

Luminex

2009 Combined IAMM & AsTeC Workshop



Multiplexed Microbead Array Detection of Aspergillosis and other invasive fungi

Frank Merante
November 16, 2009

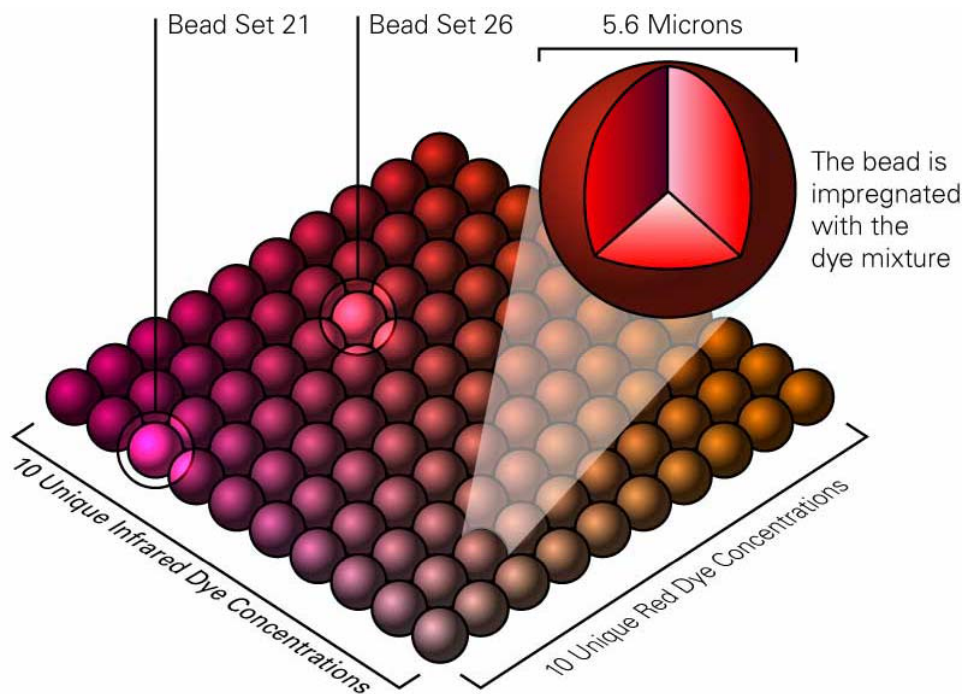
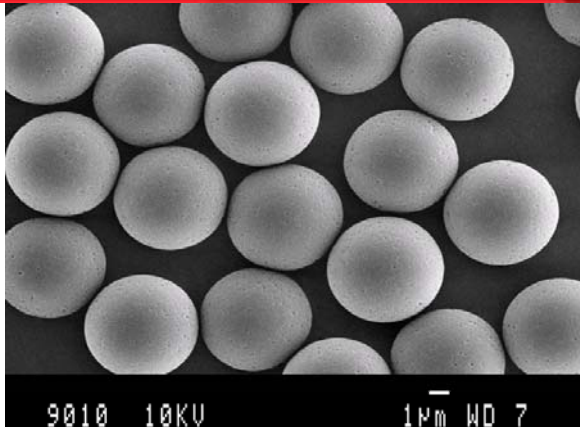
Presentation Overview

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- Luminex xMAP bead array technology
 - Multiplexed fungal panel targets
 - Performance with isolates and mycelium spiked blood
 - Guinea pig experimental model of inhalational aspergillosis
 - Conclusions
-

xMAP Bead Array Technology

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- 5.6 μM Microspheres are dyed to create 100 spectrally distinct populations with a unique spectral address.
- Additionally, each population is uniquely coupled to an oligonucleotide anti-tag complementary to a tagged PCR primer = enables 100-plex multiplexing capacity.
- Microspheres exhibit quasi-solution phase hybridization kinetics.
- Captured analytes/targets are fluorescently detected

Multiplex Fungal Panel

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Product Specifications

- Single test, easy-to-use multiplexed format
- Detect and simultaneously identify a wide spectrum of clinically relevant fungal pathogens
- For use with venous blood, serum and BAL's
- TAT (specimen-in-result-out) 3-4 hours
- High analytical sensitivity capable of detecting low “fungal loads”
- High analytical specificity for clear identification of different fungal targets

	Target	
1	<i>Aspergillus fumigatus</i>	Aspergillus
2	<i>Aspergillus terreus</i>	
3	<i>Aspergillus flavus</i>	
4	<i>Aspergillus niger</i>	
5	<i>Candida albicans</i>	Candida
6	<i>Candida glabrata</i>	
7	<i>Candida lusitanae</i>	
8	<i>Candida tropicalis</i>	
9	<i>Candida parapsilosis</i>	
10	<i>Candida krusei</i>	
11	<i>Candida guilliermondii</i>	
12	<i>Histoplasma capsulatum</i>	
13	<i>Coccidioides immitis</i>	
14	<i>Cryptococcus neoformans</i>	
15	<i>Blastomyces dermatiditis</i>	
16	<i>Scedosporium apiospermum</i>	
17	<i>Scedosporium prolificans</i>	
18	<i>Fusarium*</i>	
19	<i>Rhizopus arrhizus</i>	Zygomycetes
20	<i>Rhizopus microsporus</i>	
21	<i>Mucor indicus</i>	
22	<i>Cunninghamella bertholletiae</i>	
23	<i>Pneumocystis jirovercii</i>	
24	<i>Tremella fuciformis (internal control)</i>	

4 Aspergillus species

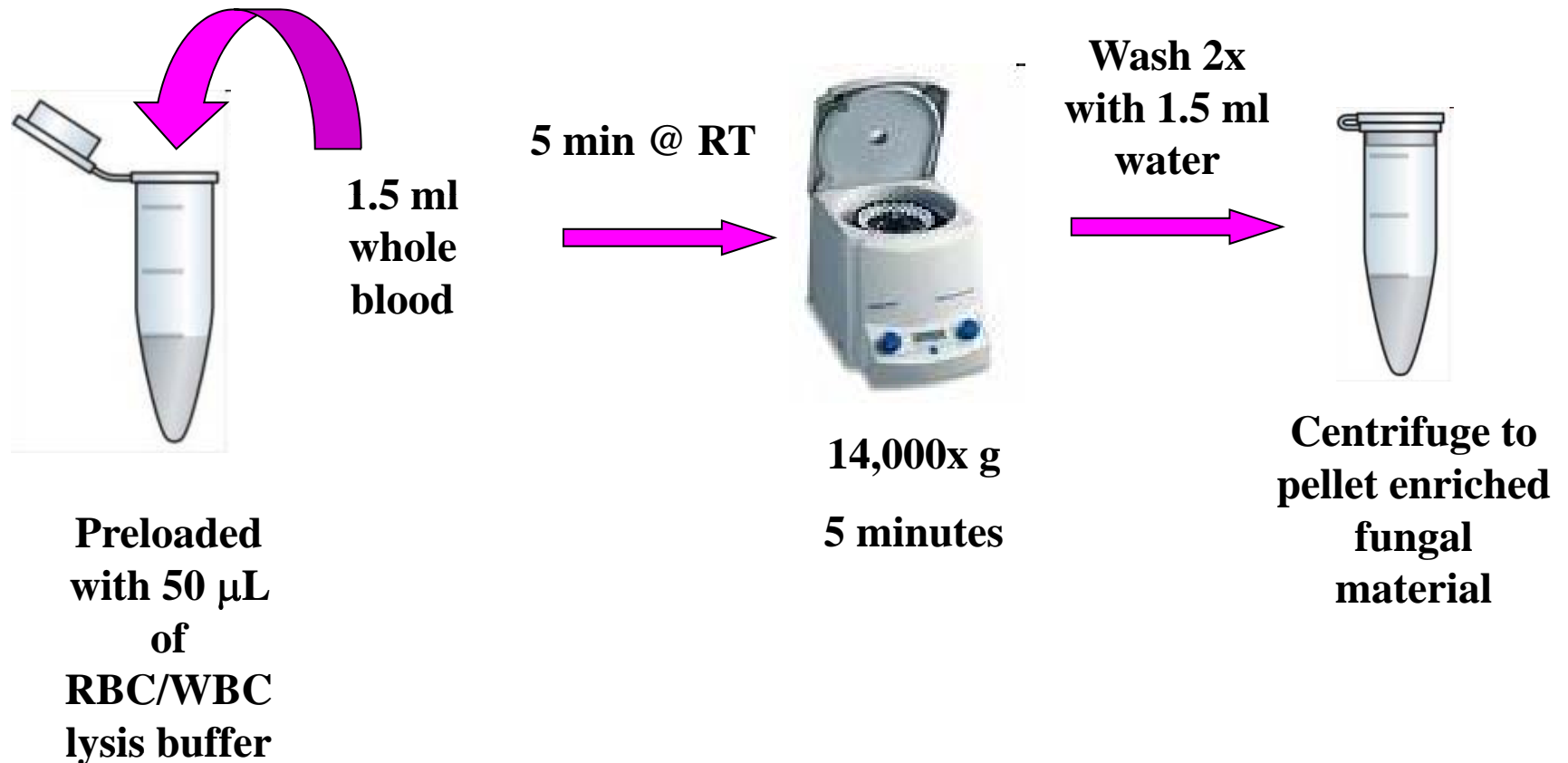
7 Candida species

* *F. oxysporum* & *solani*

4 Zygomycete species

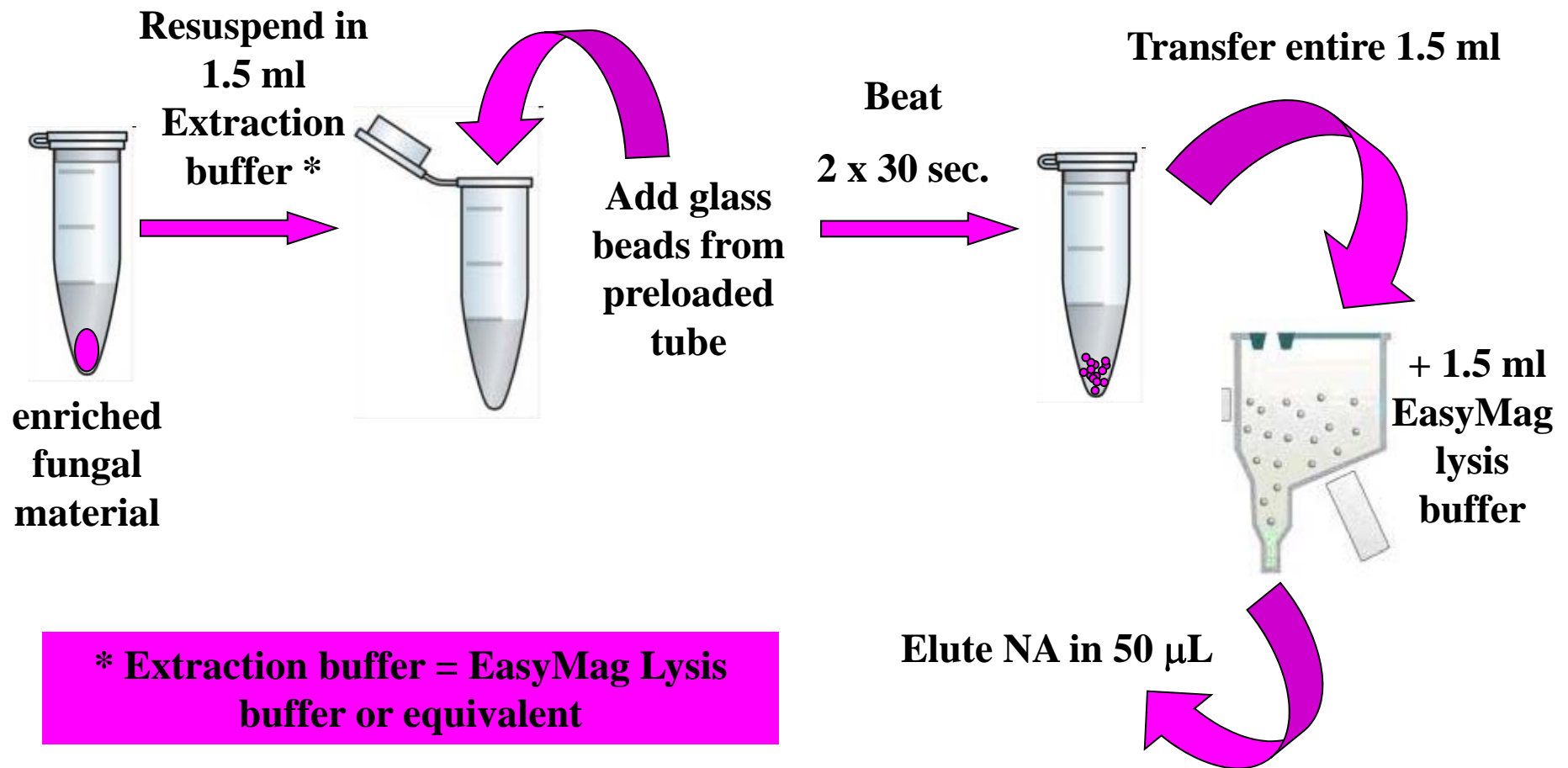
Sample Preparation: Blood Processing Protocol

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Blood Extraction Protocol

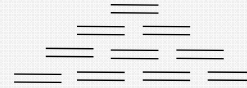
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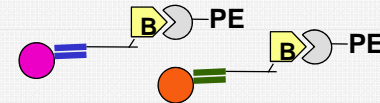
xTAG[®] Fungal Assay

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I. Multiplex PCR



II. Universal Array Sorting



III. xMAP[®] Detection



IV. Data Analysis



xTag Fungal Assay – Performance on DNA Isolates

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Sample	01-C. albicans	02-C. glabrata	03-C. lusitaniae	04- C.Tropical	05-C. parapsilosi	06-C. krusei	07-C. guilliermo	08-A. terreus	09-A. fumigatus	10-A. flavus	11-A. niger	12-H. capsulatu
Neg Con	72	98	72	73	102	67	87	61	59	74	100	76
<i>Candida albicans</i>	14684	68	78	69	102	91	76	71	63	72	79	97
<i>Candida albicans</i>	14438	69	56	70	76	65	62	61	89	55	102	54
<i>Candida glabrata</i>	95	7113	66	73	68	98	66	64	67	93	89	107
<i>Candida glabrata</i>	79	6668	82	64	54	90	84	87	78	63	83	67
<i>Candida tropicalis</i>	66	85	4378	70	76	72	152	62	76	62	76	73
<i>Candida tropicalis</i>	83	85	4484	35	69	79	90	70	86	65	63	65
<i>Candida parapsilosis</i>	62	82	76	7629	78	88	85	71	58	66	101	73
<i>Candida parapsilosis</i>	91	75	73	7159	93	78	87	64	53	98	61	73
<i>Candida krusei</i>	78	93	51	59	12517	83	89	72	66	76	56	259
<i>Candida krusei</i>	79	87	75	64	12429	63	88	64	52	65	71	68
<i>Candida lusitaniae</i>	84	98	43	63	82	8200	91	77	57	77	94	58
<i>Candida lusitaniae</i>	81	56	91	47	87	8604	62	77	76	73	70	56
<i>Candida guilliermondii</i>	73	83	82	57	71	73	13180	79	59	58	100	72
<i>Candida guilliermondii</i>	53	62	50	87	72	92	13490	90	67	53	97	60
<i>Aspergillus terreus</i>	91	89	61	46	85	120	87	11233	67	72	75	93
<i>Aspergillus terreus</i>	63	62	97	44	73	84	86	10899	72	82	92	80
<i>Aspergillus fumigatus</i>	81	79	82	62	83	85	86	58	12902	77	67	48
<i>Aspergillus fumigatus</i>	65	74	74	69	47	65	121	66	13799	61	70	80
<i>Aspergillus flavus</i>	66	91	88	67	77	95	96	63	66	10233	94	72
<i>Aspergillus flavus</i>	211	83	68	80	65	80	89	59	67	9934	75	101
Neg Con	85	84	64	84	110	67	84	89	83	73	77	77
<i>Histoplasma capsulatum</i>	95	90	62	76	95	86	81	77	82	58	83	10488
<i>Histoplasma capsulatum</i>	94	82	63	52	80	87	76	55	69	96	83	10619
<i>Aspergillus niger</i>	88	95	91	99	74	87	106	99	59	77	7418	85
Neg Con	57	81	63	68	67	105	85	73	76	70	85	74

Data representing 1 ng isolate DNA/Amplification Reaction

Example of Analytical Sensitivity for *A. fumigatus* mycelium spiked into WB

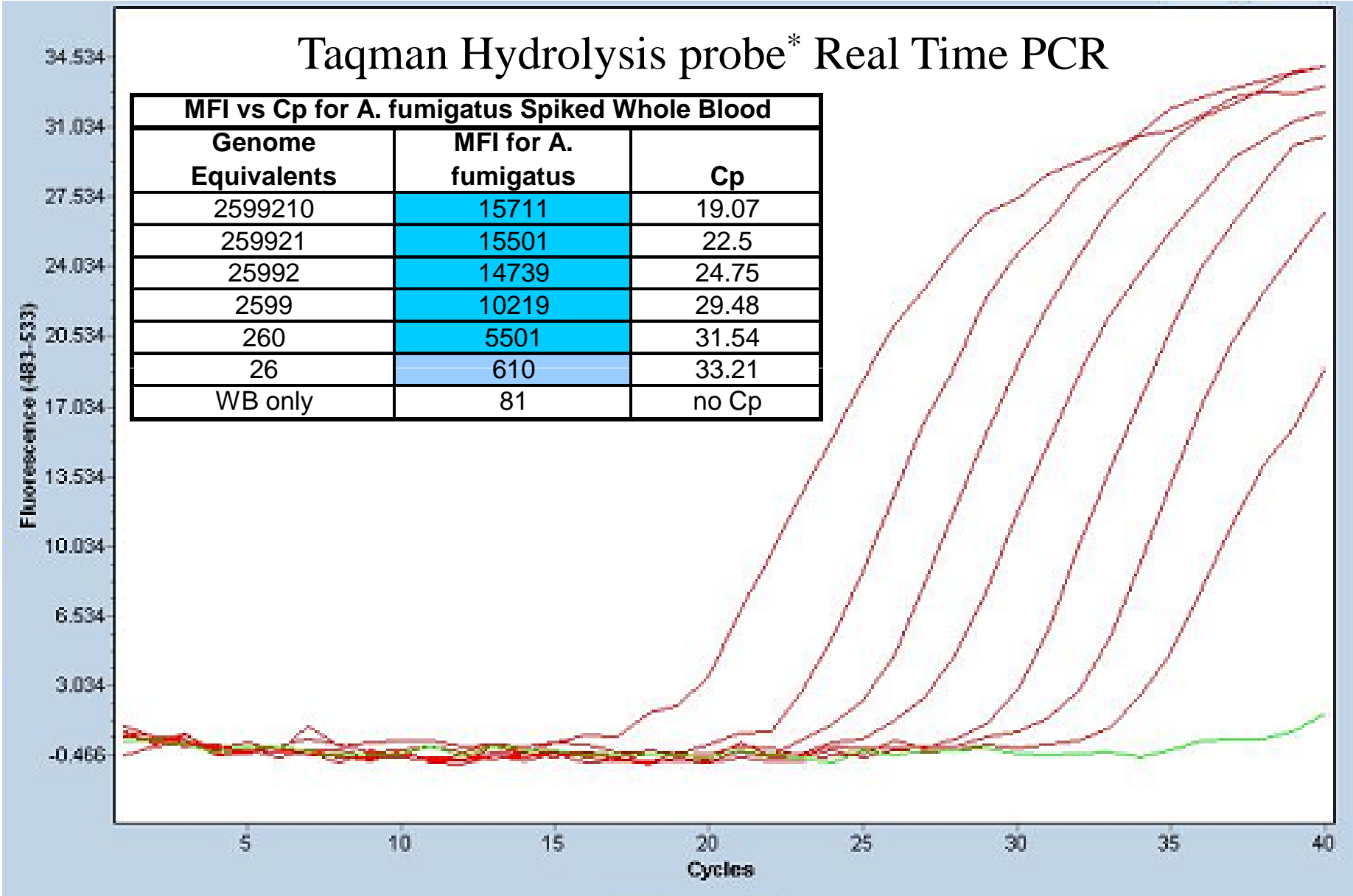
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EasyMag Extracted DNA

	01-C.	02-C.	03-C.	04-	05-C.	06-C.	07-C.	08-A.	09-A.	24-
copies/ml	albicans	glabrata	lusitaniae	C.Tropical	parapsilosi	krusei	guilliermo	terreus	fumigatus	Tremella
water blank	96	86	92	84	89	54	69	85	122	89
26.0	104	72	88	74	91	74	76	58	610	76
259.9	72	81	65	87	84	78	79	144	5501	63
2599.2	84	107	68	63	63	91	80	105	10219	72
25992.1	59	92	85	56	98	70	60	108	14739	60
259921.0	71	71	84	54	77	70	87	87	15501	55
2599210.3	75	91	75	38	79	104	89	101	15711	80
WB no tremella	55	67	54	65	86	71	78	99	81	101
*WB + tremella	69	68	45	105	44	105	52	123	79	1622
<i>A. fumigatus</i>	63	52	52	63	84	31	70	60	16458	62

* *Tremella fuciformis* = Can be used as an extraction control or internal control

Example of Real Time PCR for LOD Dilutions



* Primers from Kami et al., (2001) Clin. Infect. Dis. 33:1504-1512

Guinea Pig Immunocompromised Model of Inhalational Aspergillosis

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Specimens received from UT Health Sciences Center (San Antonio)

- ✓ Whole Blood
- ✓ Serum
- ✓ CSF

Extraction & Analysis:

- ✓ LMX Fungal Panel
- ✓ Real-time PCR

Guinea Pig BALs

Median	MFI		Infected/ uninfected	Day of collection	Group	Cp
	09-A. fumigatus	24-Tremella				
water blank	125	65	/	/	/	40.62
<i>A. fumigatus</i> Isolate	6186	77	/	/	/	18.93
<i>Tremella</i> Isolate	83	5582	/	/	/	Not tested
GP blank (<i>Tremella</i> in buffer)	100	6772	/	/	/	No Cp
GP 3	11396	9700	Infected	0	Infected control D0 (1h)	30.03
GP 4	2659	9774	Infected	0	Infected control D0 (1h)	32.3
GP 7	12825	9144	Infected	3	Infected control D3	28.94
GP 8	4364	9460	Infected	3	Infected control D3	32
GP 9	11242	8908	Infected	3	Infected control D3	29.01
GP 10	11408	8871	Infected	3	Infected control D3	29.06
GP 11	14945	8303	Infected	5	Infected control D5	27.06
GP 12	10317	9432	Infected	5	Infected control D5	29.68
GP 13	10811	8947	Infected	5	Infected control D5	29.12
GP 14	13249	8825	Infected	5	Infected control D5	27.64
GP 15	11866	9014	Infected	5	Infected control D5	28.96
GP 18	137	4556	Infected	7	Infected control D7	Not tested
GP 19	15844	6870	Infected	7	Infected control D7	26.02
GP 20	8976	7945	Infected	7	Infected control D7	30.46
GP 26	12283	8740	Infected	6	Infected control D7	28.67
GP 27	9375	8186	Infected	3	Posaconazole D3 (20 mg/kg BID)	29.97
GP 28	3200	9295	Infected	5	Posaconazole D5 (20 mg/kg BID)	32.88
GP 31	89	9891	uninfected	7	Uninfected controls D7	39.43
GP 32	62	8213	uninfected	7	Uninfected controls D7	37.15
GP 33	56	9671	uninfected	7	Uninfected controls D7	37.38
GP 34	125	9415	uninfected	7	Uninfected controls D7	No Cp
GP 35	38	9389	uninfected	7	Uninfected controls D7	No Cp
Buffer blank (no DNA control)						38.87

1 mL BAL + 1x10⁵ Cells/mL Tremella

Guinea Pig CSF

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Median Data	Sample	Day	A. fumigatus	Tremella	Cp Value
	NC	/	103	86.5	38.87
~200 mL CSF samples	GP2 CSF	1 hour	88	3713.5	38.97
	GP3 CSF	1 hour	110	2529	39.46
	GP5 CSF	1 hour	95.5	2115.5	39.43
	GP6 CSF	Day 3	36	3413	38.94
	GP7 CSF	Day 3	100.5	3325	37.90
	GP9 CSF	Day 3	67.5	3765	39.94
	GP10 CSF	Day 3	80	4191.5	39.27
	GP11 CSF	Day 5	65	1640	39.26
	GP13 CSF	Day 5	57	3356	39.23
	GP15 CSF	Day 5	63	3052	/
	GP20 CSF	Day 7	73	3050.5	39.78
	GP26 CSF	Day 7	98	177.5	/
	GP27 CSF	Day 3 + Posa	79	1637	/
GP32 CSF	Uninfected		67	303	38.99

Guinea Pig Serum “Unbeaten” to assess “Free DNA”

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Median	Sample	Day	A. fumigatus	Tremella*	Cp value
~1 ml Serum	GP11 SERUM	Day 5	66	1403.5	39.53
	GP12 SERUM	Day 5	71	2311	/
	GP13 SERUM	Day 5	92.5	775	38.89
	GP14 SERUM	Day 5	42	1333	38.49
	GP28 SERUM	Day 5 + Posa*	383.5	1067.5	/
	GP18 SERUM	Day 7	11	1401.5	35.22
	GP19 SERUM	Day 7	72.5	55.5	/
	GP20 SERUM	Day 7	62	2979	/
	WATER	/	98	86	/
	EXT	/	86.5	2308.5	/

* Greater variability resulting from “unbeaten” samples

Sample	Sampling Day	<i>A. fumigatus</i>	<i>Tremella</i>
GP 2	Day 0 (1 hr)	193	5632
GP 3	Day 0 (1 hr)	73	338
GP 4	Day 0 (1 hr)	115	4060
GP 5	Day 0 (1 hr)	51	6247
GP 6	Day 3	53	6328
GP 7	Day 3	67	5857
GP 8	Day 3	39	5159
GP 9	Day 3	80	5199
GP 10	Day 3	424	4914
GP 11	Day 5	125	5492
GP 12	Day 5	90	5104
GP 18	Day 7	215	5749
GP 19	Day 7	64	3659
GP 20	Day 7	71	4277
GP 27	Day 3 + Posa	171	6951
GP 28	Day 5 + Posa	55	5827
GP 31	Uninfected	79	6992
GP 32	Uninfected	44	5431
GP 33	Uninfected	112	6596
GP 34	Uninfected	133	6769
GP 35	Uninfected	61	5033
extraction control	/	76	4715

Guinea Pig
Whole
Blood

May 22, 2009

250 μ L Whole
Blood
(+ 1×10^5 cells
Tremella)

Sample	Sampling Day	<i>A. fumigatus</i>
GP2	Day 0 (1 hr)	76.5
GP3	Day 0 (1 hr)	51
GP4	Day 0 (1 hr)	66
GP5	Day 0 (1 hr)	85
GP6	Day 3	60
GP7	Day 3	72
GP8	Day 3	31
GP9	Day 3	46.5
GP10	Day 3	272
GP11	Day 5	57
GP12	Day 5	55.5
GP13	Day 5	55
GP14	Day 5	79.5
GP15	Day 5	52
GP18	Day 7	59
GP19	Day 7	65
GP20	Day 7	95
GP26	Day 7	67.5
GP31	Uninfected	27.5
GP32	Uninfected	78
GP33	Uninfected	65.5
ext blk 1	/	54

Guinea Pig **Whole Blood**

~ 1.5 mL Whole Blood
(NO Tremella added)

Clinical Specimens

Human Bronchial Alveolar Lavage (BAL)

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Clinical specimen	Reference method diagnosis	Detected	MFI	Co-infection	Species	MFI
BAL 1	<i>A. fumigatus</i>	YES	15196	YES	<i>C. albicans</i>	945
BAL 2	<i>A. fumigatus</i> & <i>Scedosporium ap (P.boydii)</i>	YES (both)	4303 9760	YES		
BAL3	<i>C. albicans</i>	YES	588	NO		
BAL 4	<i>Fusarium</i>	YES	16193	NO		
BAL 5	<i>C. parapsilosis</i>	YES	13512	YES	<i>C.albicans</i> <i>Scedosporium ap.</i> <i>P. jirovercii</i>	12388 2143 1893
BAL 6	<i>C. albicans</i> + other fungus	YES	553	YES	<i>C. glabrata</i>	2103

Average background MFI range: 65 - 108

Clinical Specimens – Various Sources

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Clinical specimen	Concentrated or dilute	MFI	Comments
<i>Pneumocystis</i> BAL 1	Concentrated	11607	
<i>Pneumocystis</i> BAL 2		11812	
<i>Pneumocystis</i> BAL 3		10910	
<i>Pneumocystis</i> BAL 4		10797	
<i>Pneumocystis</i> BAL 5	dilute	5955	
<i>Pneumocystis</i> BAL 6	dilute (supernatant) {free DNA}	511	
<i>Pneumocystis</i> BAL 7	?	13581	
<i>A. terreus</i> BAL 1	dilute (supernatant) {free DNA}	398	colonization
<i>A. terreus</i> BAL 1.2		463	disease
<i>A. terreus</i> BAL 1.3		510	disease
<i>A. terreus</i> BAL 1.4		910	disease
<i>A. terreus</i> BAL 1.5		2953	disease
<i>A. terreus</i> BAL 2	?	1506	
<i>A. fumigatus</i> BAL 7	dilute (supernatant) {free DNA}	963	
<i>C. albicans</i> BAL 1	dilute	620	
<i>C. albicans</i> BAL 2	?	5347	
<i>H. capsulatum</i> serum	N/A	2761	200 uL

Conclusions:

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- The multiplexed panel contains a broad selection of human - clinically - relevant fungal pathogen targets
- Demonstrates very good analytical performance (specificity & sensitivity)
- Valuable tool used to assess Aspergillosis animal model:
 - Demonstrated that *A. fumigatus* is present at high levels in BAL samples of exposed animals
 - But - we were unable to consistently detect significant levels of dissemination into the bloodstream or CSF.
- Effective detection of clinical BAL associated pathogens
- Continue to test Clinical specimens: BAL, blood & serum

Guinea Pig Model

William R. Kirkpatrick

Laura K. Najvar

Rosie Bocanegra

Nathan P. Wiederhold

Thomas F. Patterson

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Photini Pitsikas

Meredith McLaren

Richard Janeczko

Thank you for your attention

www.luminexcorp.com
