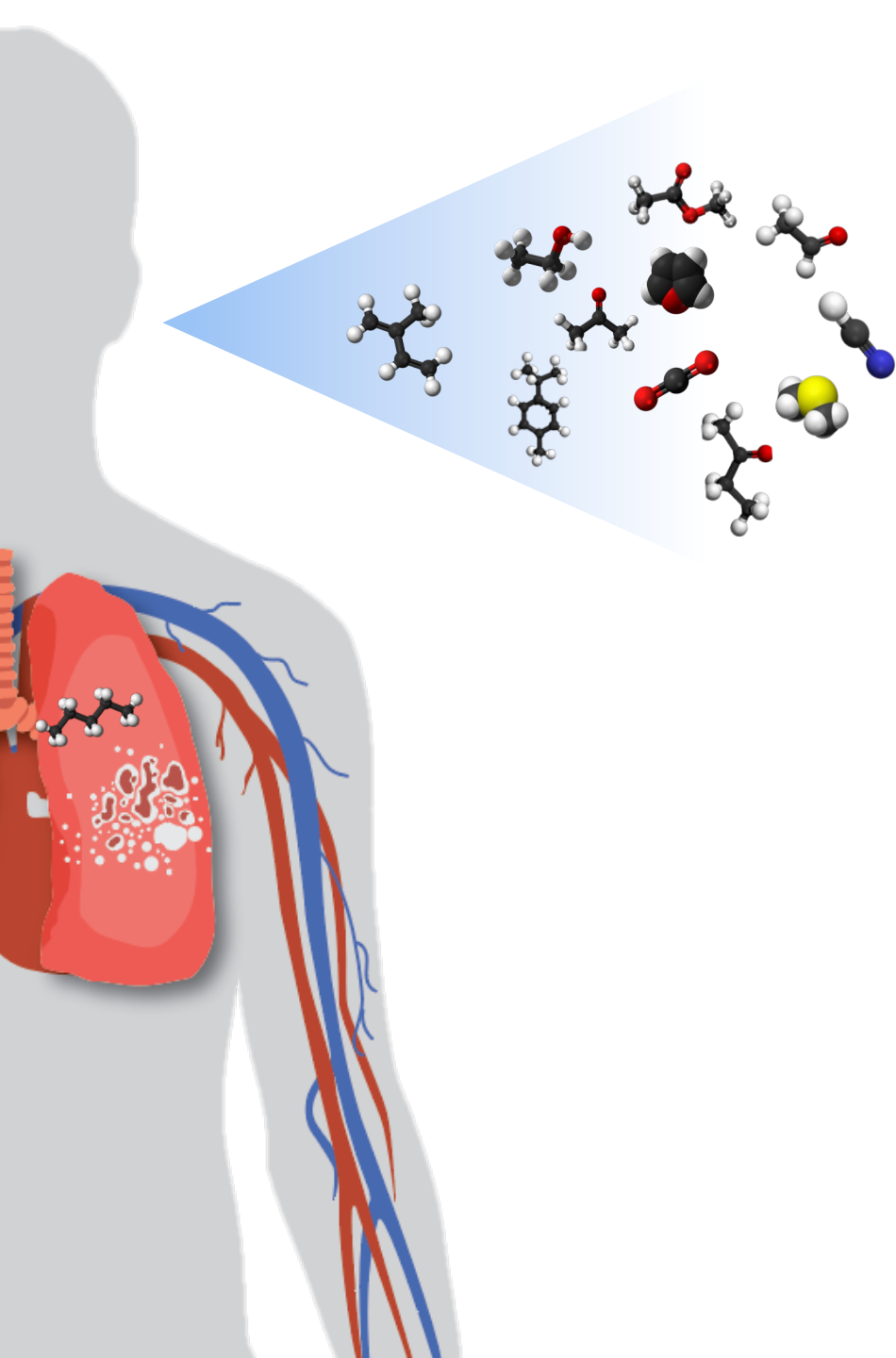


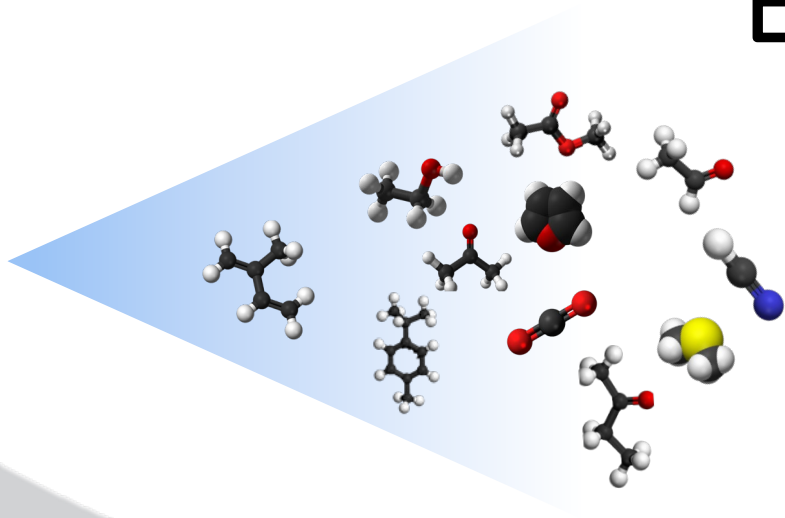
Breath tests to diagnose VF and measure disease burden

Heather D. Bean, PhD
Arizona State University

NASEM Workshop 2022



Exhaled breath



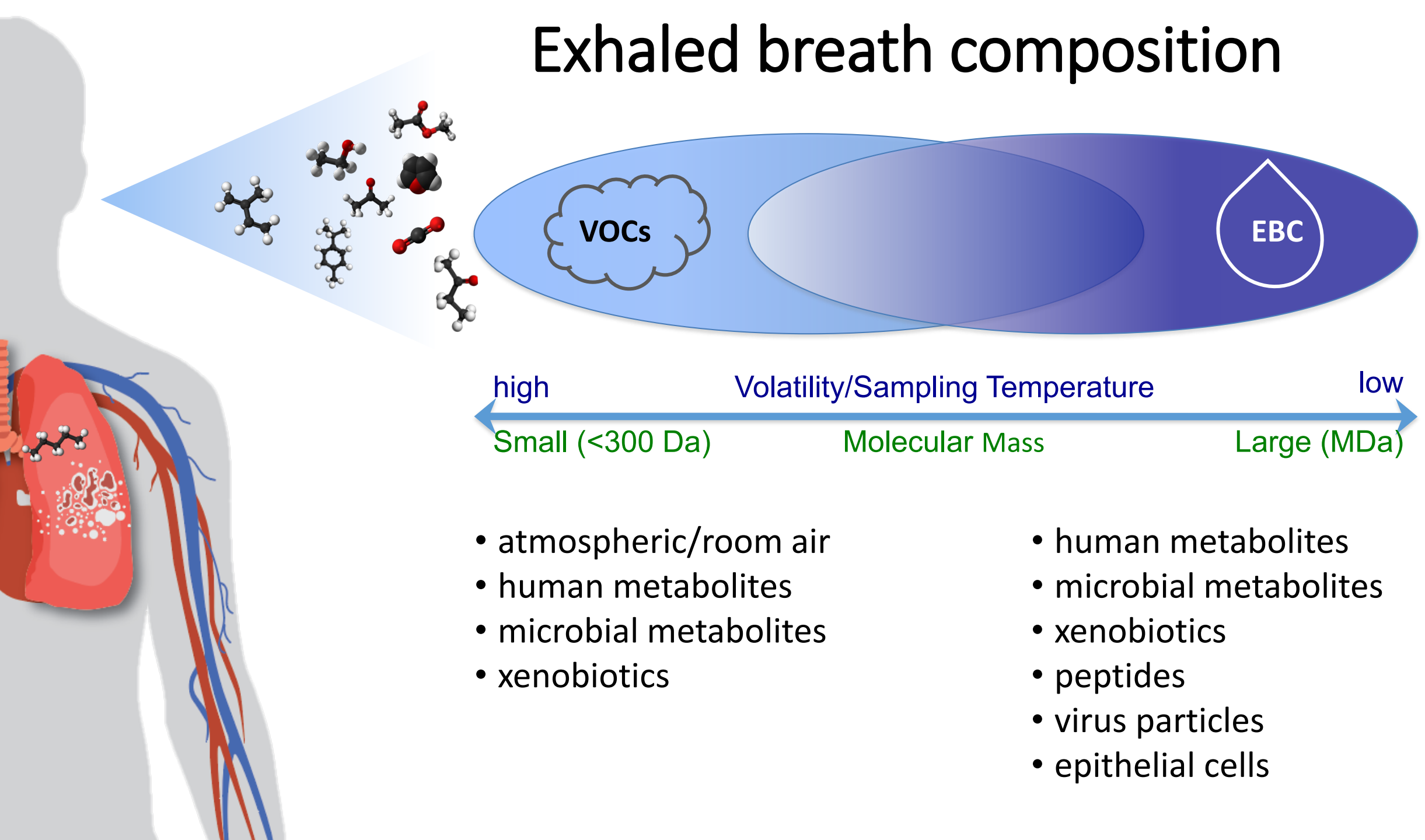
One of the three main waste streams of the body

- Solid waste → feces
- Liquid waste → urine
- Gaseous waste → breath

Headspace of the blood

- Information on health of lungs *and* other systems
- Breathing maneuvers can enrich for lung vs. blood biomarkers

Exhaled breath composition



State of the field – clinical/field use

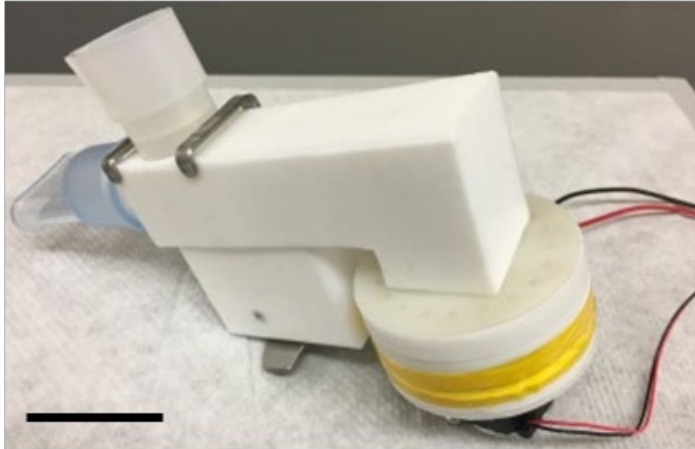
April 14, 2022: FDA issues an EUA for the first COVID-19 diagnostic breath test



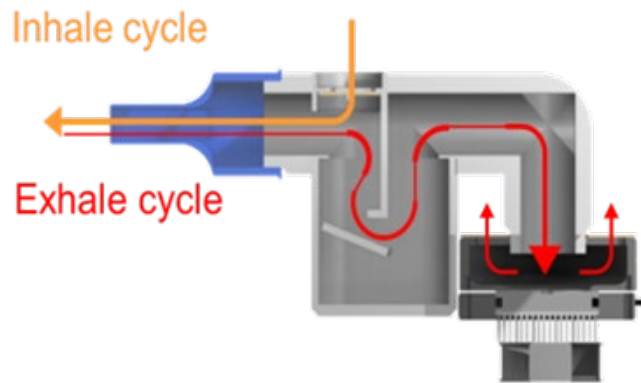
InspectIR COVID-19 Breathalyzer
91% sensitive
99% specific

State of the field – home use

Home collection (near future) & home testing (a little further out)



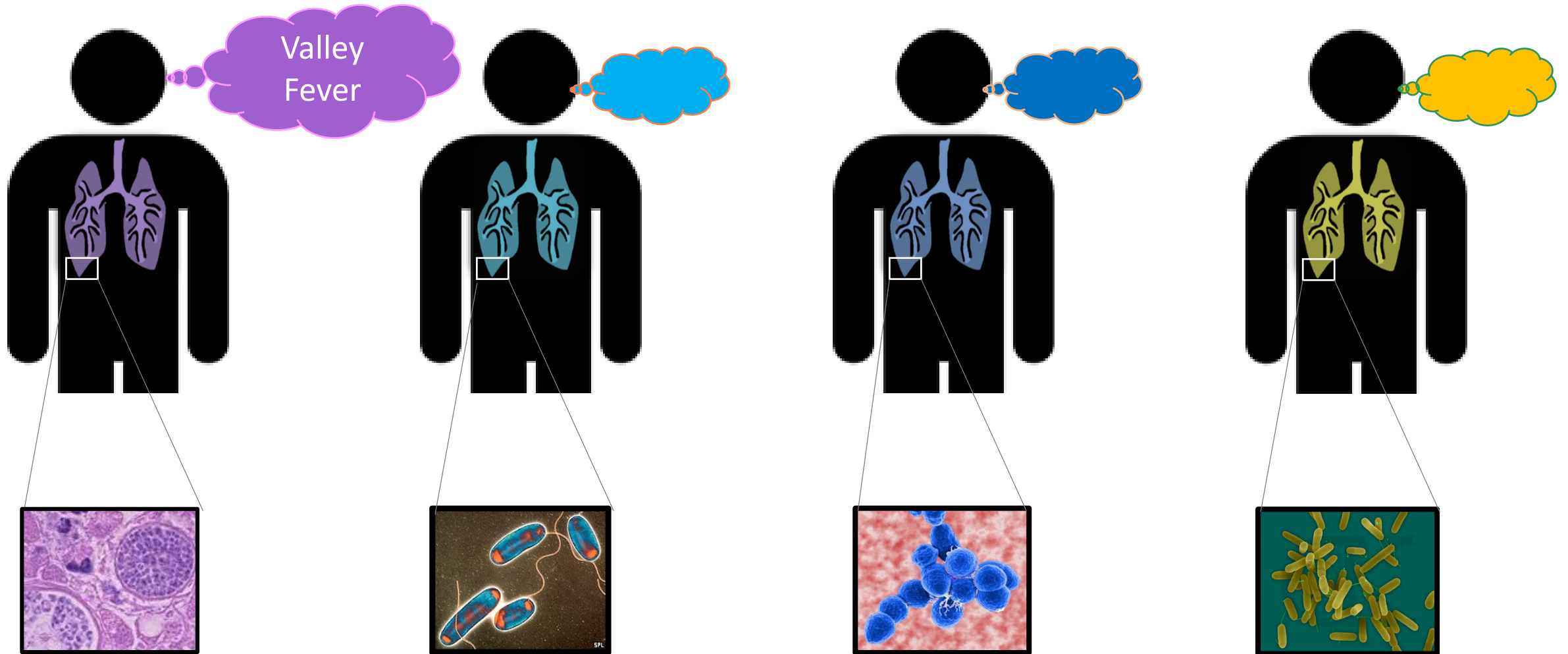
Palm-Sized Breath micro-Condenser
for at-home EBC collection



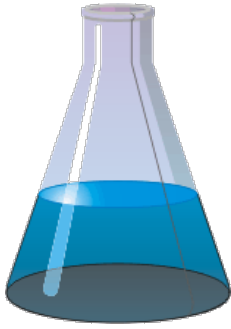
Zamuruyev KO, Bardaweel HK, Carron CJ, Kenyon NJ, Brand O, Delplanque JP, Davis CE. (2014). *Langmuir* 30(33): 10133-10142.
Zamuruyev KO, Schmidt AJ, Borrás E, Schivo M, Kenyon NJ, Delplanque JP, Davis CE. (2018) *Journal of Breath Research* 12: 036020.
Schmidt AJ, Borrás E, Nguyen A, Yeap D, Kenyon NJ, Davis CE. (2020) *Journal of Breath Research* 14(2): 026001.

Developing a breath test for Valley fever

Hypothesis:



Developing a breath test for Valley fever



Study:

In vitro cultures

C. immitis &

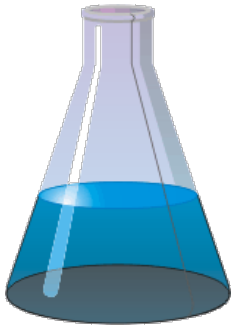
C. posadasii

EA Higgins Keppler et al. 2021

mSphere 6:e00040-21

Collaborators: (ASU) Emily Higgins Keppler, Doug Lake, Mitch Magee; (Mayo) Tom Gryns; (NAU) Bridget Barker, Heather Mead, Marley Caballero Van Dyke

Developing a breath test for Valley fever



Study:

In vitro cultures

C. immitis &
C. posadasii

EA Higgins Keppler et al. 2021
mSphere 6:e00040-21

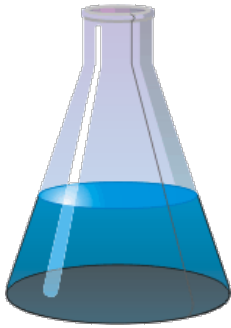
Study:
Murine
model lung
infections



EA Higgins Keppler et al.
In revision

Collaborators: (ASU) Emily Higgins Keppler, Doug Lake, Mitch Magee; (Mayo) Tom Gryns; (NAU) Bridget Barker, Heather Mead, Marley Caballero Van Dyke

Developing a breath test for Valley fever



Study:

In vitro cultures

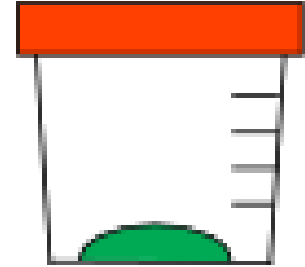
C. immitis &
C. posadasii

EA Higgins Keppler et al. 2021
mSphere 6:e00040-21

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Murine
model lung
infections



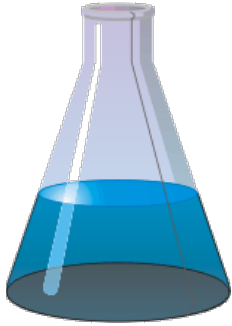
EA Higgins Keppler et al.
In revision



Study:
Human
specimens

In preparation

Collaborators: (ASU) Emily Higgins Keppler, Doug Lake, Mitch Magee; (Mayo) Tom Gryns; (NAU) Bridget Barker, Heather Mead, Marley Caballero Van Dyke



Study: *In vitro* cultures

Medium: RPMI 1640 with 10% FBS

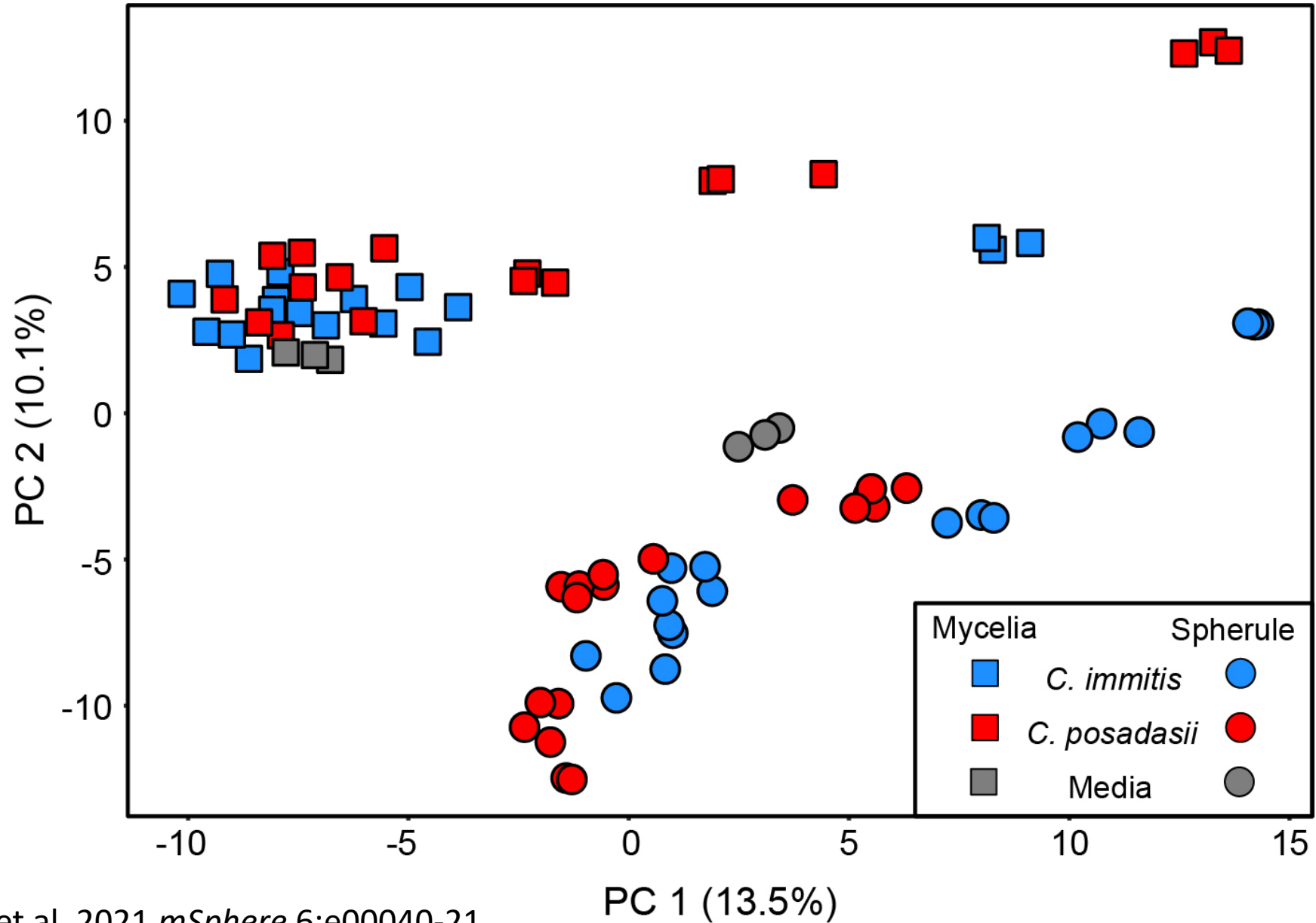
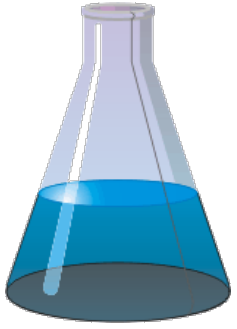
Spherules ○ : 39°C, 10% CO₂, 96 h

Mycelia □: 30°C, normoxic, 96 h

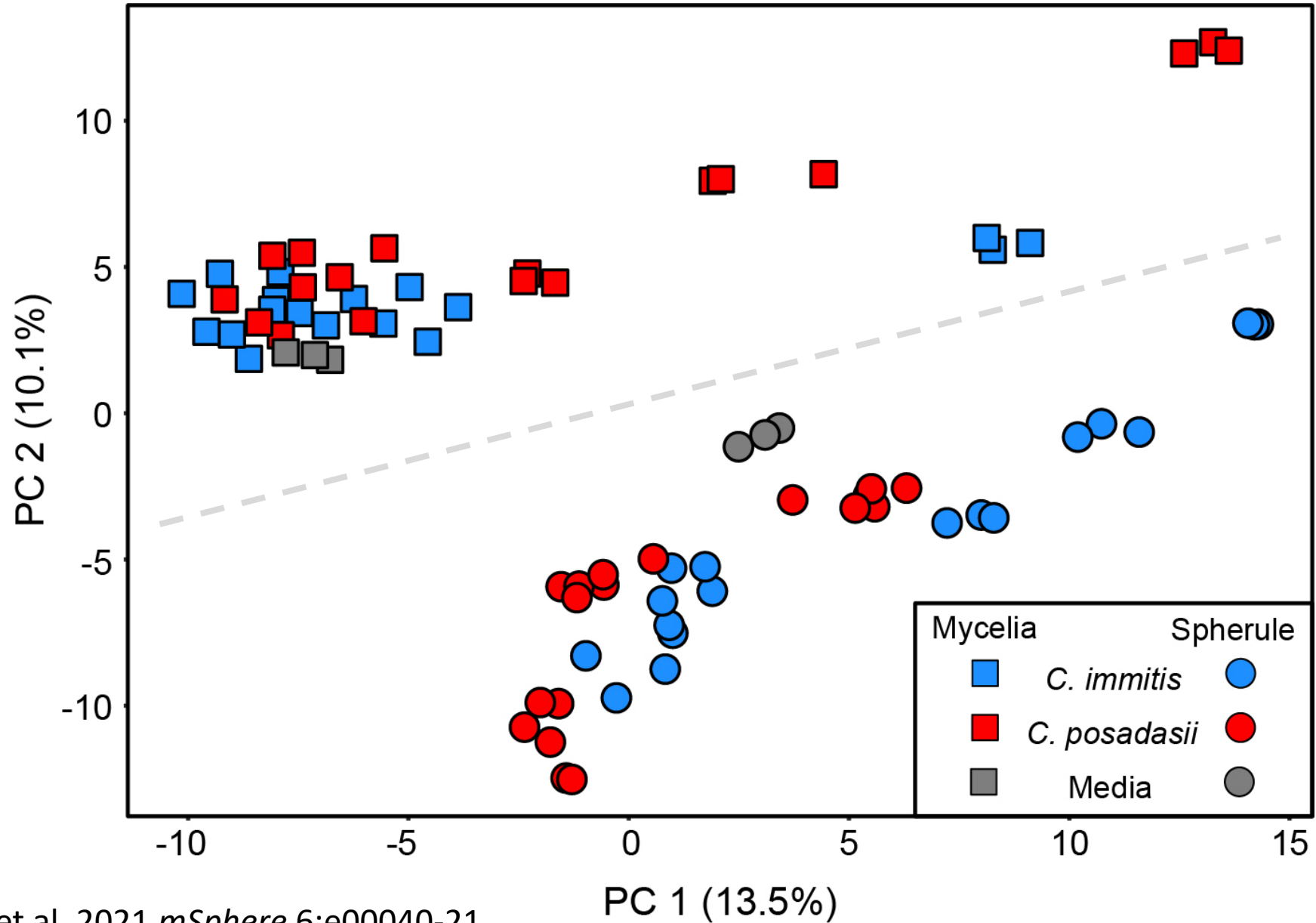
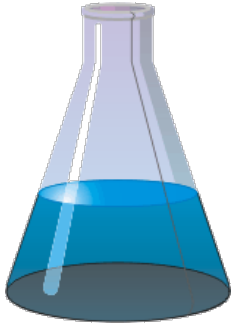
Samples collected in triplicates

<i>C. posadasii</i>		<i>C. immitis</i>	
AZ	TX/MEX/SA	SJV	SD/MX
Silveira	RMSCC2343	RMSCC2006	RS
B3221	RMSCC3506	RMSCC2009	RMSCC2395
B3222	GT-166	RMSCC2010	RMSCC3505

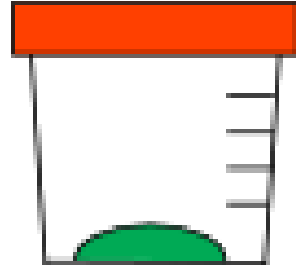
VOC are not species-specific



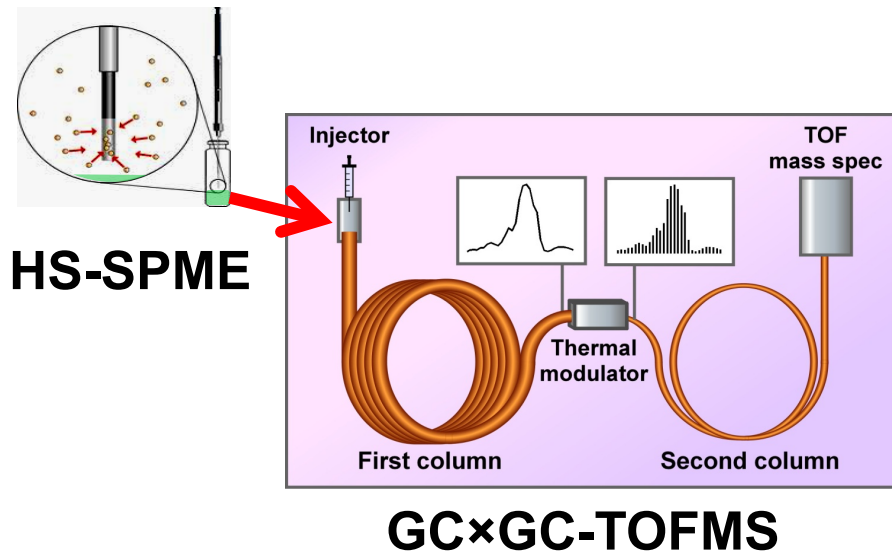
VOC are not species-specific



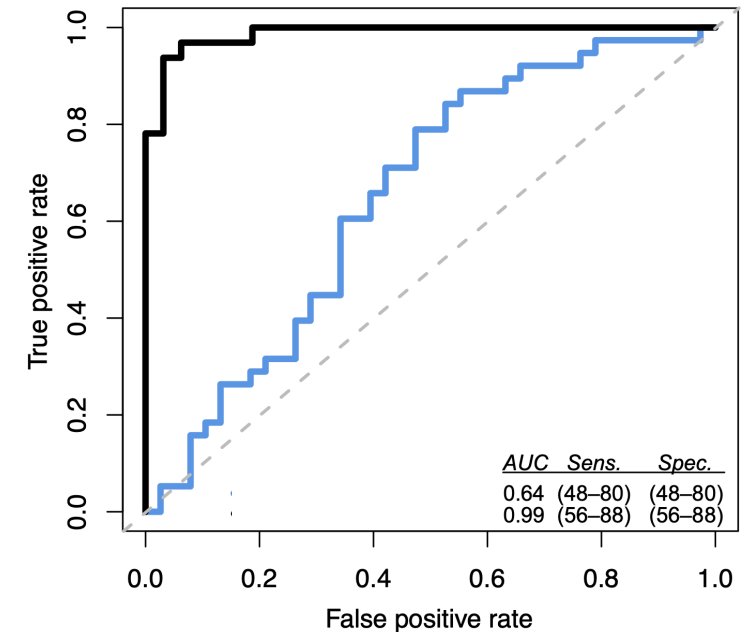
Study: Human bronchoalveolar lavage fluid



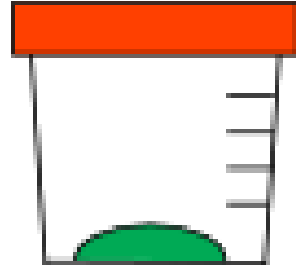
BALF from 55 subjects
Triplicate samples (200 μ l)



Random Forest
feature
selection



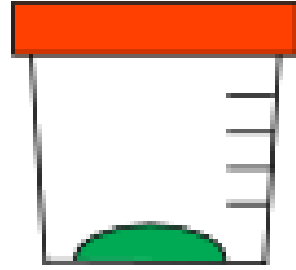
Study: Human bronchoalveolar lavage fluid



BALF from 55 subjects
Triplicate samples (200 μ l)

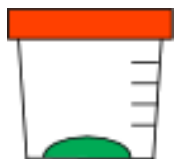
Infection Category	Subjects	Samples
No infection	12	36
Viral	3	9
Bacterial (+NTM & <i>Nocardia</i>)	21	62
Cocci	17	49
Other fungal	2	6

Study: Human bronchoalveolar lavage fluid



BALF from 55 subjects
Triplicate samples (200 μ l)

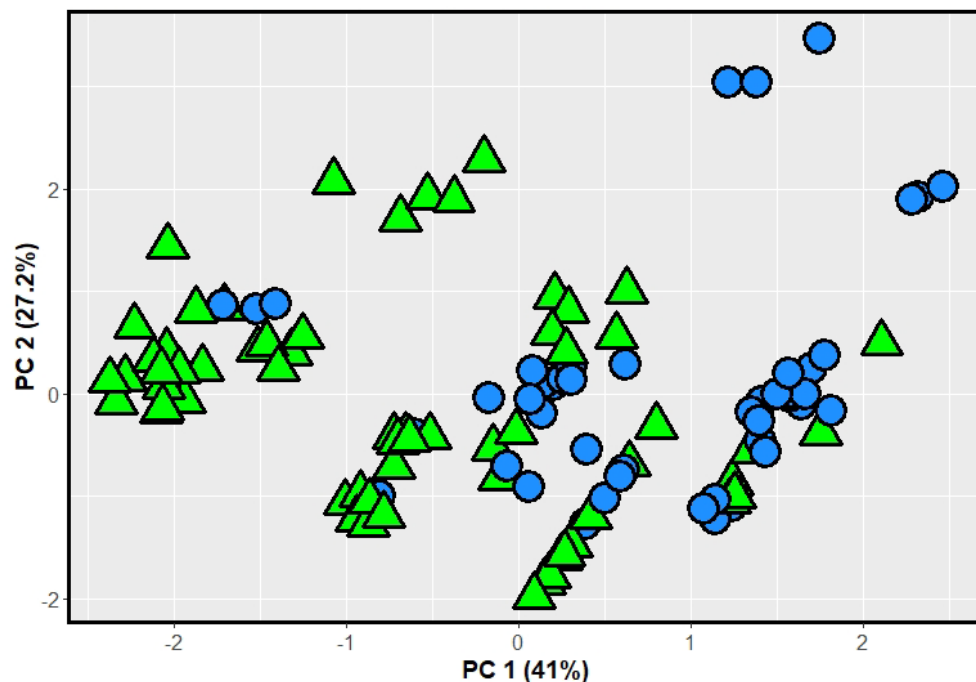
Infection Category	Subjects	Samples
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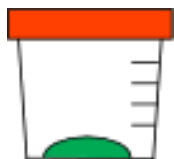
Lung VOCs classify Cocci (Fungal) vs. Bacterial infections

49 Cocci vs. 62 Bacterial

V150, 536, 98, 255



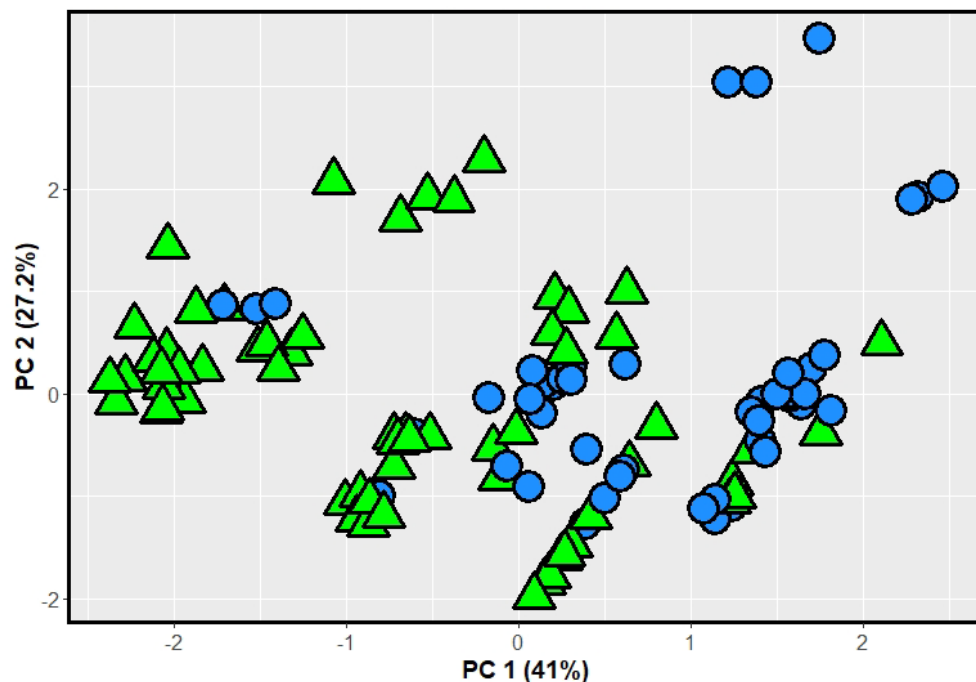
	P(C)	P(B)	Class Error
C	42	7	0.143
B	1	61	0.016



Lung VOCs classify Cocci (Fungal) vs. Bacterial infections

49 Cocci vs. 62 Bacterial

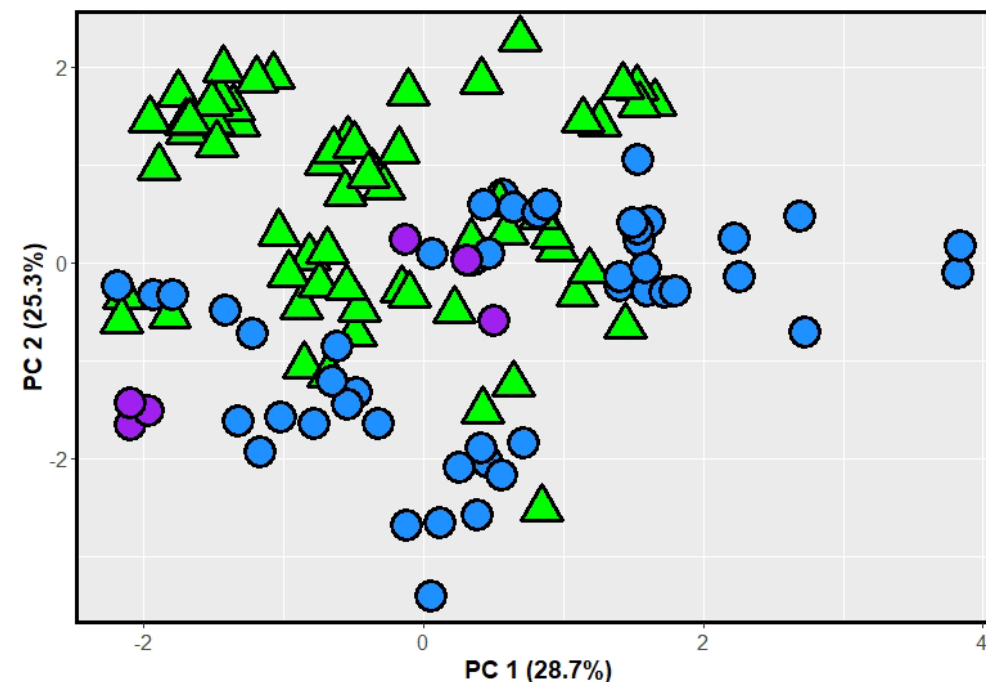
V150, 536, 98, 255



	P(C)	P(B)	Class Error
C	42	7	0.143
B	1	61	0.016

49 Cocci + 6 Fungal vs. 62 Bacterial

V150, 536, 98, 109, 255, 41



	P(C+F)	P(B)	Class Error
C+F	52	3	0.055
B	1	61	0.016



Female C57BL/6 mice

Study: Murine infections

Intranasal inoculation of 100 conidia in PBS

6 mice *C. immitis* RS

6 mice *C. posadasii* Silveira

4 mice PBS vehicle control

~2 mL BALF collected at 10 days PI



Female C57BL/6 mice

Study: Murine infections

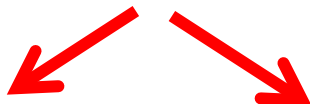
Intranasal inoculation of 100 conidia in PBS

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6 mice *C. posadasii* Silveira

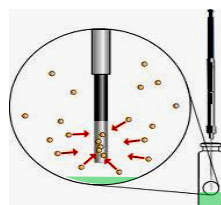
4 mice PBS vehicle control

~2 mL BALF collected at 10 days PI

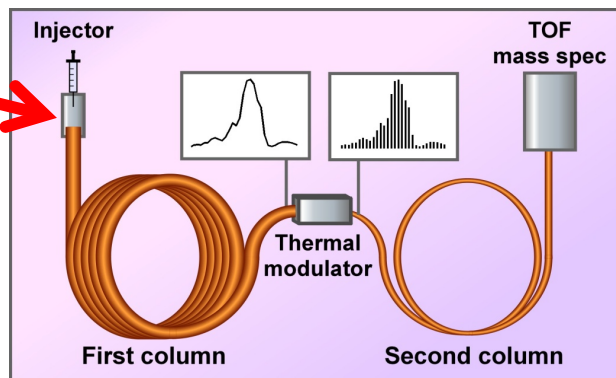


Volatile biomarkers

1 mL BALF split into
technical triplicates



HS-SPME



GCxGC-TOFMS

Cytokines

1 mL BALF analyzed with
26-Plex Mouse
ProcartaPlex™ panel





Female C57BL/6 mice

Study: Murine infections

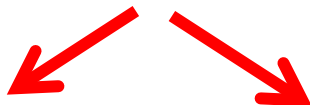
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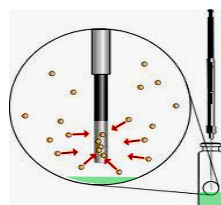
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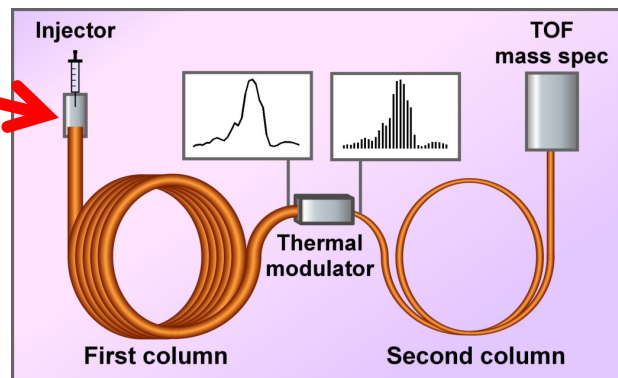


Volatile biomarkers

1 mL BALF split into technical triplicates



HS-SPME



GCxGC-TOFMS

Cytokines

1 mL BALF analyzed with
26-Plex Mouse
ProcartaPlex™ panel



Disseminated disease

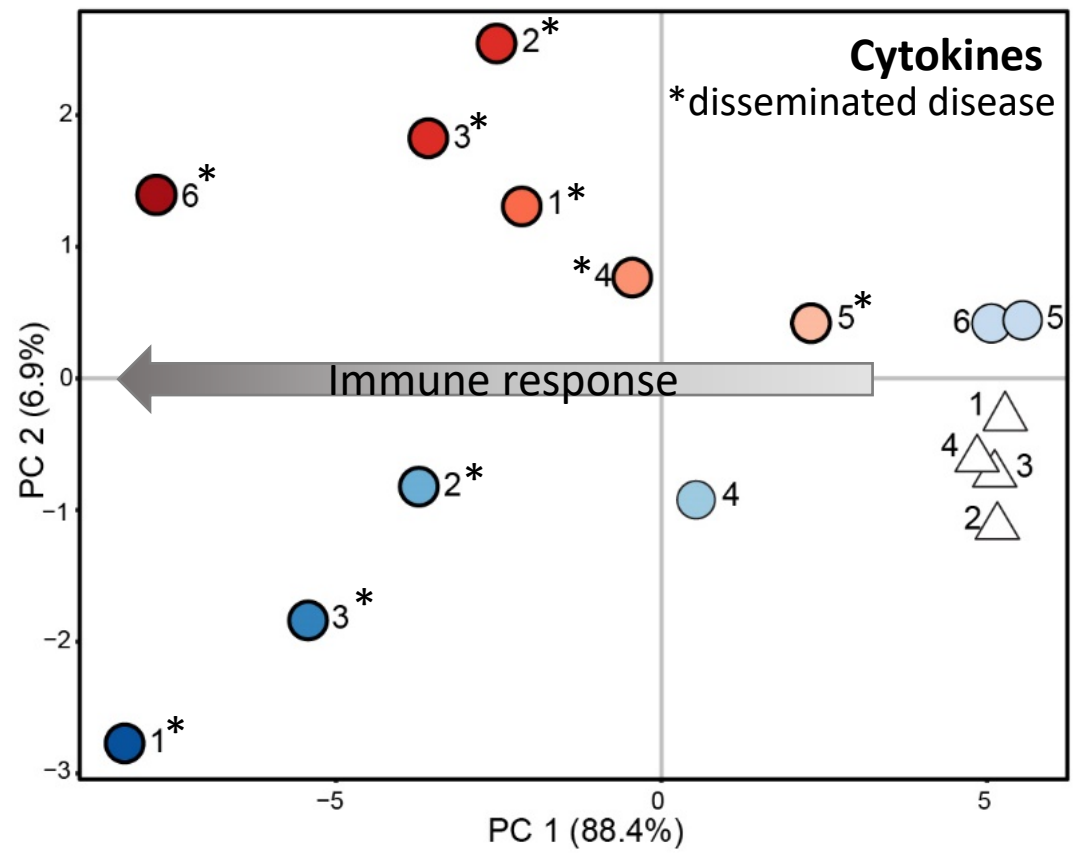
Mouse spleen & brain
quantified for fungal
dissemination





Lung VOCs correlate to immune response (& severity?)

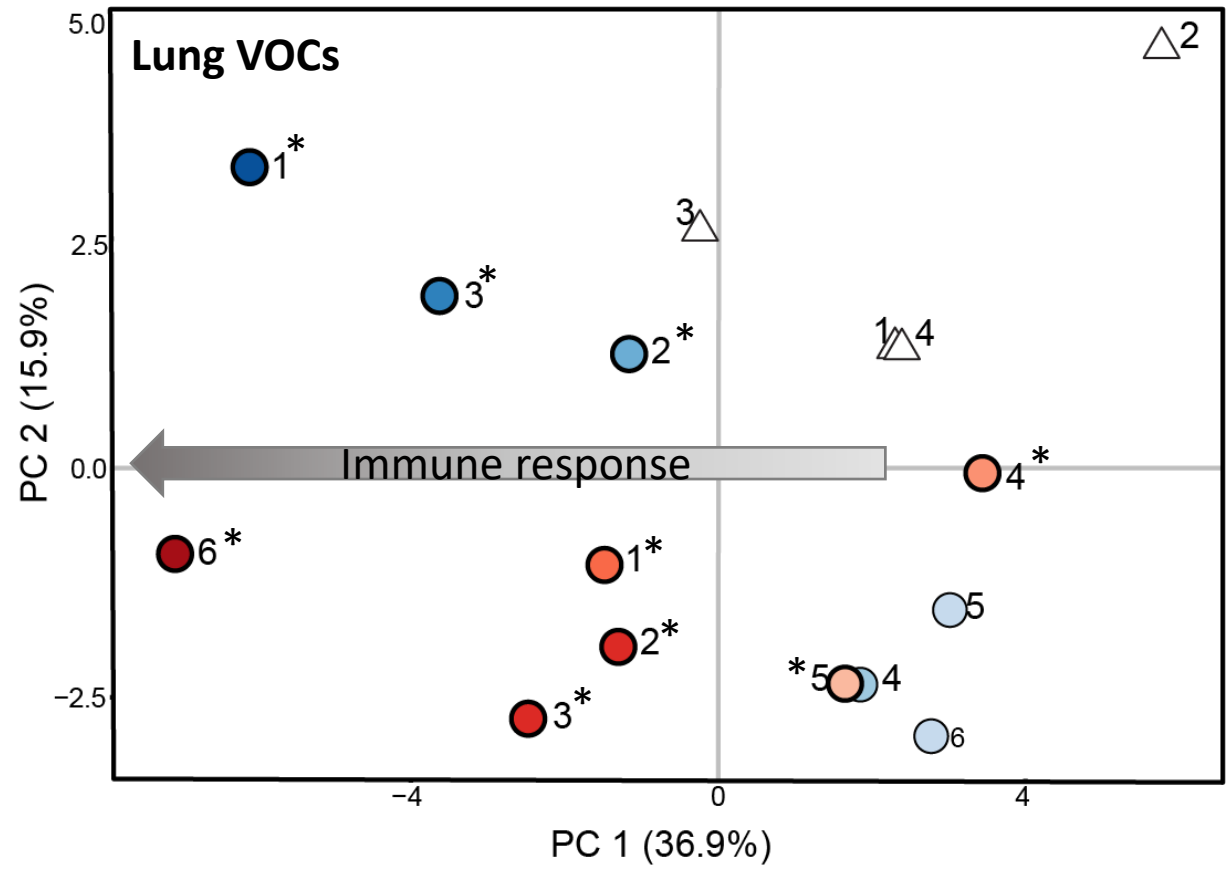
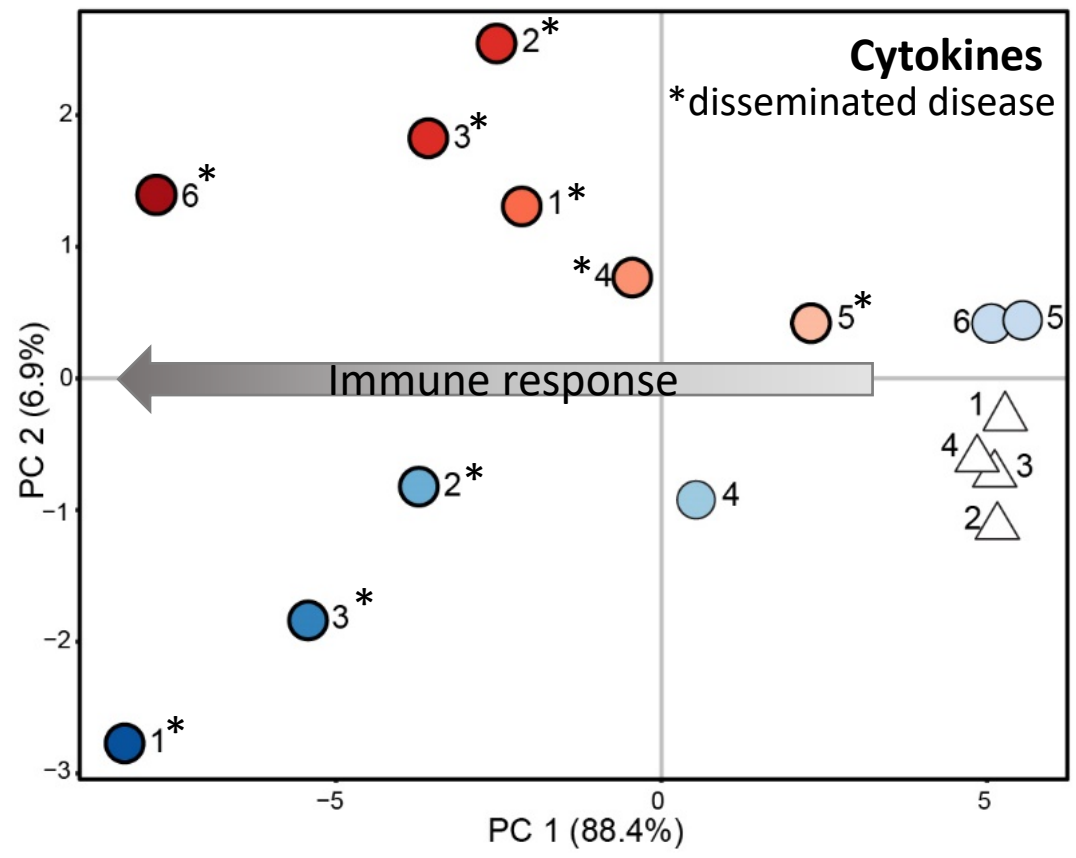
- 6 mice with *C. posadasii*
- 6 mice with *C. immitis*
- △ 4 controls



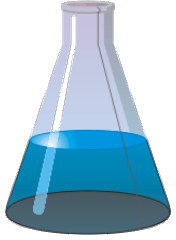


Lung VOCs correlate to immune response (& severity?)

- 6 mice with *C. posadasii*
- 6 mice with *C. immitis*
- △ 4 controls

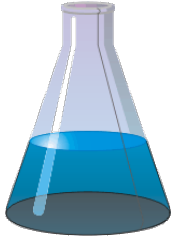


Developing a breath test for Valley fever

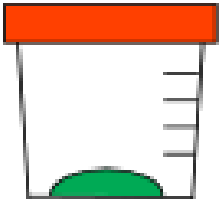


- *Coccidioides* does not have a species-specific VOC profile
 - ✓ Universal breath test for VF is feasible

Developing a breath test for Valley fever

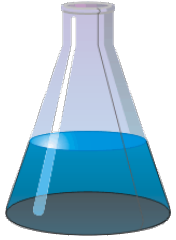


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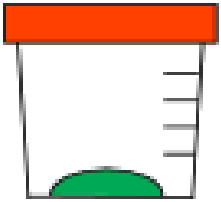


- VOCs differentiate Cocci (fungal) vs. bacterial pneumonia
 - ✓ Reduce improper diagnosis and antibiotic use

Developing a breath test for Valley fever



- *Coccidioides* does not have a species-specific VOC profile
 - ✓ Universal breath test for VF is feasible



- VOCs differentiate Cocci (fungal) vs. bacterial pneumonia
 - ✓ Reduce improper diagnosis and antibiotic use



- Valley fever VOCs correlate to immune response
- May correlate to disease severity
 - ✓ Breath test may facilitate antifungal stewardship

Thank you!



Emily Higgins Keppler
D. Mitch Magee, Ph.D.
Douglas Lake, Ph.D.



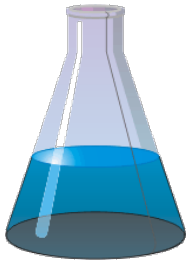
Thomas Grys, Ph.D.



Bridget M. Barker, Ph.D.
Heather L. Mead, Ph.D. (TGen North)
Marley C. Caballero Van Dyke, Ph.D. (UTSW)



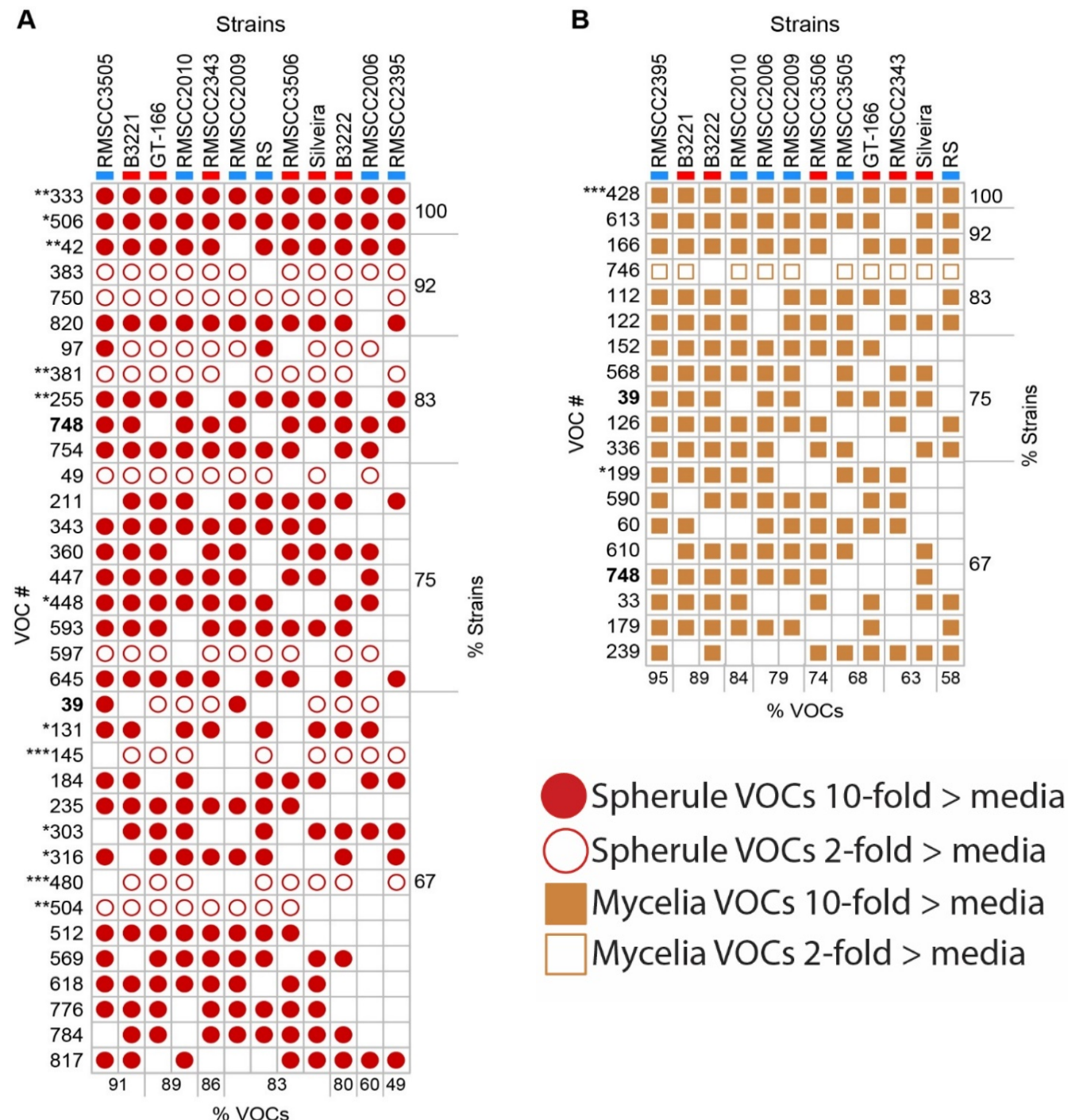
ABRC New Investigator Award



VOCs in 2/3 of strains

35 of 272 (13%)
spherule VOCs

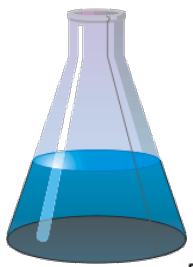
19 of 224 (8%)
mycelia VOCs



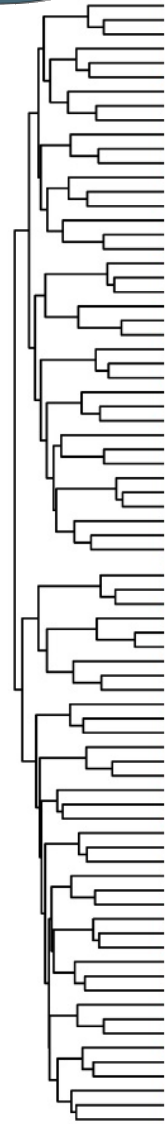


Samples





Samples

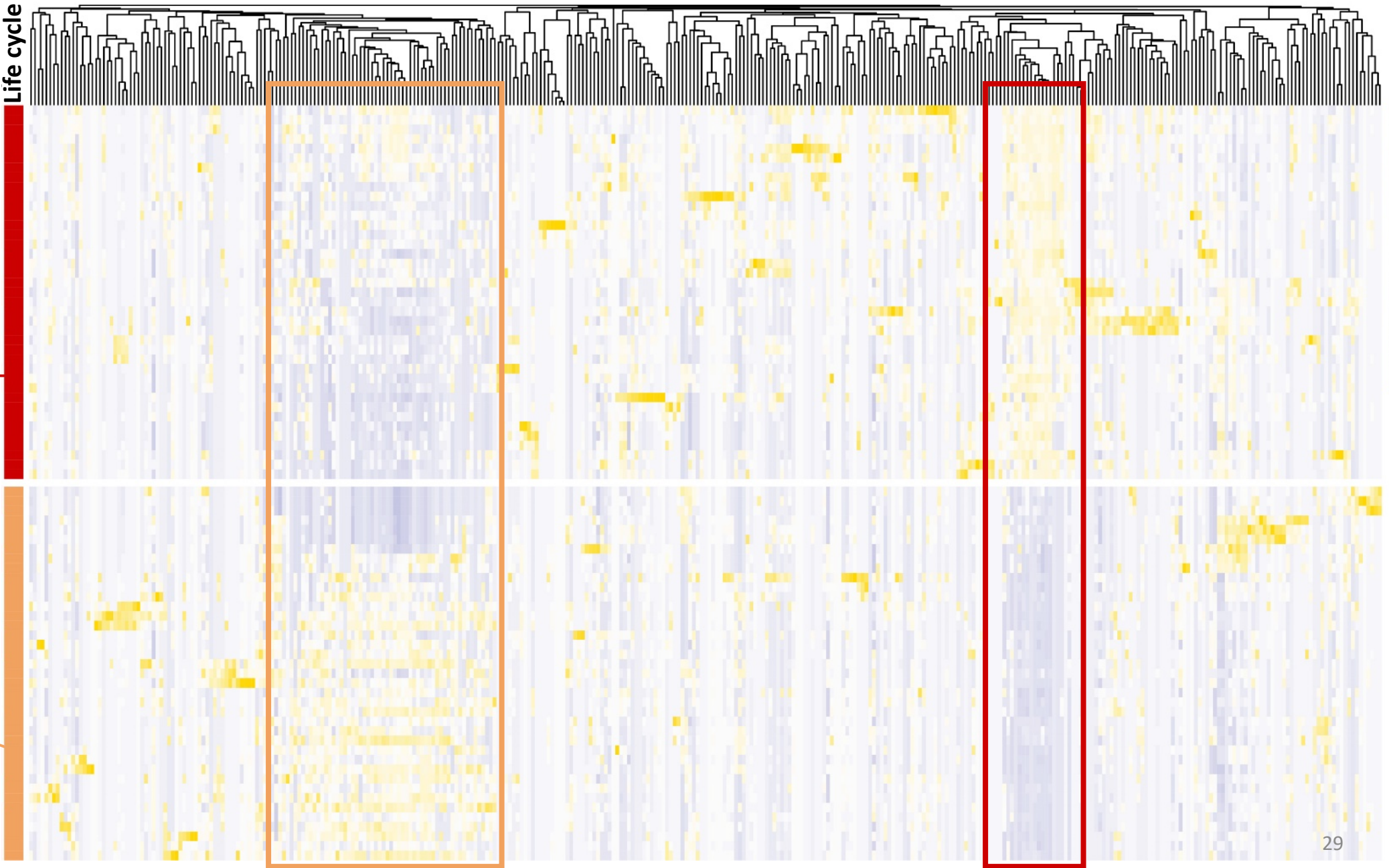


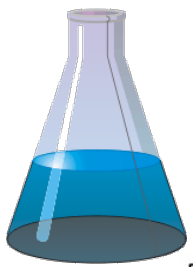
Mycelia

Spherule

Life cycle

Volatiles





Samples

Volatiles

