Recent articles on analysis of volatile compounds and breath tests.

Phillips M, Basa-Dalay V, Blais J, Bothamley G, Chaturvedi A, et al. (2012) Point-of-care breath test for biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb) ahead of print.

Rationale: Volatile organic compounds (VOCs) in breath provide biomarkers of tuberculosis (TB) because Mycobacterium tuberculosis manufactures VOC metabolites that are detectable in the breath of infected patients. Objectives: We evaluated breath VOC biomarkers in subjects with active pulmonary TB, using an internet linked rapid point-of-care breath test. Methods: 279 subjects were studied at four centers in three countries, Philippines, UK, and India, and data was analyzed from 251 (130 active pulmonary TB, 121 controls). A point-of-care system collected and concentrated breath and air VOCs, and analyzed them with automated thermal desorption, gas chromatography, and surface acoustic wave detection. A breath test was completed in 6 min. Chromatograms were converted to a series of Kovats Index (KI) windows, and biomarkers of active pulmonary TB were identified by Monte Carlo analysis of KI window alveolar gradients (abundance in breath minus abundance in room air).Measurements and main results: Multiple Monte Carlo simulations identified eight KI windows as biomarkers with better than random performance. Four KI windows corresponded with KI values of VOCs previously identified as biomarkers of pulmonary TB and metabolic products of M. tuberculosis, principally derivatives of naphthalene, benzene and alkanes. A multivariate predictive algorithm identified active pulmonary TB with 80% accuracy (area under curve of receiver operating characteristic curve), sensitivity ¼ 71.2%, and specificity ¼ 72%. Accuracy increased to 84% in age-matched subgroups. In a population with 5% prevalence, the breath test would identify active pulmonary TB with 98% negative predictive value and 13% positive predictive value. Conclusions: A six-minute point-of-care breath test for volatile biomarkers accurately identified subjects with active pulmonary TB.

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Nawrath T, Mgode GF, Weetjens B, Kaufmann SH, Schulz S (2012) The volatiles of pathogenic and nonpathogenic mycobacteria and related bacteria. Beilstein J Org Chem 8: 290-299.

Abstract: Volatiles released by pathogenic and nonpathogenic mycobacteria, as well as by mycobacteria-related Nocardia spp., were analyzed. Bacteria were cultivated on solid and in liquid media, and headspace samples were collected at various times during the bacterial lifecycle to elucidate the conditions giving optimal volatile emission. Emitted volatiles were collected by using closed-loop stripping analysis (CLSA) and were analyzed by gas-chromatography-mass-spectrometry. A wide range of compounds was produced, although the absolute amount was small. Nevertheless, characteristic bouquets of compounds could be identified. Predominantly aromatic compounds and fatty-acid derivatives were released by pathogenic/nonpathogenic mycobacteria, while the two Nocardia spp. (N. asteroides and N. africana) emitted the sesquiterpene aciphyllene. Pathogenic Mycobacterium tuberculosis

strains grown on agar plates produced a distinct bouquet with different volatiles, while liquid cultures produce less compounds but sometimes an earlier onset of volatile production because of their steeper growth curves under this conditions. This behavior differentiates M. tuberculosis from other mycobacteria, which generally produced fewer compounds in seemingly lower amounts. Knowledge of the production of volatiles by M. tuberculosis can facilitate the rational design of alternative and faster diagnostic measures for tuberculosis.

Full article: http://www.beilstein-journals.org/bjoc/single/articleFullText.htm?publicId=1860-5397-8-31&vt=f&bpn=latest

Mgode GF, Weetjens BJ, Nawrath T, Cox C, Jubitana M, et al. (2012) Diagnosis of tuberculosis by trained African giant pouched rats and confounding impact of pathogens and microflora of the respiratory tract. J Clin Microbiol 50: 274-280.

Trained African giant-pouched rats (Cricetomys gambianus) can detect Mycobacterium tuberculosis and show potential for the diagnosis of tuberculosis (TB). However, rats' ability to discriminate between clinical sputum containing other Mycobacterium spp. and nonmycobacterial species of the respiratory tract is unknown. It is also unknown whether nonmycobacterial species produce odor similar to M. tuberculosis and thereby cause the detection of smear-negative sputum. Sputum samples from 289 subjects were analyzed by smear microscopy, culture, and rats. Mycobacterium spp. were isolated on Lowenstein-Jensen medium, and nonmycobacterial species were isolated on four different media. The odor from nonmycobacterial species from smear- and M. tuberculosis culture-negative sputa detected by >/=2 rats ("rat positive") was analyzed by gas chromatography-mass spectrometry and compared to the M. tuberculosis odor. Rats detected 45 of 56 confirmed cases of TB, 4 of 5 suspected cases of TB, and 63 of 228 TB-negative subjects (sensitivity, 80.4%; specificity, 72.4%; accuracy, 73.9%; positive predictive value, 41.7%; negative predictive value, 93.8%). A total of 37 (78.7%) of 47 mycobacterial isolates were M. tuberculosis complex, with 75.7% from rat-positive sputa. Ten isolates were nontuberculous mycobacteria, one was M. intracellulare, one was M. avium subsp. hominissuis, and eight were unidentified. Rat-positive sputa with Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus spp., and Enterococcus spp. were associated with TB. Rhodococcus, Nocardia, Streptomyces, Staphylococcus, and Candida spp. from rat-positive sputa did not produce M. tuberculosis-specific volatiles (methyl nicotinate, methyl para-anisate, and ortho-phenylanisole). Prevalence of Mycobacterium-related Nocardia and Rhodococcus in smear-negative sputa did not equal that of smear-negative mycobacteria (44.7%), of which 28.6% were rat positive. These findings and the absence of M. tuberculosis-specific volatiles in nonmycobacterial species indicate that rats can be trained to specifically detect M. tuberculosis.

Full artlce: http://jcm.asm.org/content/50/2/274.full.pdf+html

Mgode GF, Weetjens BJ, Cox C, Jubitana M, Machang'u RS, et al. (2012) Ability of Cricetomys rats to detect Mycobacterium tuberculosis and discriminate it from other microorganisms. Tuberculosis (Edinb) 92: 182-186.

Abstract: Trained African giant pouched rats (Cricetomys gambianus) have potential for diagnosis of tuberculosis (TB). These rats target volatile compounds of Mycobacterium tuberculosis (Mtb) that cause TB. Mtb and nontuberculous mycobacteria (NTM) species are related to Nocardia and Rhodococcus spp., which are also acid-fast bacilli and can be misdiagnosed as Mtb in smear microscopy. Diagnostic performance of C. gambianus on in vitro-cultured mycobacterial and related pulmonary microbes is unknown. This study reports on the response of TB detection rats to cultures of reference Mtb, clinical Mtb, NTM, Nocardia; Rhodococcus; Streptomyces; Bacillus; and yeasts. Trained rats significantly discriminated Mtb from other microbes ($p < 0.008$, Fisher's exact test). Detection of Mtb cultures was age-related, with exponential and early stationary phase detected more frequently than early log phase and late stationary phase ($p < 0.001$, Fisher's test) (sensitivity = 83.33%, specificity = 94.4%, accuracy = 94%). The detection of naturally TB-infected sputum exceeded that of negative sputum mixed with Mtb, indicating that C. gambianus are conditioned to detect odours of TB-positive sputum better than spiked sputum. Although further studies on volatiles from detectable growth phases of Mtb are vital for identification of Mtb-specific volatiles detected by rats, our study underline the potential of C. gambianus for TB diagnosis.
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McNerney R, Mallard K, Okolo PI, Turner C (2012) Production of volatile organic compounds by mycobacteria. FEMS Microbiol Lett 328: 150-156.

Abstract: The need for improved rapid diagnostic tests for tuberculosis disease has prompted interest in the volatile organic compounds (VOCs) emitted by Mycobacterium tuberculosis complex bacteria. We have investigated VOCs emitted by Mycobacterium bovis BCG grown on Lowenstein-Jensen media using selected ion flow tube mass spectrometry and thermal desorption-gas chromatography-mass spectrometry. Compounds observed included dimethyl sulphide, 3-methyl-1-butanol, 2-methyl-1-propanol, butanone, 2-methyl-1-butanol, methyl 2-methylbutanoate, 2-phenylethanol and hydrogen sulphide. Changes in levels of acetaldehyde, methanol and ammonia were also observed. The compounds identified are not unique to M. bovis BCG, and further studies are needed to validate their diagnostic value. Investigations using an ultra-rapid gas chromatograph with a surface acoustic wave sensor (zNose) demonstrated the presence of 2-phenylethanol (PEA) in the headspace of cultures of M. bovis BCG and Mycobacterium smegmatis, when grown on Lowenstein-Jensen supplemented with glycerol. PEA is a reversible inhibitor of DNA synthesis. It is used during selective isolation of gram-positive bacteria and may also be used to inhibit mycobacterial growth. PEA production was observed to be dependent on growth of mycobacteria. Further study is required to elucidate the metabolic pathways involved and assess whether this compound is produced during in vivo growth of mycobacteria.
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Kolk AH, van Berkel JJ, Claassens MM, Walters E, Kuijper S, et al. (2012) Breath analysis as a potential diagnostic tool for tuberculosis. Int J Tuberc Lung Dis 16: 777-782.

Abstract; SETTING: Cape Town, South Africa. OBJECTIVES: We investigated the potential of breath analysis by gas chromatography-mass spectrometry (GC-MS) to discriminate between samples collected prospectively from patients with suspected tuberculosis (TB). DESIGN: Samples were obtained in a TB-endemic setting in South Africa, where 28% of culture-proven TB patients had Ziehl-Neelsen (ZN) negative sputum smear. A training set of breath samples from 50 sputum culture-proven TB patients and 50 culture-negative non-TB patients was analysed using GC-MS. We used support vector machine analysis for classification of the patient samples into TB and non-TB. RESULTS: A classification model with seven compounds had a sensitivity of 72%, a specificity of 86% and an accuracy of 79% compared with culture. The classification model was validated with breath samples from a different set of 21 TB and 50 non-TB patients from the same area, giving a sensitivity of 62%, a specificity of 84% and an accuracy of 77%. CONCLUSION: This study shows that GC-MS breath analysis is able to differentiate between TB and non-TB breath samples even among patients with a negative ZN sputum smear but a positive culture for Mycobacterium tuberculosis. We conclude that breath analysis by GC-MS merits further research.
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Crespo E, de Ronde H, Kuijper S, Pol A, Kolk AH, et al. (2012) Potential biomarkers for identification of mycobacterial cultures by proton transfer reaction mass spectrometry analysis. Rapid Commun Mass Spectrom 26: 679-685.

Abstract: RATIONALE: Several mycobacterial species can produce serious infections in humans, and the treatment required depends on the infecting species. Fast identification, ideally with minimal manipulation of the infecting species, is therefore critical; here, we propose a method potentially allowing cultures to be identified by headspace analysis and use it to screen for differences between mycobacterial species based on the volatiles released during growth. METHODS: Short-chain volatile organic compound emissions from two non-tuberculosis slow growing mycobacterial species, Mycobacterium avium and Mycobacterium kansasii, and a non-pathogenic fast growing species, Mycobacterium smegmatis, in Middlebrook M7H9 culturing media were followed online with a proton transfer reaction quadrupole mass spectrometer. RESULTS: Measurable differences between the headspace of the two slow growing mycobacteria M. kansasii and M. avium were found, as well as differences with respect to the faster growing mycobacteria M. smegmatis. Three compounds, attributed to sulfur-containing volatiles--dimethyl sulfide, propanethiol and dimethyl disulfide--were found to be specific to M. avium. CONCLUSIONS: Clear differences were detected in the low molecular weight volatile emissions compounds of the mycobacterial species under study, without the need for sample manipulation. Further studies with other mycobacterial species will reveal if the differences observed are specific to the species studied here. Furthermore, the use of an ion trap as a mass analyzer

with the same ionization technique, allowing molecular detection over a wider molecular range, could allow the detection of additional biomarkers thus capturing a wider molecular range.
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Chambers ST, Scott-Thomas A, Epton M (2012) Developments in novel breath tests for bacterial and fungal pulmonary infection. Curr Opin Pulm Med 18: 228-232.

PURPOSE OF REVIEW: Breath testing has developed over the last 20 years. New techniques that can identify fingerprints for specific diseases and specific markers of respiratory pathogens have been applied to breath analysis. This review discusses the recent advances in breath analysis for the diagnosis of bacterial and fungal lower respiratory tract infections. RECENT FINDINGS: The current techniques continue to develop rapidly, but preconcentration techniques are needed to analyse many target volatile organic compounds for most systems. Breath testing with an electronic nose is promising for the diagnosis of tuberculosis (TB), and specific volatiles identifiable by gas chromatography with mass spectrometry have been identified in breath for Mycobacterium tuberculosis, Pseudomonas aeruginosa and Aspergillus fumigatus, but are found at very low concentrations in breath. Contamination from the environment is an ongoing confounding influence. Exhaled breath condensate (EBC) is disappointing as a diagnostic sample. SUMMARY: Careful attention needs to be paid to the sensitivity and specificity of a technique and confounding from the environment. The role of technologies such as selected ion flow tube-mass spectrometry is emerging. The electronic nose requires further validation for TB. The identification of specific microbial biomarkers aids the quest for improved accuracy. EBC is currently of limited value.
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2011

Van Beek SC, Nhung NV, Sy DN, Sterk PJ, Tiemersma EW, et al. (2011) Measurement of exhaled nitric oxide as a potential screening tool for pulmonary tuberculosis. Int J Tuberc Lung Dis 15: 185-192.

Suckling DM, Sagar RL (2011) Honeybees Apis mellifera can detect the scent of Mycobacterium tuberculosis. Tuberculosis (Edinb) 91: 327-328.

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Poling A, Weetjens B, Cox C, Beyene N, Durgin A, et al. (2011) Tuberculosis Detection by Giant African Pouched Rats. Behav Anal 34: 47-54.

Mahoney AM, Weetjens BJ, Cox C, Beyene N, Mgode G, et al. (2011) Using giant African pouched rats to detect tuberculosis in human sputum samples: 2010 findings. Pan Afr Med J 9: 28.

Koo CK, Wang S, Gaur RL, Samain F, Banaei N, et al. (2011) Fluorescent DNA chemosensors: identification of bacterial species by their volatile metabolites. Chem Commun (Camb) 47: 11435-11437.

Jain P, Thaler DS, Maiga M, Timmins GS, Bishai WR, et al. (2011) Reporter phage and breath tests: emerging phenotypic assays for diagnosing active tuberculosis, antibiotic resistance, and treatment efficacy. J Infect Dis 204 Suppl 4: S1142-1150.

Crespo E, Cristescu SM, de Ronde H, Kuijper S, Kolk AH, et al. (2011) Proton Transfer Reaction Mass Spectrometry detects rapid changes in volatile metabolite emission by Mycobacterium smegmatis after the addition of specific antimicrobial agents. J Microbiol Methods 86: 8-15.

Banday KM, Pasikanti KK, Chan EC, Singla R, Rao KV, et al. (2011) Use of urine volatile organic compounds to discriminate tuberculosis patients from healthy subjects. Anal Chem 83: 5526-5534.

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Poling A, Weetjens BJ, Cox C, Mgode G, Jubitana M, et al. (2010) Using giant African pouched rats to detect tuberculosis in human sputum samples: 2009 findings. Am J Trop Med Hyg 83: 1308-1310.

Phillips M, Basa-Dalay V, Bothamley G, Cataneo RN, Lam PK, et al. (2010) Breath biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb) 90: 145-151.

McNerney R, Wondafrash BA, Amena K, Tesfaye A, McCash EM, et al. (2010) Field test of a novel detection device for Mycobacterium tuberculosis antigen in cough. BMC Infect Dis 10: 161.

Kolk A, Hoelscher M, Maboko L, Jung J, Kuijper S, et al. (2010) Electronic-nose technology using sputum samples in diagnosis of patients with tuberculosis. J Clin Microbiol 48: 4235-4238.

Jassal MS, Nedeltchev GG, Lee JH, Choi SW, Atudorei V, et al. (2010) 13[C]-urea breath test as a novel point-of-care biomarker for tuberculosis treatment and diagnosis. PLoS One 5: e12451.

Weetjens BJ, Mgode GF, Machang'u RS, Kazwala R, Mfinanga G, et al. (2009) African pouched rats for the detection of pulmonary tuberculosis in sputum samples. Int J Tuberc Lung Dis 13: 737-743.

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Syhre M, Manning L, Phuanukoonnon S, Harino P, Chambers ST (2009) The scent of Mycobacterium tuberculosis--part II breath. Tuberculosis (Edinb) 89: 263-266.

Spooner AD, Bessant C, Turner C, Knobloch H, Chambers M (2009) Evaluation of a combination of SIFT-MS and multivariate data analysis for the diagnosis of Mycobacterium bovis in wild badgers. Analyst 134: 1922-1927.

This list was prepared for the STOPTB New Diagnostics Working Group by Ruth McNerney in June 2012. Articles are presented in reverse chronological order. NDWG take no responsibility for errors or ommissions.