Recent articles on analysis of volatile compounds and breath tests.


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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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


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. hominisuis, 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 article: http://jcm.asm.org/content/50/2/274.full.pdf+html

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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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 Löwenstein-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 Löwenstein-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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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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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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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


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


2010


2009


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