ANALÝZA DECHU

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Ústav Chemie, PřF, MU

1

DECH fakta

Člověk dýchá od narození

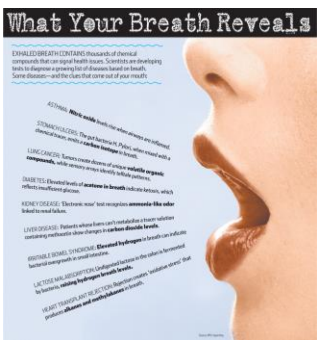
Denní výměna vzduchu 10.000 L / den

Celý život : 70 let X 365 dní 255 500 000 L = 255 000 m3

Srovnání objemu:

Rodinný dům : 1000 m3

Menší mrakodrap ~ 255 000 m3



http://online.wsj.com/news/interactive/HEALTHCOL1009?ref=SB10000872396390444024204578044401470426998

- Úvod
- Důležitost klinické analýzy
- Příklady – krevní vyšetření
- POC analýza
- Glukometr
- Test moči
- Těhotenský test

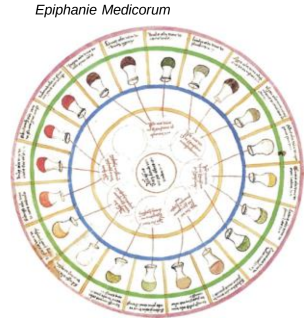
Neinvazivní dávkování

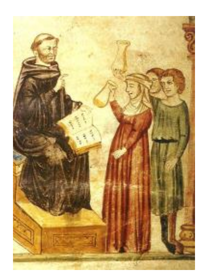
INVAZIVNÍ vs. NEINVAZIVNÍ ODBĚR

KREV	MOČ
CSF (cerebrospinal fluid)	SLINY
BIOPSIE	
TKÁŇOVÝ ODBĚR	
	DECH

1506 by Ulrich Pinder

Epiphanie Medicorum

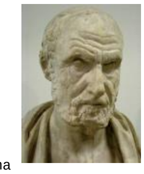




Testování moči - uroskopie

Sladká	- diabetes
Hnědavá	- žloutenka
Červené zbarvení, pěna	- ledviny
Krev	- rakovina močového ústrojí

HISTORIE ANALÝZY DECHU



Hippokrates (460 BC – c. 370 BC)

fetor oris and *fetor hepaticus* in his treatise on breath aroma and disease

Hippocrates, The Corpus: The Hippocratic Writings, Kaplan Publishing, New York, 2008.

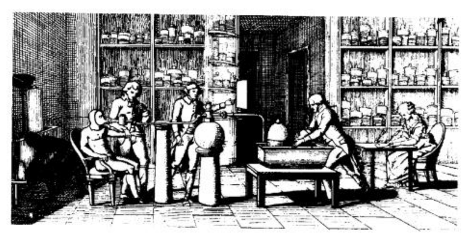
The Hippocratic Corpus (Latin: *Corpus Hippocraticum*) is a collection of around seventy early medical works from Alexandrian Greece.It is written in Ionic Greek

Paličkovité prsty u pacienta s Eisenmengerovým syndromem

Poprvé popsány Hipokratem, "Hippocratic fingers". jsou důsledkem dlouhodobé hypoxie periferie u cyanotických srdečních vad.

Paličkové prsty jsou připisovány chorobám plicním: rakovina plic, CF, komplikovaná TBC, srdečním chorobám

Antoine Lavoisier 1784



"Experiments on Animal Respiration and the Changes Occurring When Air Passes Through the Lungs"

1874 Anstie made an observation that small amounts of alcohol were excreted in breath

1897 Nebelthau jedinci, kteří trpí cukrovkou mají v dechu aceton

1927 Bogen první alko tester

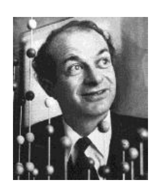
McNalley Breathalyzer

1936 Patent Harger

MODERNÍ ÉRA ANALÝZY DECHU

1971 Linus Pauling (nositel Nobelovy ceny 1954) analyzoval v dechu ca 250 různých VOC

V současnosti identifikováno ca 400 látek



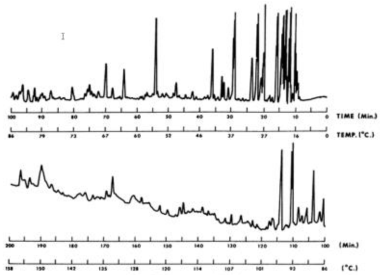


Fig. 2. Chromatogram of breath.

-poprvé analyzoval kondenzát dechu
- použil kondenzaci a zmražení v ocelové trubici ledové lázni

Další práce na téma EBC:

1979

T.V. Larson, D.S. Covert, R. Frank, J. Appl. Physiol. 46 (1979) 603–607.

1980

G.I. Sidorenko, E.I. Zborovskii, D.I. Levina, Ter. Arkh. 52 (1980) 65–68.

1987

M.V. Kurik, L.V. Rolik, N.V. Parkhomenko, L.I. Tarakhan, N.V. Savitskaia, Vrach Delo 7 (1987) 37–39.

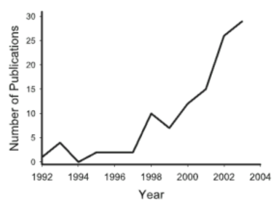


Fig. 1. The number of exhaled breath condensate (EBC) papers published each year has increased dramatically over the past several years (Horvath I, Hunt J, and Barnes PJ, unpublished observations).

METODY ANALÝZY

DECH

TĚKAVÉ LÁTKY: IMS, SIFT MS, GC/MS

NETĚKAVÉ LÁTKY: Spektrofotometrie, enz. reakce, HPLC, GC, CE

ANALÝZA TĚKAVÝCH LÁTEK

Iontová mobilitní spektrometrie

Selected ion flow tube mass spectrometry

Plynová chromatografie s MS

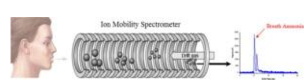
Velké množství látek, malé koncentrace

Nutnost zakoncentrování

Kontaminace z okolí

Využití statistických metod pro analýzu dat

IMS příklad



ANALYSIS OF HUMAN BREATH USING IMS

V. Ruzsanyi, J.I. Baumbach

ISAS - Institute for Analytical Sciences, Bunsen-Kirchhoff-Str. 11, 44139 Dortmund, Germany

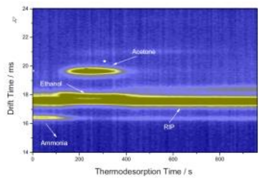


Figure 1: IMS-Chromatogram of the breath of a non-smoker

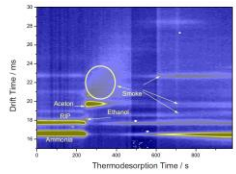
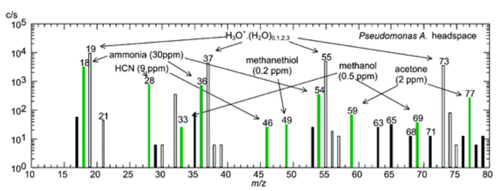
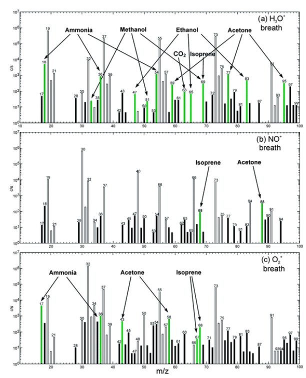


Figure 2: IMS-Chromatogram of the breath of a smoker

SIFT MS příklad

Selected ion flow tube mass spectrometry, SIFT-MS, is a technique for real-time measurement of concentrations of trace gases and vapours of volatile compounds in humid air including exhaled breath. It is based on chemical ionization using H_3O^+, NO^+, and O_2^+ precursor (reagent) ions via ion/molecule reactions proceeding during an accurately defined time. Absolute concentrations of trace gases can be calculated from the ratios of ion count rates using the know reaction rate constants with a limit of detection being typically 1 parts-per-billion, ppb. SIFT-MS is used in several areas of research including non-invasive breath analysis for clinical diagnosis and for therapeutic monitoring, environmental research and security related research.





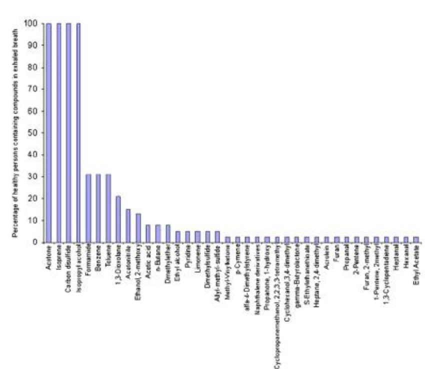
GC/MS příklad

The main compounds contained in exhaled breath samples of healthy volunteers are unsaturated hydrocarbons, ketones, alcohols and hydrocarbons. Usually, breath samples of smoking persons contain a high concentration of acetonitrile and furans, as well as aromatic hydrocarbons (e.g., benzene, toluene).

J. Breath Res. 2 (2008) 046006 (8pp) doi:10.1088/1752-7155/2/4/046006

The analysis of healthy volunteers' exhaled breath by the use of solid-phase microextraction and GC-MS

T Ligor[1,2,3], M Ligor[1,2,3,5], A Amann[1,2], C Ager[1,2], M Bachler[1], A Dzien[4] and B Buszewski[2,3]



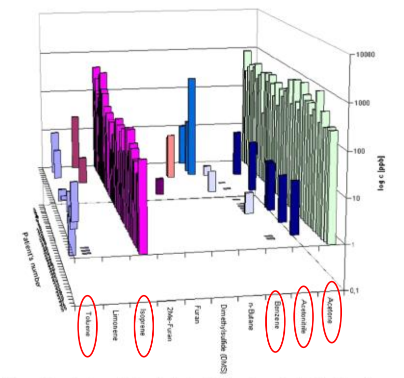


Figure 3. Results of quantitative analysis of exhaled breath samples for 39 healthy volunteers.

ANALÝZA NETĚKAVÝCH LÁTEK

KONDENZÁT VYDECHOVANÉHO VZDUCHU

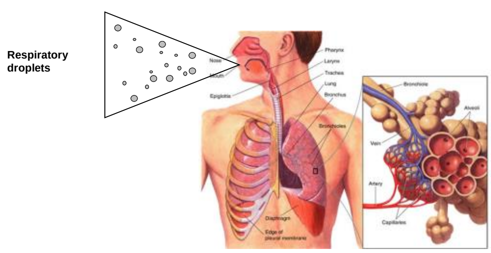
EBC

- Spektrofotometrie
- Enzymatické reakce
- HPLC, HPLC/MS
- GC, GC/MS
- CE

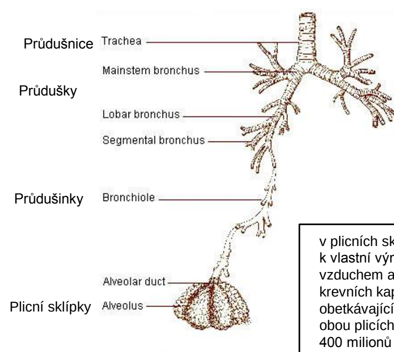
Relativně méně látek, také malé koncentrace

Látky rozpustné ve vodě

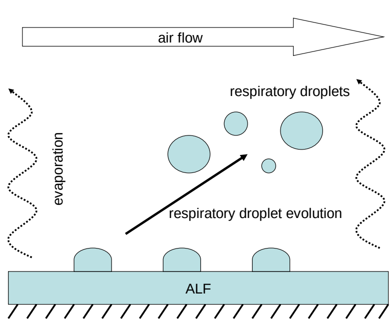
PLÍCE A DÝCHÁNÍ



Plíce jsou houbovitý šedorůžový orgán, který vyplňuje převážnou část hrudníku. Svým tvarem připomínají křídla. Povrch plic je krytý blánou zvanou poplicnice. Máme pravou a levou plíci. Pravá je větší, skládá se ze tří laloků. Levá má pouze dva laloky.



v plicních sklípcích dochází k vlastní výměně plynů mezi vzduchem a krví v sítích krevních kapilár obetkávajících alveoly. V obou plicích je celkem 300 – 400 milionů alveolů, jejichž celková plocha činí při vdechu 55 – 80 m2.



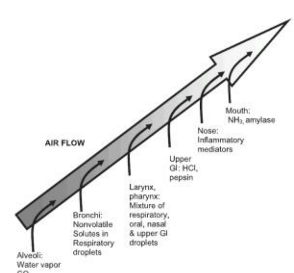
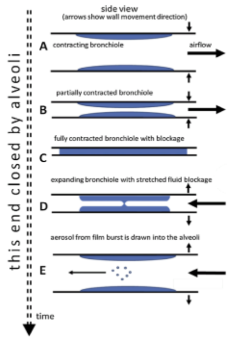
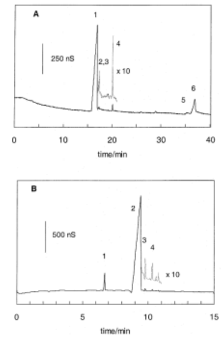


Fig. 2. Aerosol droplets can be derived from multiple sites when collected from the mouth. Some of the principal ingredients that are derived from each site are indicated.

Bronchiole fluid film burst (BFFB) model

G.J. Johnson, L. Morawska, J. Aersol. Med. Pulm. Drug Deliv. 22 (2009) 229–237





Analysis of small-scale biological compartments by capillary electrophoresis

K. Govindaraju, D.K. Lloyd*

Fig. 3. Analysis of anions (A) and cations (B) in rat ASF. Separation conditions: 90 cm long fused-silica capillary (50 μm I.D.) with end-capillary conductivity detection cell with (A) BGE, 100 m*M* CHES, 40 m*M* LiOH (pH 9.3)–2-propanol (92:8, v/v) (propanol improves resolution of nitrate/nitrite from chloride) with 80 μ*M* spermine additive to eliminate EOF; field strength, -278 V cm^{-1}: (1) Cl^-, (2) NO_2^-, (3) NO_3^-, (4) SO_4^{2-}, (5) PO_4^{2-}, (6) HCO_3^-. (B) BGE, 100 m*M* MES, 100 m*M* histidine, pH 5.6, with 20 m*M* α-hydroxyisobutyric acid as a complexing agent to modify selectivity; field strength, 222 V cm^{-1}: (1) K^+, (2) Na^+, (3) Ca^{2+}, (4) Mg^{2+}. Reprinted with permission from Ref. [41], copyright 1997, American Chemical Society.

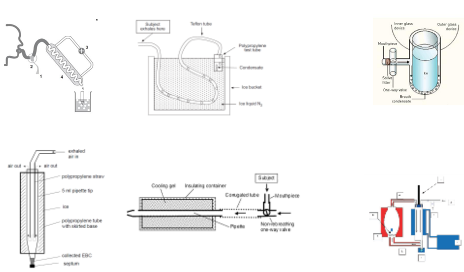
DÁVKOVÁNÍ EBC

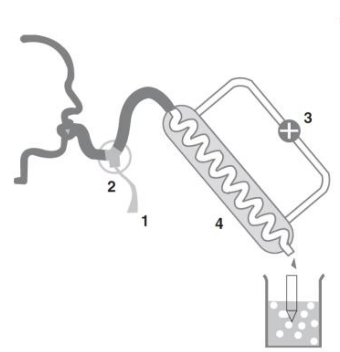
Principem je kondenzace vydechovaného vzduchu po ochlazení

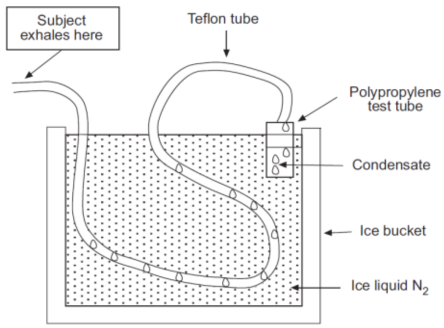
Přístroje
- komerční
- vědecké
- jednoduché přenosné

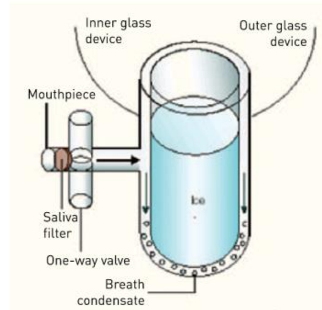
KOMERČNÍ PŘÍSTROJE

VĚDECKÉ PŘÍSTROJE









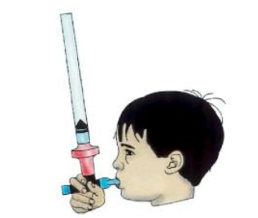
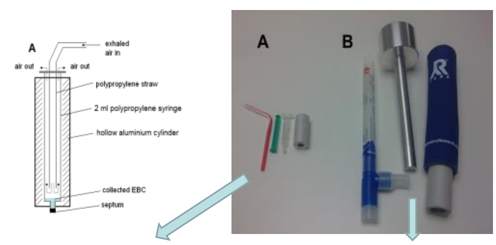


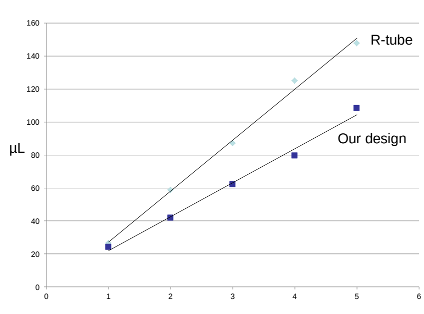
FIG 2. A commercially available collection device. The RTube collection system is portable and disposable, and it can collect a sample in as little as 1 minute.

SROVNÁNÍ

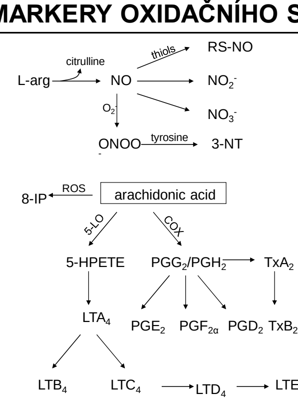


CENA: 1 Kč
Objem EBC/1 výdech: 20 uL

CENA: 500 Kč
Objem EBC/1 výdech: 30 uL

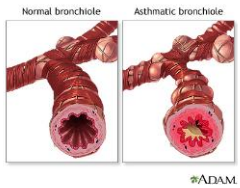


MARKERY OXIDAČNÍHO STRESU

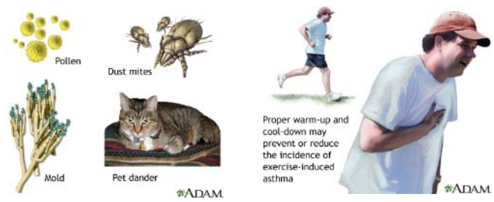


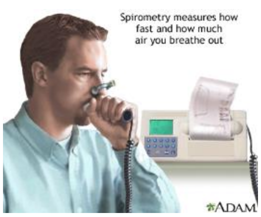
ASTHMA

Asthma is a disease that causes the airways of the lungs to swell and narrow, leading to wheezing, shortness of breath, chest tightness, and coughing.



http://www.ncbi.nlm.nih.gov/pubmedhealth/aboutnlm/





A peak flow meter is commonly used by a person with asthma to measure the amount of air that can be expelled from the lungs. If the airways become narrow or blocked due to asthma, peak flow values will drop because the person cannot blow air out of the lungs as well. A peak flow meter can be a useful aid in monitoring a person's asthma over time and can also be used to help determine how well a patient's medications are working.

NO MONITORING

www.nioxmino.com, www.aerocrine.com

COPD

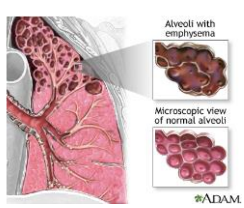
Chronic obstructive pulmonary disease (COPD) is one of the most common lung diseases. It makes it difficult to breathe.

There are two main forms of COPD:

Chronic bronchitis, which involves a long-term cough with mucus.

Emphysema, which involves destruction of the lungs over time.

Most people with COPD have a combination of both conditions.



CF – cystická fibróza

lidská dědičná nemoc, která postihuje převážně dýchací a trávicí soustavu.

Jde o autozomální recesivní vrozené onemocnění způsobené mutací genu produkujícího protein CFTR (anglicky Cystic fibrosis transmembrane conductance regulator).

Léčba se v současnosti soustřeďuje zejména na léčbu dýchacího ústrojí – inhalace, fyzioterapie, antibiotika. Nemocní užívají trávicí enzymy v kapslích, tedy na odstraňování důsledků onemocnění. Jeho příčina je léčitelná, ale zatím nevyléčitelná.[1]

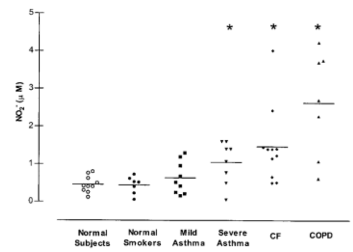
Polovina nemocných se dnes v České republice dožívá alespoň 32 let. Předpokládá se, že každý 25. člověk v populaci je přenašečem postiženého genu, ale jelikož má jednu kopii poškozenou a druhou v pořádku, onemocnění se u něho neprojevuje

PŘÍKLADY MONITOROVÁNÍ MARKERů OX. STRESU

Increased Nitrosothiols in Exhaled Breath Condensate in Inflammatory Airway Diseases

MASSIMO CORRADI, PAOLO MONTUSCHI, LOUISE E. DONNELLY, ALBERTO PESCI, SERGEI A. KHARITONOV, and PETER J. BARNES

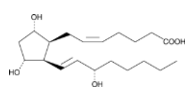
Am J Respir Crit Care Med Vol 163. pp 854–858, 2001
Internet address: www.atsjournals.org



Exhaled Breath Condensate Isoprostanes Are Elevated in Patients With Acute Lung Injury or ARDS*

Chace T. Carpenter, MD; Patricia V. Price, BS; and Brian W. Christman, MD, FCCP

CHEST / 114 / 6 / DECEMBER, 1998 1653



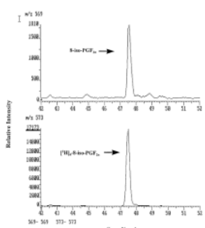


FIGURE 1. Selected ion current profile used for the quantification of 8-iso-PGF$_{2\alpha}$. In the lower tracing (m/z 573), the peak represents the derivatized internal standard introduced for sample measurement. The upper tracing (m/z 569) shows a peak co-eluting with the internal standard. This represents the endogenously generated 8-iso-PGF$_{2\alpha}$. m/z = mass to charge ratio.

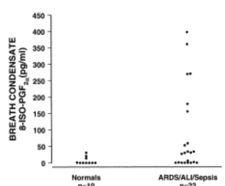


FIGURE 2. 8-iso-PGF$_{2\alpha}$ levels in the breath condensate of patients with or at risk for ALI/ARDS and patients undergoing minor surgical procedures (normal subjects).

3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children

E. Baraldi, G. Giordano, M. F. Pasquale, S. Carraro, A. Mardegan, G. Bonetto, C. Bastardo, F. Zacchello, S. Zanconato

Allergy 2006: 61: 90–96

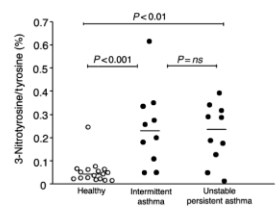


Figure 3. Nitrotyrosine levels in exhaled breath condensate of unstable asthmatic children treated with inhaled corticosteroid, steroid-naive, stable asthmatic children and healthy controls. Horizontal bars represent median values.

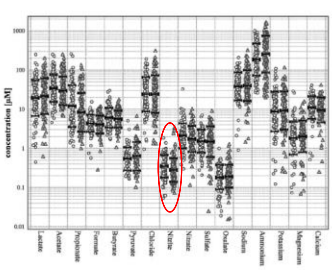


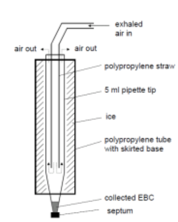
Fig. 2. Concentration of ionic species in non-deaerated EBC samples (N = 246). The central bars are the mean concentrations while the upper and lower bars are standard deviations (calculated according to lognormal distributions). Circles are before exercise values, triangles are after exercise values.

Pediatric Pulmonology 44:768–777 (2009)

Ionic Determinants of Exhaled Breath Condensate pH Before and After Exercise in Adolescent Athletes

Roby Greenwald, PhD, Jill M. Ferdinands, PhD, and W. Gerald Teague, MD

PŘÍKLAD ANALÝZY EBC POMOCÍ KAPILÁRNÍ ELEKTROFORÉZY



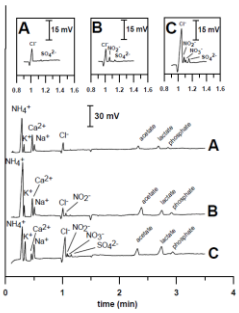


Figure 4.

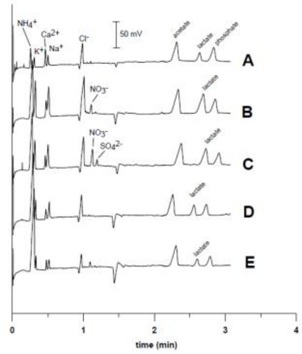


Figure 6.

Brit. J. Sports Med. – Vol. 15, No. 3, September 1981, pp. 172-176

BLOOD LACTATE CONCENTRATION FOLLOWING MAXIMUM EXERCISE IN TRAINED SUBJECTS

G. C. GASS, PhD,* S. ROGERS, DipPhty,** and R. MITCHELL, MSc†

Venous blood lactate concentration has long been considered a useful measurement in quantifying the extent of anaerobic metabolism during exercise and may be used as an objective criterion of the degree of exhaustion, (Åstrand et al. 1963). The extent to which venous blood

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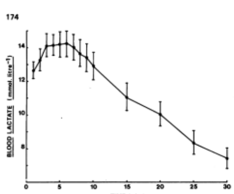
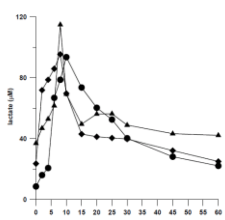


Figure 1: Blood lactate concentration following maximum exercise.

Měření laktátu v EBC



STANOVENÍ MARKERU RAKOVINY PLIC

Diagnosing lung cancer in exhaled breath using gold nanoparticles

Gang Peng[1,2], Ulrike Tisch[1,2], Orna Adams[1], Meggie Hakim[1], Nisrean Shehada[1], Yoav Y. Broza[1], Salem Billan[3], Roxolyana Abdah-Bortnyak[3], Abraham Kuten[3,4] and Hossam Haick[1,2]*

Conventional diagnostic methods for lung cancer[1,2] are unsuitable for widespread screening[2,3] because they are expensive and occasionally miss tumours. Gas chromatography/mass spectrometry studies have shown that several volatile organic compounds, which normally appear at levels of 1–20 ppb in healthy human breath, are elevated to levels between 10 and 100 ppb in lung cancer patients[4–6]. Here we show that an array of sensors based on gold nanoparticles can rapidly distinguish the breath of lung cancer patients from the breath of healthy individuals in an atmosphere of high humidity. In combination with solid-phase microextraction[7], gas chromatography/mass spectrometry was used to identify 42 volatile organic compounds that represent lung cancer biomarkers. Four of these were used to train and optimize the sensors, demonstrating good agreement between patient and simulated breath samples. Our results show that sensors based on gold nanoparticles could form the basis of an inexpensive and non-invasive diagnostic tool for lung cancer.

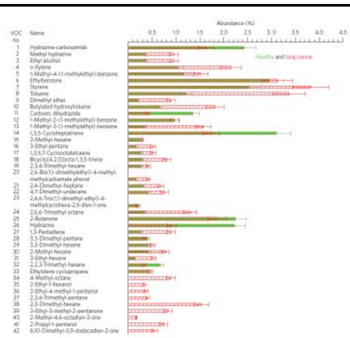


Figure 1 | Gas chromatography/mass spectrometry (GC-MS) analysis of healthy and lung cancer breath. Average abundance ratio of the 42 lung cancer biomarkers identified with solid-phase microextraction (SPME)-aided GC-MS. Thirty-three common VOCs and nine uncommon VOCs were found in at least 83% of the patients and in fewer than 83% of the healthy subjects, respectively. The variance of a specific VOC in either the healthy group or the lung cancer group was relatively small, most probably due to the relatively 'homogeneous' population included in this study. Note: setting a cutoff value of 100% (rather than ~83%, which considered a typical value in the literature) would give lower variances than those presented in the current figure. The variances are indicated by the error bars.

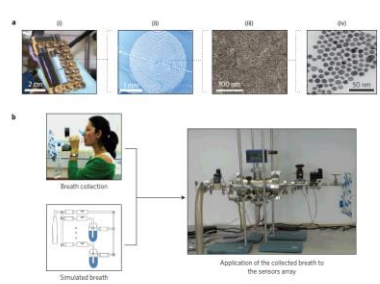


Figure 2 | Illustration of the diagnosis of lung cancer using breath testing. a, A photograph of the array of chemiresistors (**i**), a scanning electron microscopy image for the chemiresistor (**ii**), a scanning electron microscopy image of a gold nanoparticles film located between two adjacent electrodes (**iii**), and a transmission electron micrograph (TEM) of the monolayer-capped gold nanoparticles (**iv**). Note that in the TEM image, the gold nanoparticles appear as dark dots and the capping organic molecules appear as a bright medium between the adjacent dark dots. In these films, the metallic particles provide the electrical conductivity and the organic film component provides sites for the sorption of analyte molecules. **b**, Testing the exhaled breath (collected from patients) and simulated breath (that is, a mixture of representative VOCs at concentrations similar to those determined by GC-MS analysis of exhaled patient's breath) using the array of gold nanoparticle sensors.

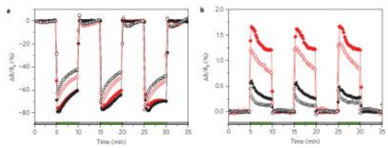


Figure 3 | Typical responses of the chemiresistors to real breath samples. a, Typical responses, $\Delta R/R_b$ (where R_b is the baseline resistance of the sensor in the absence of analyte and ΔR is the baseline-corrected steady-state resistance change upon exposure of the sensor to analyte), of 11-mercapto-1-undecanol-gold nanoparticles (red circles) and decanethiol-gold nanoparticles (black squares) upon exposure to healthy breath (filled symbols) and lung cancer breath (open symbols), as representative examples for sensors having positive responses. The sensors show a decrease in resistance, most likely due to increase in the permittivity of the organic matrix surrounding the metal cores[23,24]. **b**, Typical responses of 2-mercaptobenzoxazole-gold nanoparticles (red diamonds) and tert-dodecanethiol-gold nanoparticles (black triangles) upon exposure to headspace of healthy breath (filled symbols) and lung cancer breath (open symbols), as representative examples for sensors having negative responses. The sensors show an increase in resistance, most likely due to swelling that may increase the interparticle tunnel distance[23,24]. The grey bars on the *x*-axis indicate that the sensors are under vacuum. The green bars on the *x*-axis indicate that the sensors are exposed to either healthy or lung cancer breath.

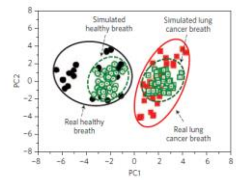


Figure 4 | Principal component analysis (PCA) of the dataset of real and simulated breath. Each data point corresponds to the multidimensional $\Delta R/R_b$ (where R_b is the baseline resistance of the sensor in the absence of analyte and ΔR is the baseline-corrected steady-state resistance change upon exposure of the sensor to analyte) of one breath sample and is the averaged response of 3-5 exposures. The results yield well-defined clusters for cancer states and healthy states, thus allowing fast, reliable and non-invasive lung cancer diagnosis. All data points were obtained using the same nine-sensor array described in the text and *Methods* section.

ZÁVĚR

Analýza dechu se jeví jako slibný diagnostický nástroj

Než bude možné jej použít v klinické praxi, je třeba provést standardizaci jak dávkování tak obsahu biomarkerů u zdravé populace

Dech je komplexní vzorek a analýza jednotlivých markerů není jednoduchá, je třeba vyvinout nové, citlivé metody analýzy

Aplikace v point of care diagnostice.

Využití statistických metod a chemometrie.

A JEDNA PERLIČKA NA KONEC...

The Dog's Nose Knows

Certified police dogs, on average, performed slightly better than the experimental set. However, they displayed greater variability: the best and the worst dogs in our trial were both from the police set

26 McCulloch M, Jezierski T, Broffman M, *et al.* Diagnostic accuracy of canine scent detection in early-and late-stage lung and breast cancers. *Integr Cancer Ther* 2006; 5: 30–39.

The year 2003 has seen a new direction of canine detection research at our Department. According to some anecdotal reports dogs are able to detect the presence of human cancers on the basis of odorous volatile organic compounds produced by cancer tissue.

The trained dogs were able to **distinguish breath samples taken from patients with lung or breast cancer** versus healthy volunteers with a **sensitivity and specificity of about 97%!**

This is a significantly higher rate than that achieved using gas chromatography and mass spectroscopy.

Děkuji za pozornost !