Use of sniffing animals in diagnostic microbiology

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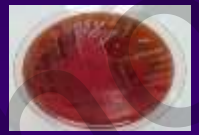
No conflict of interest

What do you smell in a microbiology laboratory?

As a microbiologist, were you taught with smelling?

- To recognize
  - *Pseudomonas aeruginosa*
  - *Clostridium difficile*
  - *Haemophilus influenzae*
  - *Streptococcus milleri*
  - *Staphylococcus aureus*
  - *Mycobacterium sp.* rapid growers (*M.smegmatis, M. chelonae*…)
  - *Cancida albicans*
  - Anaerobes





Animal sniffing is an innate behavior

- Dogs olfactory acuity is over 100 000 times stronger than human
- Animal sniffing (dog, rats) serves for
  - detecting bombs
  - detecting persons
  - detecting drugs

Dogs snif

- Dogs snif cancer nodules !
  - 90% sensititivy and specificity
  - biases as insufficient controls, blindness,
- Dogs may snif stools positive with *Clostridium difficile*
  - 2 dogs
  - 300 samples: 30% positive, 70% negative samples.
  - response as either positive (dog sits) or negative (dog does not sit)
  - Kappa 0.52 only

Sensitivity (95% CI)	Specificity (95% CI)
77.6 (67.3–86.0)	85.1 (79.6–89.6)
92.6 (84.6–97.2)	84.5 (79.0–89.0)

Bomers MK 2012, 2014; Guirao A 2018; Taylor MT 2018

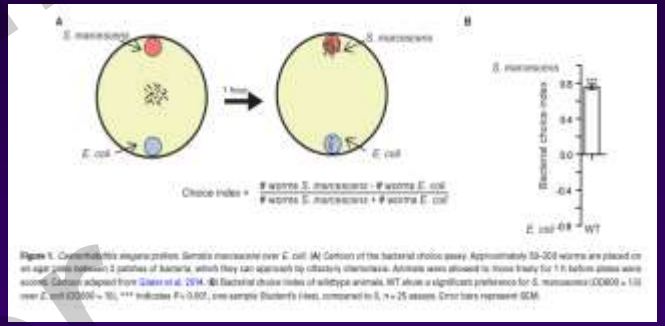
Dogs scenting UTI

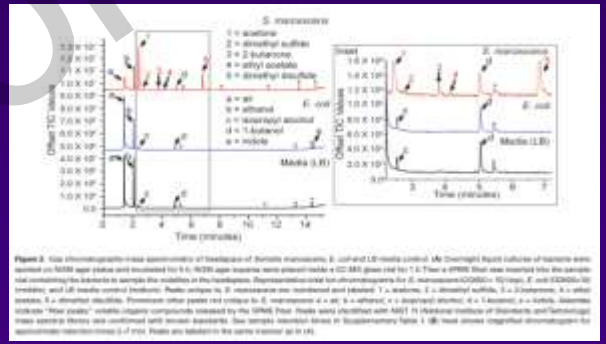
- Double blind, case-control study
- 687 persons, 5 dogs and handlers
- 10^5/ml culture-positive *(E. coli, K. pneumoniae, S. aureus, E. faecalis)*:
  - sensitivity 99.6%
  - specificity 91.5%

Maurer M et al. Open Forum Infectious Diseases 2016; 9:ofw051.

Worms may snif as well!

- *Caenorhabditis elegans* is more attracted by *Serratia marscescens* than *Escherichia coli* due to olfactory preferences
- GC-MS analysis: 5 VOCs specific for Serratia





Worthy SE et al., 2018

Rare applications so far

African giant-pouched rats
Cricetomys gambianus

- Started in 2007 in Tanzania
- conducted byAPOPO (Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling) with rats trained for detecting landmines
- Trained rats can snif 100 samples per 20 min
- Rats were rewarded with banana if they paused for at least 5 s to sniff TB-positive sputum

Figure 2 A trained rat indicating a positive sample by keeping the nose in the sniffing hole with the test sample underneath.

Weetjens B. et al. 2009; Mgode GF, 2012

Rats and tuberculosis

- Over 100,000 samples were tested
- trained rats
- Overall performances:
  - sensitivity : 60%
  - Specificity: 80%

Table 3 Sensitivity of detection rats compared to Ziehl-Neelsen smear microscopy

	TP	FN	Sensitivity *(95% CI)*
Group 1	748	56	93.0 *(91.0–94.7)*
Group 2	845	55	93.9 *(92.0–95.3)*

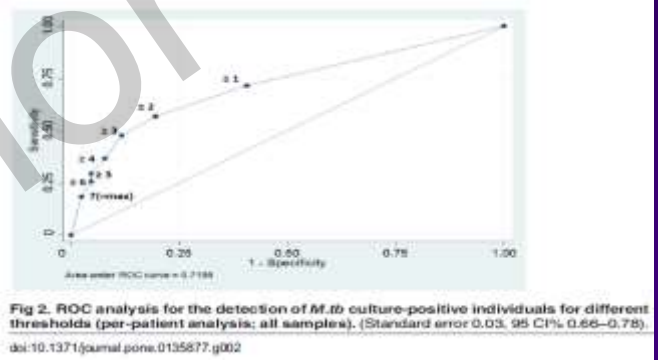


Fig 2. ROC analysis for the detection of *M.tb* culture-positive individuals for different thresholds (per-patient analysis; all samples). (Standard error 0.03, 95 CI% 0.66–0.78).

doi:10.1371/journal.pone.0135877.g002

Weetjens B. et al. 2009; Mgode GF et al. 2012; Poling A et al. 2015, Reither K. et al. 2015, www.apopo.org

Reproducibility of Giant rats sniffing TB-positive sputum

- 12,000 samples, 22 rats, 18 months, 10% TB+
- Good reproducibility if rats are well trained
- Good if intra-rat (0.7-0.97)
- Only fair if inter-rat (0.23-0.34)

Ellis H et al. BMC 2017

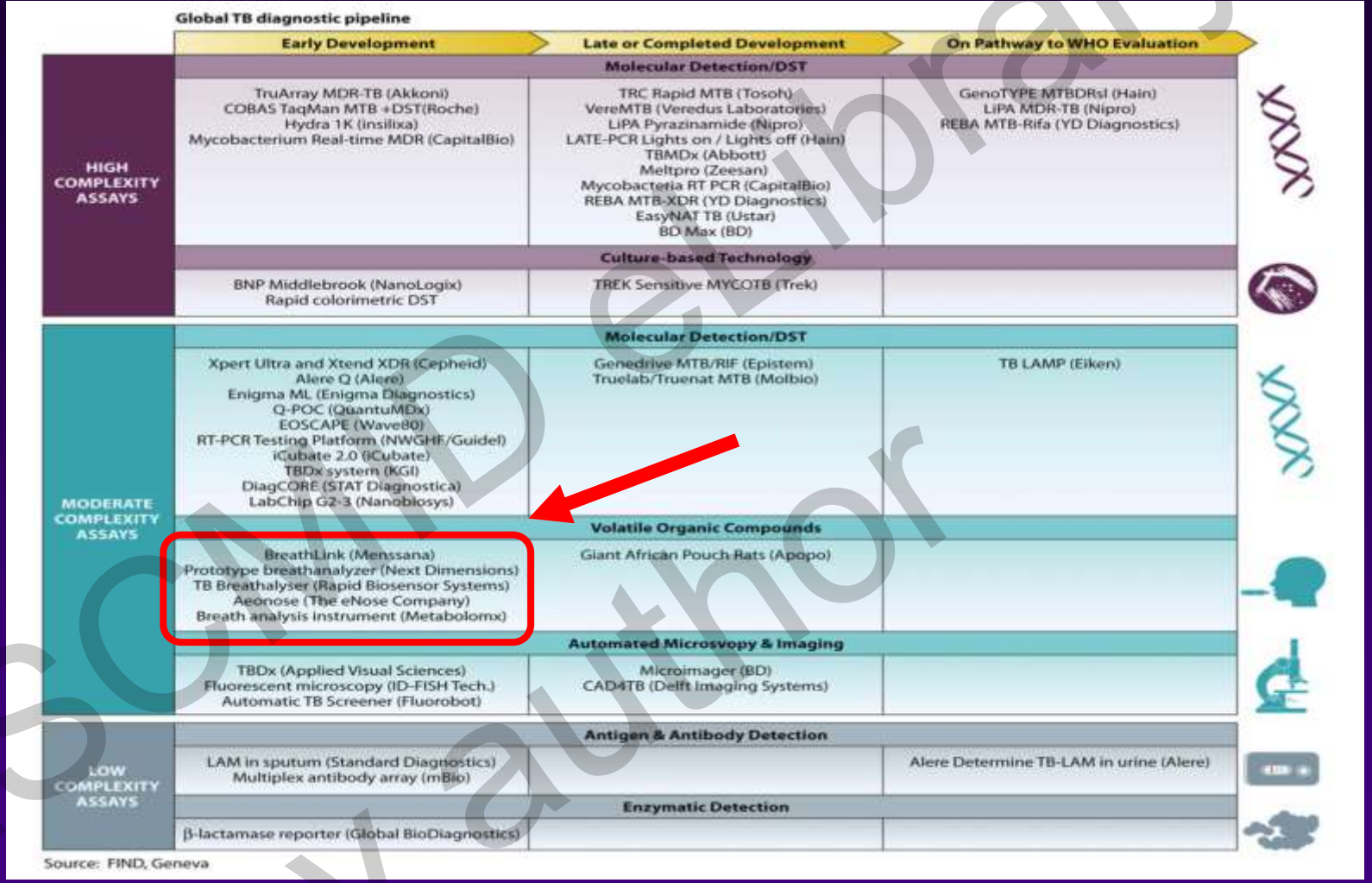
Specificity of the volatile compounds for *M. tuberculosis*?

TABLE 3 Volatile compounds of isolates from sputum samples and reference *M. tuberculosis*, *Nocardia* spp., and *Streptomyces* spp.

Compound[a]	Microorganism tested (no. of isolates)								
	Mycobacterium tuberculosis (35)	*Rhodococcus* isolate (2)	*Staphylococcus* isolate (2)	*Candida* isolate (2)	*Nocardia asteroides* (4)	*Nocardia africana* (6)	*Streptomyces coelicolor* (3)	*Streptomyces antibioticus* (3)	*Streptomyces griseoflavus* (4)
Dimethyl disulfide			X						
Dimethyl trisulfide		X	X	X			X	X	X
Dimethyl tetrasulfide		X					X	X	
Methyl methanethiosulfonate		X							
2,3-Dimethyl-5-isopentylpyrazine		X	X	X					
Unknown pyrazine		X	X	X					
Camphor	X						X		
Linalyl acetate							X		
Isobornyl acetate			X						
Aciphyllene					X	X			
Unknown diterpenoid					X	X			
2-Hydroxy-3-butanone							X	X	X
2-Hydroxy-3-pentanone	X						X	X	X
2,5-Dimethylthiopene								X	X
1-Hexanol	X								X
1-Octanol									X
4-Methyl-2-pentanone							X		X
4-Methylpent-3-*en*-2-one							X		
γ-Methylbutyrolactone	X				X				
2-Phenylethanol	X						X	X	
Ethyl phenylacetate							X		X
Methyl phenylacetate*	X								
Methyl nicotinate*	X								
Methyl *para*-anisate*	X								
ortho-Phenylanisole*	X								

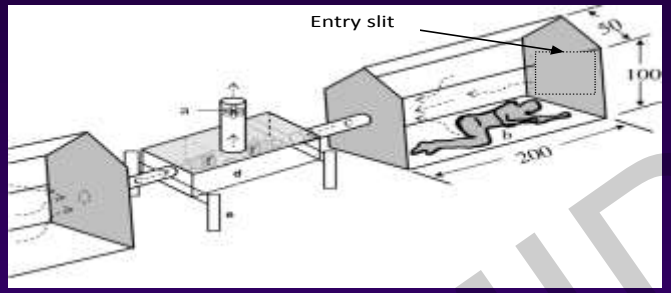
[a] *, See Syhre and Chambers (33).

Mgode GF, Journal of Clinical Microbiology 2012; 50: 274-280



Pai M, Schito M. J Infect Dis 2015;211:S21-8.

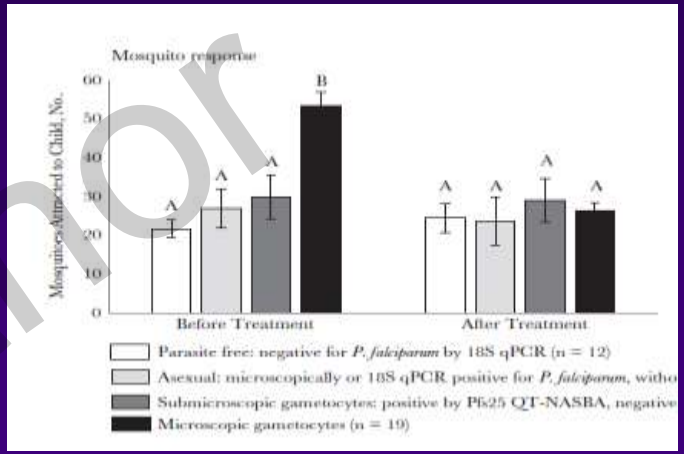
Mosquitoes snif Malaria



Researchers developing a new device to diagnose malaria in children by detecting volatile organic biomarkers in expired breath won $10 000 to advance their technology.

Busula AO, et al. 2017

- Dual choice olfactometer measuring the attraction of *Anopheles gambiae*
- 50 children: negative, carriers for gametocytes or not, 3h in a tent



=> Attraction of mosquitoes for gametocyte-carriers

Breath pattern in malaria-infected children

- Descriptive prospective case-control study
- 17 Pf+
- 18 Pf-

Schaber CH et al. 2018

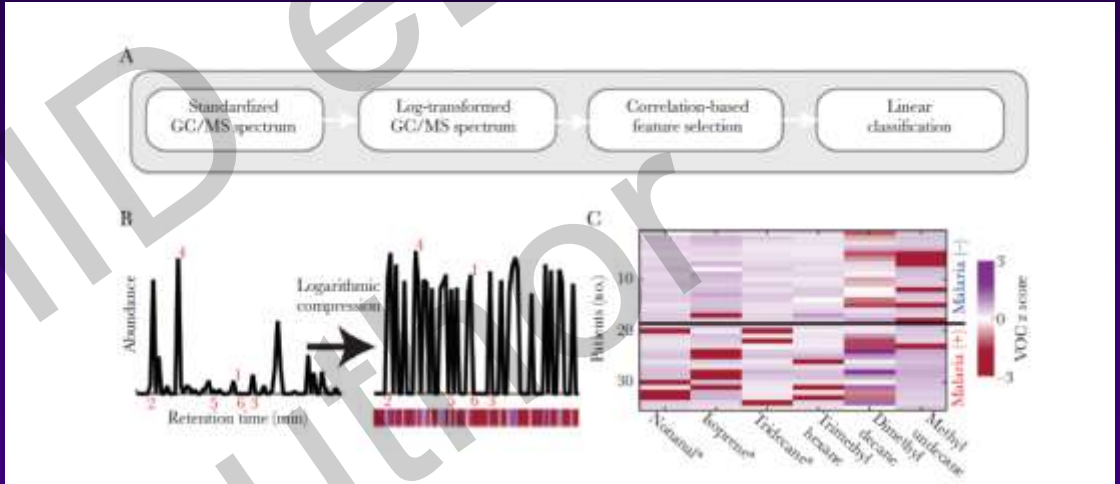


Figure 1. Presence of malarial infection corresponds with an altered breath VOC profile. *A*, Schematic of the analytical approach followed to classify breath samples. *B*, Representative total ion chromatograph determined by gas chromatography–mass spectrometry (GC/MS). After removal of contaminants and normalization, a $\log_2$ operator was used to compress the abundance values obtained from each subject. For visualization, the abundance of each volatile organic compound (VOC) is represented as a color bar shown below the GC/MS chromatograph. Red numbers indicate the 6 VOCs with the largest absolute correlations. *C*, *z* score abundances for the 6 VOCs with the highest absolute correlation with malarial infection status are shown. Compound structures and properties are specified in Supplementary Table 1. –, negative; +, positive. *Compound identity confirmed by comparison to pure standard.

Differences in abondance of VOCs in Pf+ and Pf- children (eg isoprene, acetone)

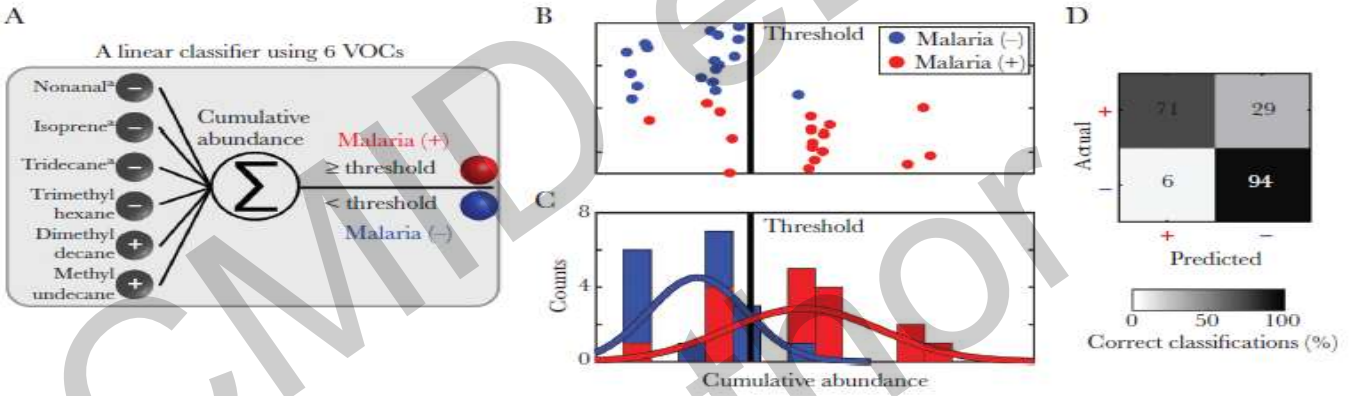


Figure 2. Accurate classification of falciparum malarial infection status achieved with 6 volatile organic compounds (VOCs) from breath specimens. *A*, Schematic of the classification approach. The internal standard normalized abundance values of the 6 VOCs are linearly combined to create a cumulative abundance metric. Negatively correlated VOCs are subtracted rather than added. *B*, Cumulative abundance of the 6 VOCs across all subjects shows clear separation between the 2 populations. *C*, Distribution of cumulative abundance of biomarkers from children with (red) or without (blue) falciparum malaria. *D*, Confusion matrix of actual and predicted malarial infection status. Displayed are the percentages of patients in each class. A total of 83% of classifications were correct, with a specificity of 94% and sensitivity of 71%. [a]Compound identify confirmed by comparison to pure standard.

« Electronic nose » detecting volatil organic compounds (VOC)

- Studies started in the 1990's
- Identification of bacterial cultures
- AirSense Portable Electronic Nose (PEN2) system
- Electrospray isonization- mass spectrometry (SESI-MS)
- gas chromatography–mass spectrometry (GC-MS)
- Headspace sorptive extraction (HSSE)

Moens M 2006, Zhu J 2010, Bos LD 2013, SethiS 2013, Devaraj H 2018

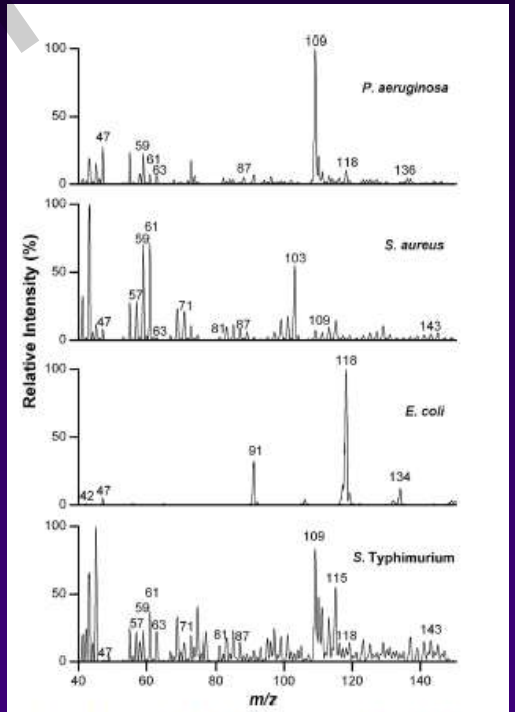


FIG. 1. Positive-ion-mode full-scan spectra (*m/z* of 40 to 150) of bacterial culture headspace for *P. aeruginosa*, *S. aureus*, *E. coli*, and *S.* Typhimurium grown aerobically in TSB at 37°C for 24 h. Every spectrum represents an average of spectrum values for nine samples (three biological replicates, each with three technical replicates), with the media blank subtracted and normalization to the peak of greatest intensity.

Specificity of VOCs / main bacterial pathogen

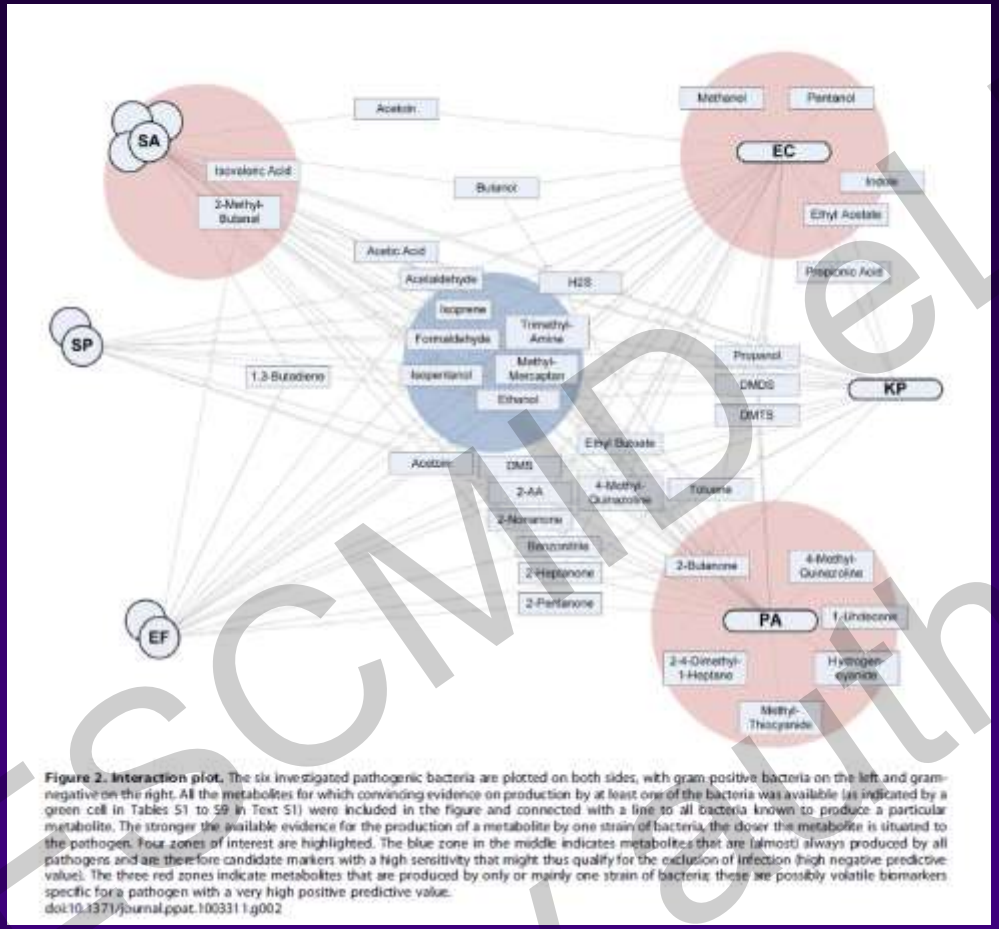


Figure 2. Interaction plot. The six investigated pathogenic bacteria are plotted on both sides, with gram-positive bacteria on the left and gram-negative on the right. All the metabolites for which convincing evidence on production by at least one of the bacteria was available (as indicated by a green cell in Tables S1 to S9 in Text S1) were included in the figure and connected with a line to all bacteria known to produce a particular metabolite. The stronger the available evidence for the production of a metabolite by one strain of bacteria, the closer the metabolite is situated to the pathogen. Four zones of interest are highlighted. The blue zone in the middle indicates metabolites that are (almost) always produced by all pathogens and are therefore candidate markers with a high sensitivity that might thus qualify for the exclusion of infection (high negative predictive value). The three red zones indicate metabolites that are produced by only or mainly one strain of bacteria; these are possibly volatile biomarkers specific for a pathogen with a very high positive predictive value.
doi:10.1371/journal.ppat.1003311.g002

BOS LD et al. 2013, Plos Pathogens review

Trapping the « volatilome»

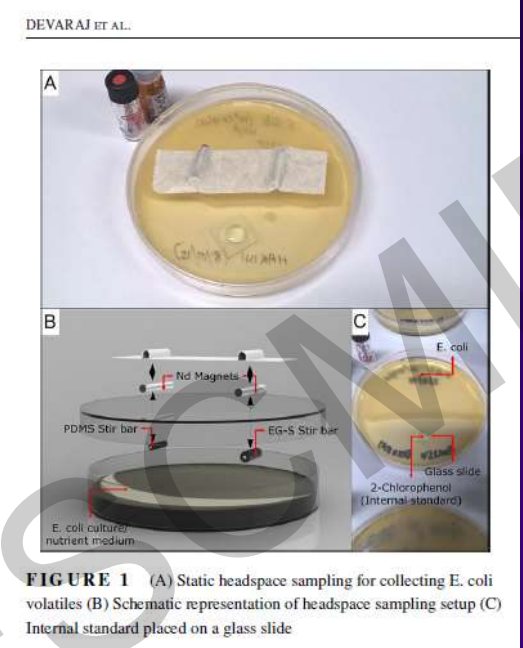


FIGURE 1 (A) Static headspace sampling for collecting E. coli volatiles (B) Schematic representation of headspace sampling setup (C) Internal standard placed on a glass slide

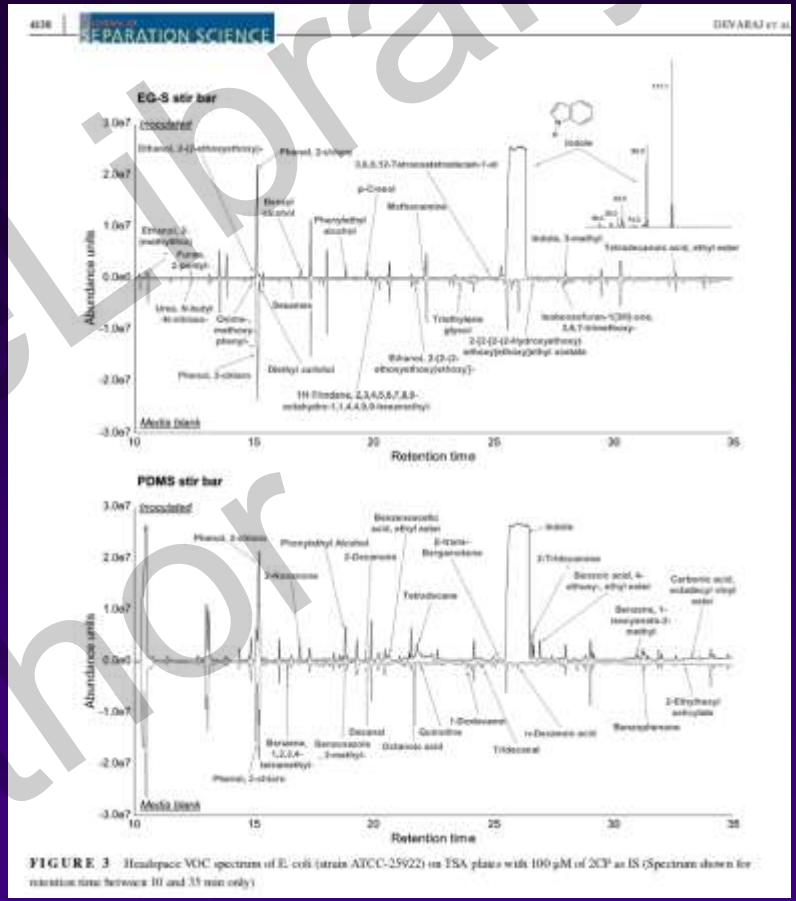


FIGURE 3 Headspace VOC spectrum of E. coli (strain ATCC-25922) on TSA plates with 100 µM of 2CP as IS (Spectrum shown for retention time between 10 and 35 min only)

Devaraj H et al. 2018, J Sep Sci. 2018

A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis

Sophia Koo,[1,2,3,a] Horatio R. Thomas,[1,3,a] S. David Daniels,[1] Robert C. Lynch,[1] Sean M. Fortier,[1] Margaret M. Shea,[1] Preshious Rearden,[4] James C. Comolli,[4] Lindsey R. Baden,[1,2,3] and Francisco M. Marty[1,2,3]

[1]Division of Infectious Diseases, Brigham and Women's Hospital, [2]Dana-Farber Cancer Institute, [3]Harvard Medical School, Boston, and [4]Draper Laboratory, Cambridge, Massachusetts

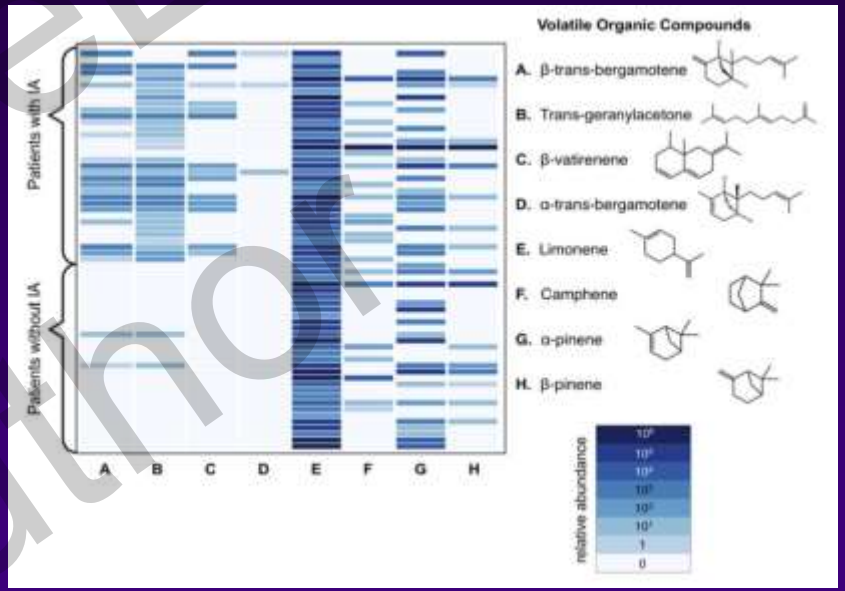
Top article award in 2014

Clin Infect Dis 2014;59:1733-40

- Thermal desorption GC-SM

- 64 prospectively collected breath samples
  - 34 patients with proven or probable invasive aspergillosis vs
  - 30 patients without aspergillosis

- 94% sensitivity (95% CI, 81%–98%)
- 93% specificity (95% CI, 79%–98%)



International Journal of Molecular Sciences

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Int. J. Mol. Sci. 2017, 18, 449

Article

Exhaled Breath Metabolomics for the Diagnosis of Pneumonia in Intubated and Mechanically-Ventilated Intensive Care Unit (ICU)-Patients

Pouline M. P. van Oort [1,*,†], Sanne de Bruin [1,†], Hans Weda [2], Hugo H. Knobel [2], Marcus J. Schultz [1] and Lieuwe D. Bos [1] On Behalf of the MARS Consortium‡

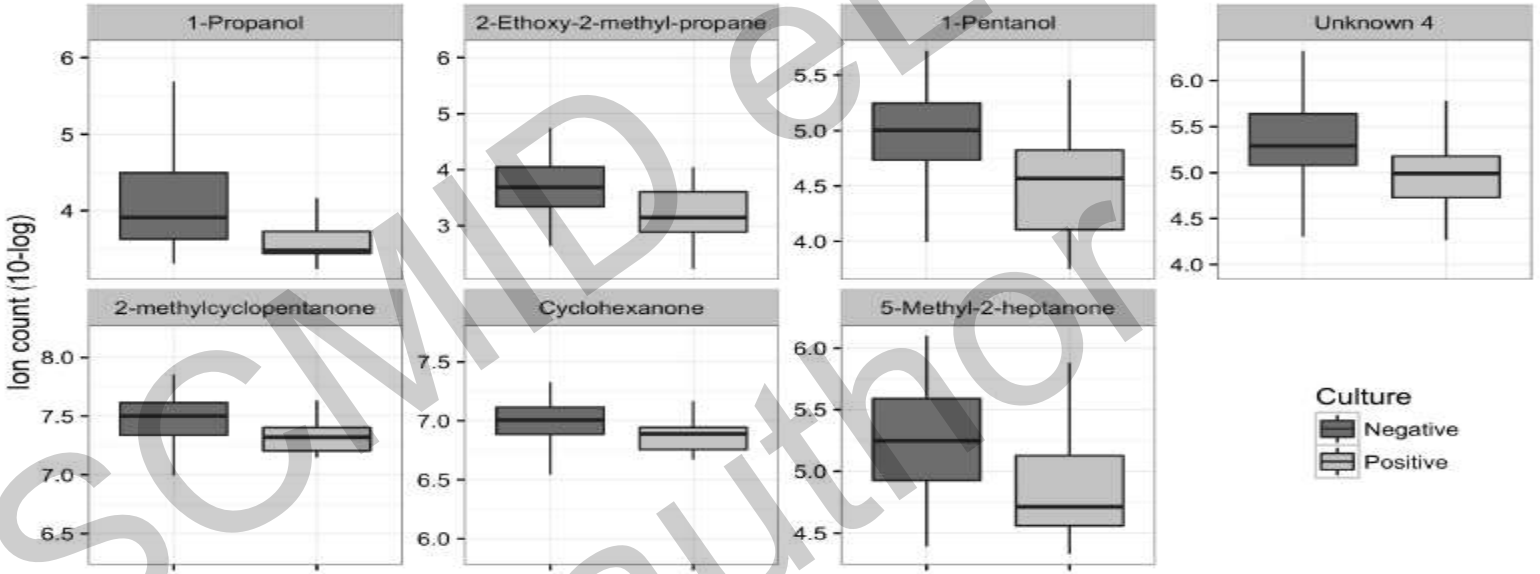

Figure 5. Ion count of VOCs that showed a p-value < 0.001.

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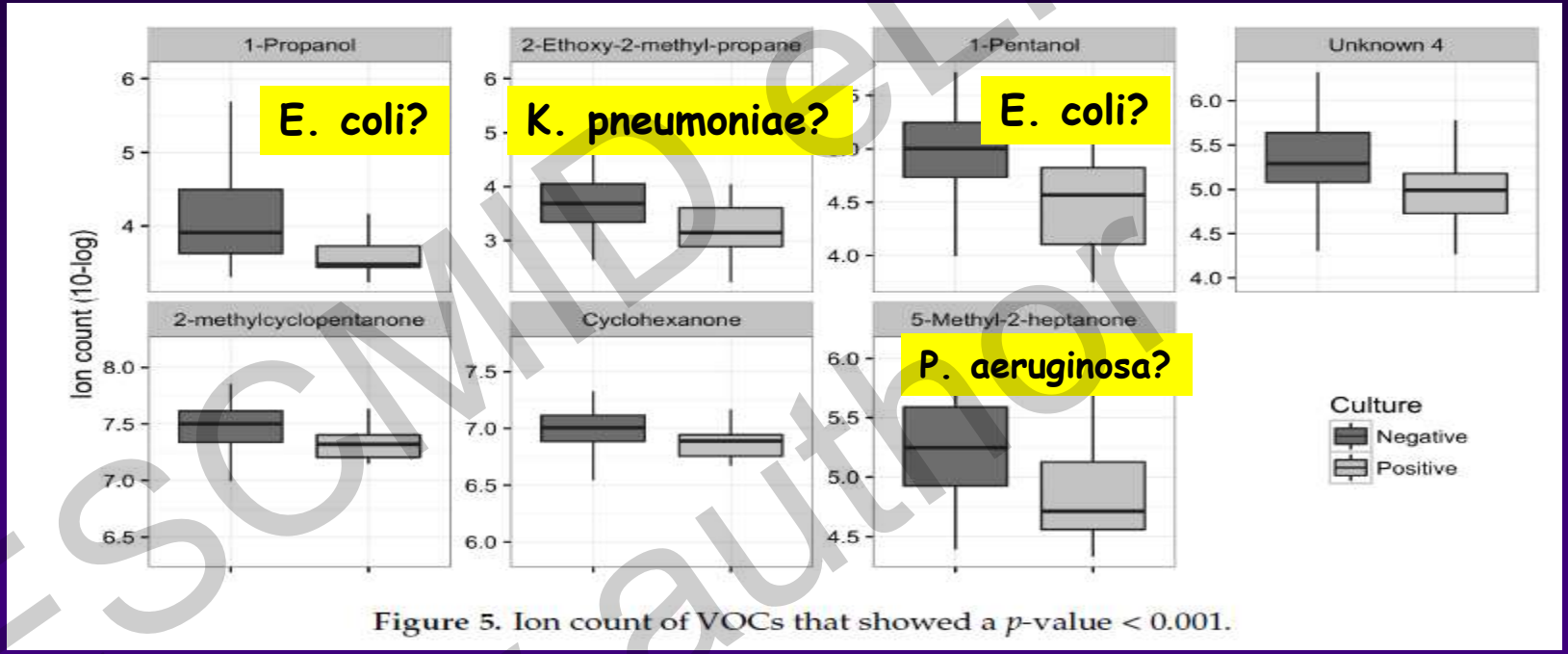

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Conclusions

- Past: animal sniffing was tested to screen for specific infectious diseases
- Present: detection of volatile compounds produced by microbes
- Future: could we use human smellling (safety)?
- Future: can we improve volatilome detection ?