urinary bladder.

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Figure S5: The mean Fe-FC concentrations detected in rat urine in the HD infection model.

Time after inoculation	Fe-FC		Fe-TAFC	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
4 h	100 (7/7)	100 (3/3)	0 (0/7)	100 (3/3)
24 h	100 (7/7)	100 (3/3)	57 (4/7)	100 (3/3)
30 h	100 (7/7)	100 (3/3)	71 (5/7)	100 (3/3)
48 h	100 (5/5)	100 (3/3)	80 (4/5)	100 (3/3)
54 h	100 (4/4)	100 (3/3)	100 (4/4)	100 (3/3)

**Supplementary Table S1:** The sensitivity and specificity of LC-CASI-FTMS determination of Fe-FC and Fe-TAFC kinetics in the high-dose model.

**Supplementary Table S2:** Urine BDG testing in rats LD5 new and HD6 indicating falsepositive results for the data points preceding the inoculation. Note: The BDG assay was not fully standardized for urine analysis.

LD5		HDe	<u>,</u>
Data point	pg/mL	Data point	pg/mL
-25 h	>617.2	-25 h	>617.2
-19 h	>617.2	- 19 h	>617.2
-1 h	>617.2	-1 h	>617.2
4 h	420.3	4 h	>617.2
24 h	>617.2	24 h	>617.2
30 h	>617.2	30 h	>617.2
48 h	478.4	48 h	>617.2
54 h	>617.2	54 h	>617.2
D3	>617.2		
D4	>617.2		
D6	>617.2		
D6	>617.2		
D7	>617.2		
D7	>617.2		
D8	>617.2		
D8	>617.2		
D9	236.5		
D9	>617.2		
D10	>617.2		

**Supplementary Table S3:** Technical variability expressed as the coefficient of variation (CV) for each group of technical replications.

	Fe-FC	Fe-TAFC
Low dose	$0.047 \pm 0.010$	0.071±0.033
High dose	$0.051 \pm 0.007$	0.065±0.016

**Supplementary Table S4:** The variability in biological replicates expressed as the coefficient of variation (CV) in the high-dose-infected animals. SEM: the standard error of the mean.

Time after inoculation	Siderophore	Mean (ng/mL)	±SEM (ng/mL)	CV
4 h	Fe-FC	4.3	1.3	0.783
	Fe-TAFC	0.0	0.0	NAN
24 h	Fe-FC	794.5	500.6	1.667
	Fe-TAFC	3.1	1.8	1.493
30 h	Fe-FC	3 019.4	1 296.3	1.136
	Fe-TAFC	36.0	23.1	1.698
48 h	Fe-FC	4 743.1	2 134.0	1.006
	Fe-TAFC	442.7	247.5	1.250
54 h	Fe-FC	12 284.5	5 239.4	0.853
	Fe-TAFC	1 470.1	849.0	1.155