ANALYTAL CHEMISTRY IN POINT-OF-CARE (POC) DIAGNOSTICS

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Point-of-care (POC) diagnostics

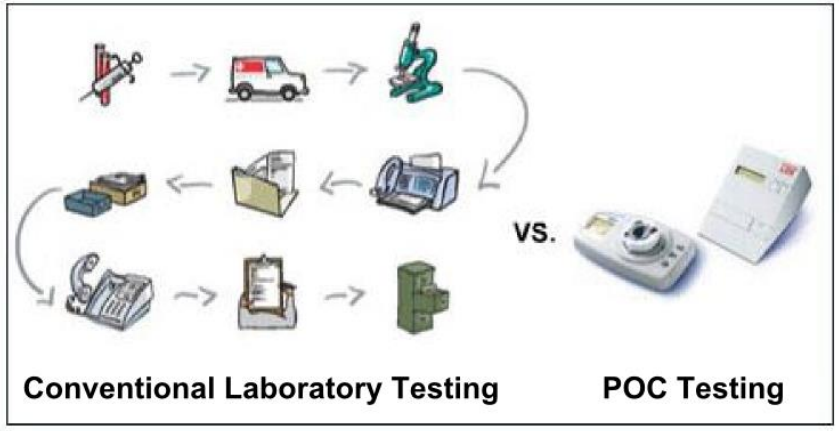
Diagnostic technologies that allow analysis of patients samples outside the clinical laboratory (doctors office, at bedside, in ambulance vehicles etc.)

Usually small, simple instruments/devices that are able to analyse important biomarkers in clinical samples (blood, saliva, urine)

The time of analysis is decreased, no need to transport the samples.

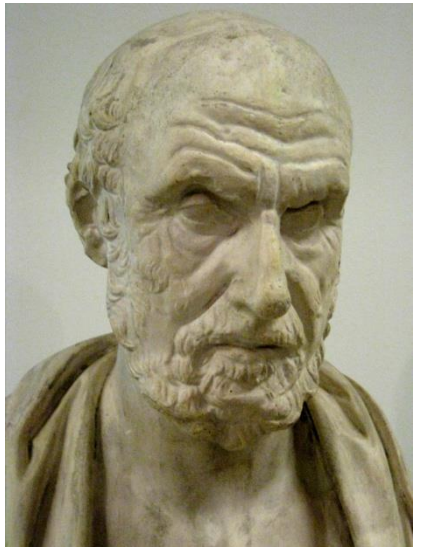
POC diagnostika - terminologie

analýzy na místě (point-of-care testing)
analýzy mimo laboratoř (off-site testing)
analýzy u pacienta (near patient testing)



SOME HISTORY

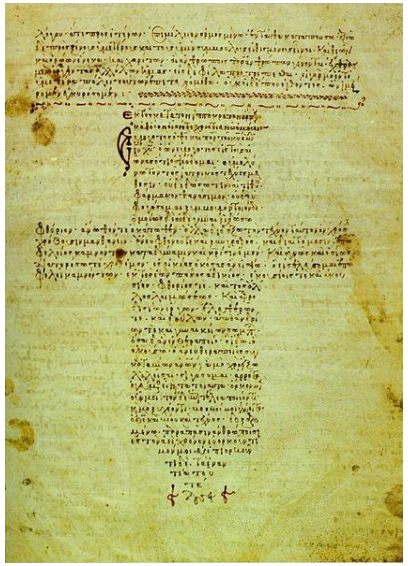
BREATH ANALYSIS



Hippokrates (460 BC – c. 370 BC)

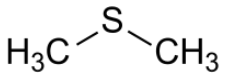
fetor oris and *fetor hepaticus* in his treatise on breath aroma and disease

fetor oris = **halitosis (anaerobic bacteria in the mouth)**



fetor hepaticus = **breath of the dead**

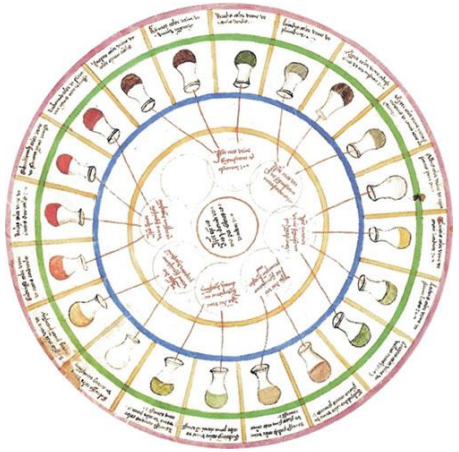
(liver failure)



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

SOME HISTORY

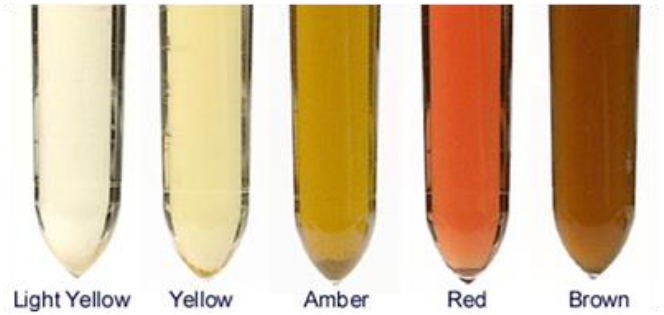
URINE WHEEL – POC diagnostics in 15th century

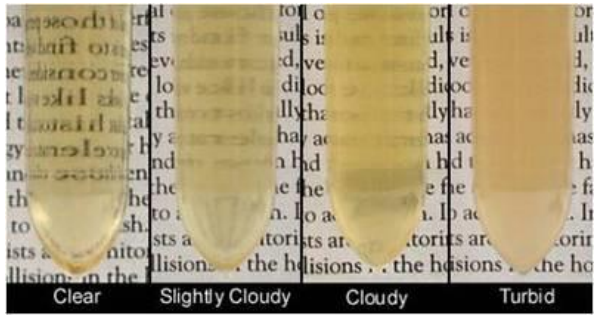


1506 by Ulrich Pinder *Epiphanie Medicorum*

Urine testing - uroscopy

sweet	- diabetes
brownish	- jaundice/hepatitis
Red, foam	- kidneys
blood	- urinary tract cancer





10 Colors That Suggest Urine Trouble

Written By: Scott LaFee

Share This Article:

Nobody talks about urine in polite company, but it says a lot about you. Its odor, consistency and color are all tell-tale indicators of your lifestyle and well-being, ranging from what you've been eating and drinking lately to diseases you might not know you have.

No color. Possibly long term kidney disease or uncoltrolled diabetes.

Amber or honey. Possibly dehydrated.

Light orange. Possibly dehydrated, but may also be caused by liver or bile duct problems

Dark orange or brown. A possible symptom of jaundice, rhabdomyolysis or Gilbert's syndrome

Red.Blood in the urine, called hematuria, possible lead or mercury poisoning

Blue. metabolic disorder known as familial hypercalcemia or "blue diaper syndrome"

Dark brown or black.

White or milky.

http://health.ucsd.edu/news/features/Pages/2014-04-21-colors-that-suggest-urine-trouble.aspx

1874 Anstie – there is alcohol even in breath

1897 Nebelthau - those with diabetes have acetone in their breath

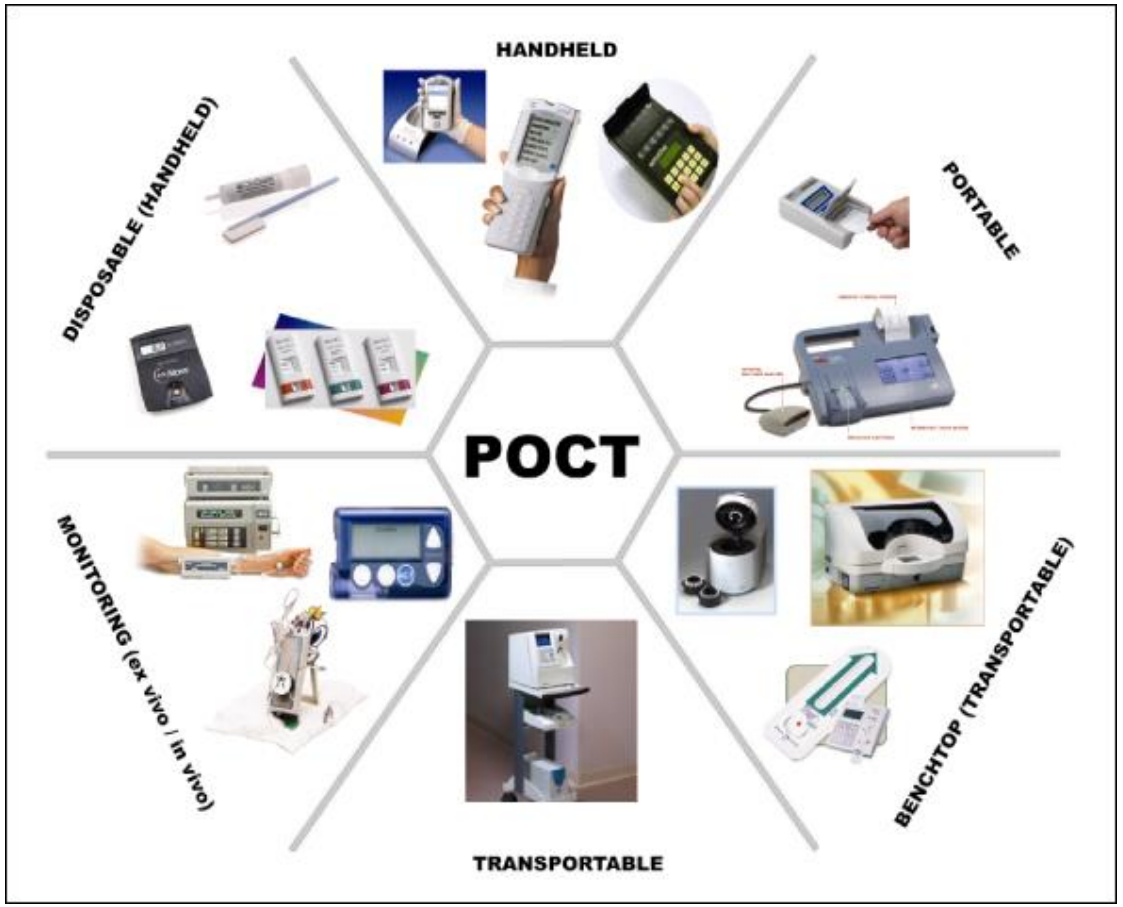
1927 Bogen first Alco Tester

McNalley Breathalyzer

1936 Patent Harger

Dräger

EXAMPLES OF POC DEVICES



Single use/disposable
Manual
Portable
Laboratory (portable)

TYPES OF POC DEVICES

Indicator papers (analytes in urine, pH, etc.)

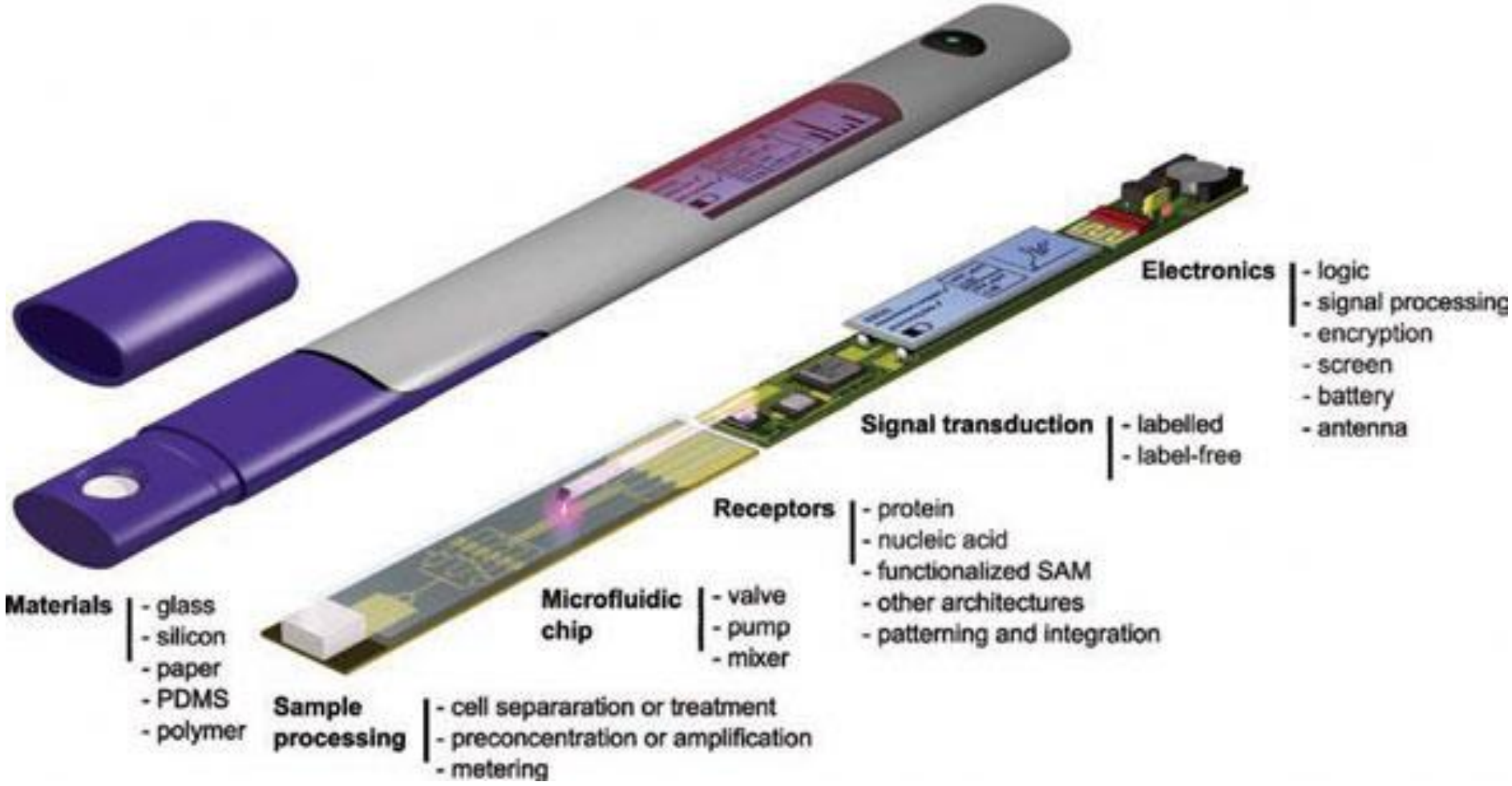
Paper microfluidics

Lateral flow assays

Microfluidic analyzers

Smartphone based devices

IDEAL POC DEVICE



http://www.nanowerk.com/spotlight/spotid=21383.php

INDICATOR PAPERS

Suitable range indicator papers (pH 5.0 – 9.0) are used for approximate pH measurements, for instance to detect the acidification of an organism

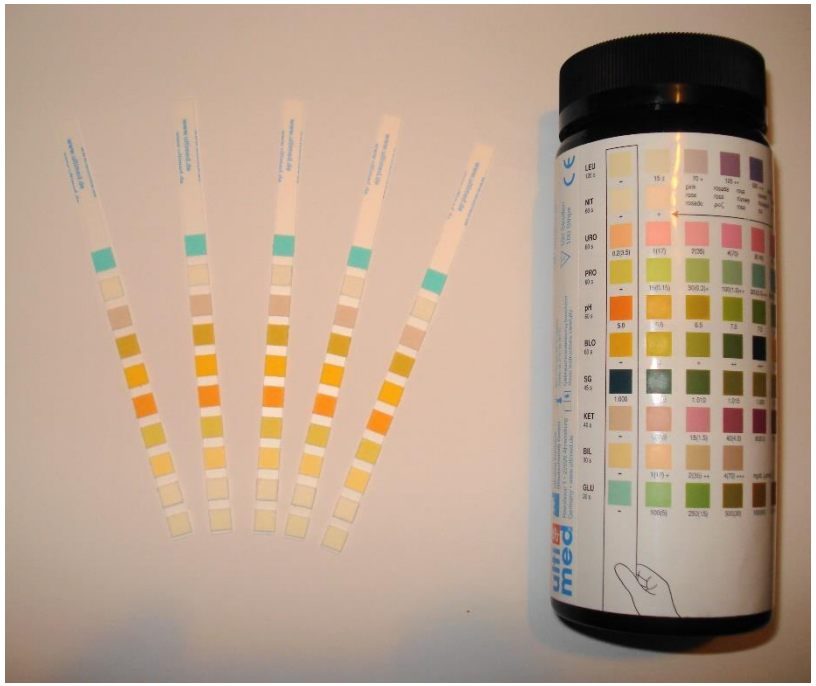
Normal: 4.6-8.0[1]

Abnormal: Some foods (such as citrus fruit and dairy products) and medicines (such as antacids) can affect urine pH.

A high (alkaline) pH can be caused by severe vomiting, a kidney disease, some urinary tract infections, and asthma

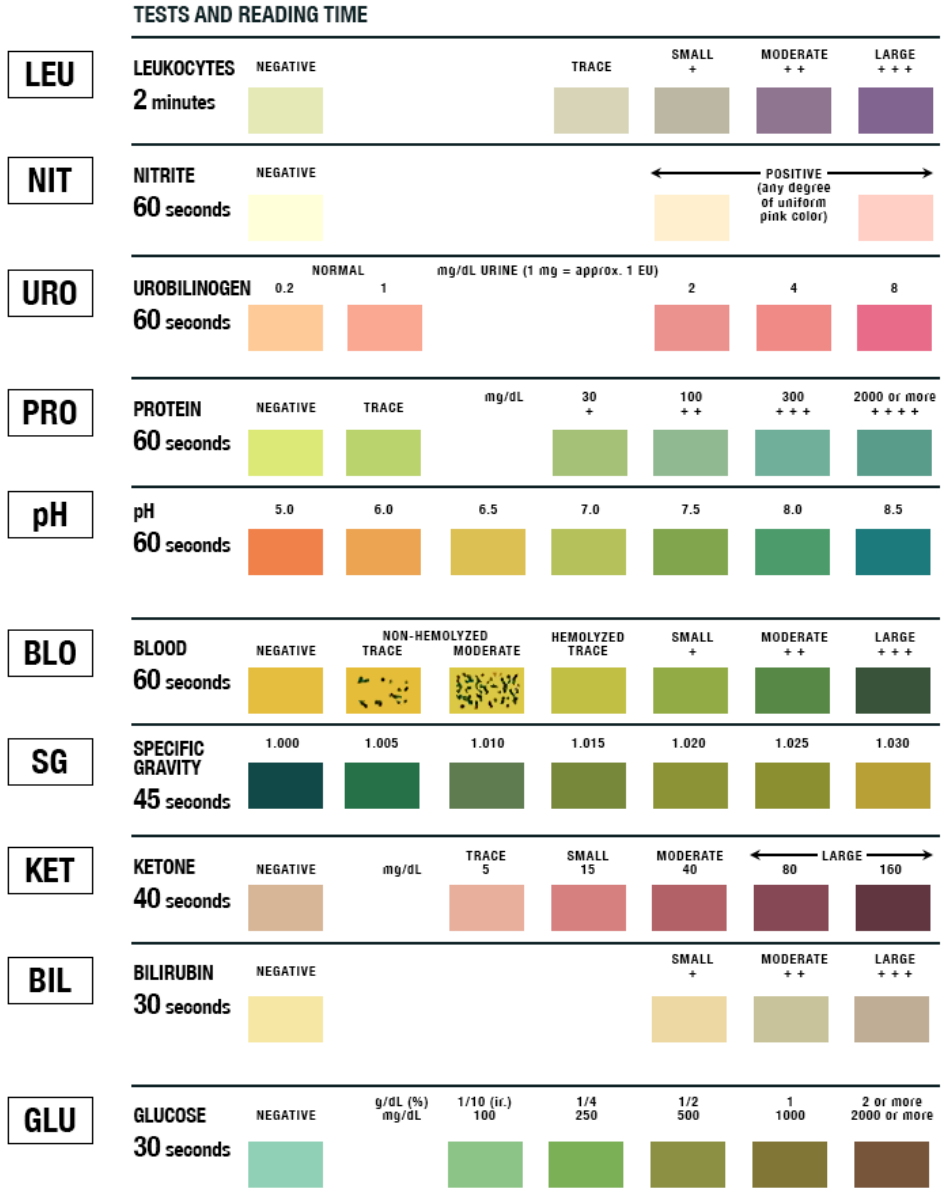
A low (acidic) pH may be caused by severe lung disease (emphysema), uncontrolled diabetes, aspirin overdose, severe diarrhea, dehydration, starvation, drinking too much alcohol, or drinking antifreeze (ethylene glycol).

Diabetes monitoring



Analytes:

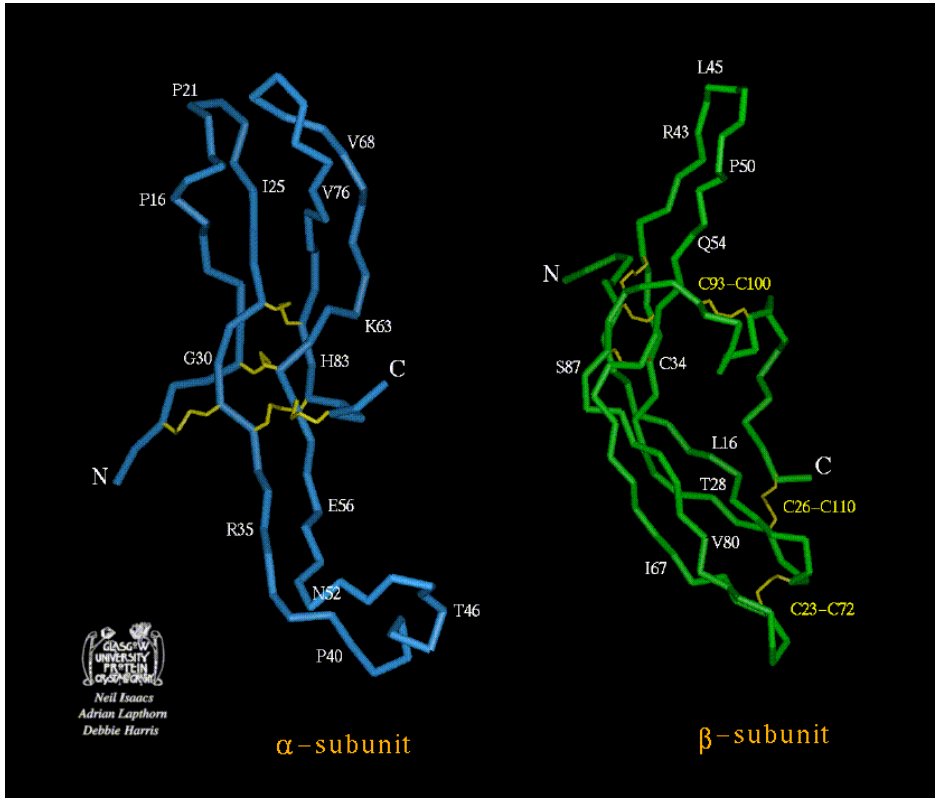
WBC	Urinary tract infection
nitrite	
proteins	
pH	
blood	hematurai
ketones	ketonuria
urobilinogen	liver diseases
bilirubin	
glucose	diabetes

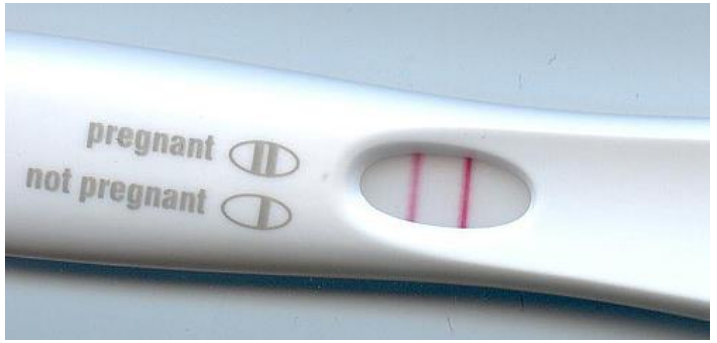


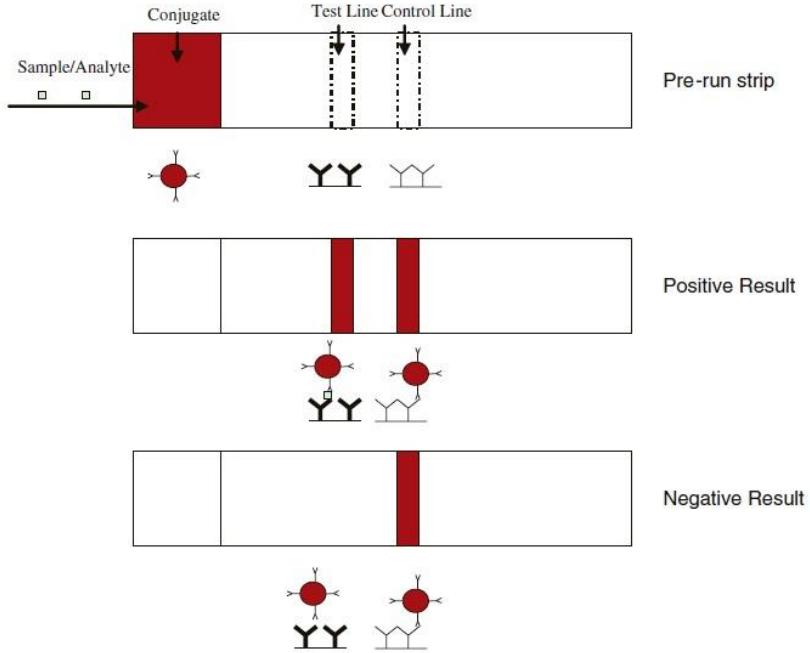
PREGNANCY TEST

hCG – human choriogonadotropine – hormone

hCG was discovered in the urine of pregnant women in 1927 and today's detection in majority of the pregnancy tests relies on detection of this compound







hCG glykoproteine hormone hCG can be determined in urine

hCG is formed from 2 subunits, α a β

α subunit of hCG = non-specific

β subunit of hCG = specific

hCG levels double about every two days in a pregnant woman, so the test is much more reliable two weeks after conception than one week later.

The tests work by binding the hCG hormone, from urine, to an antibody and an indicator. The antibody will only bind to hCG; other hormones will not give a positive test result. The usual indicator is a pigment molecule, present in a line across a home pregnancy urine test.

The most sensitive tests are able to detect 10 mIU/ml, usually though 20mIU/ml.

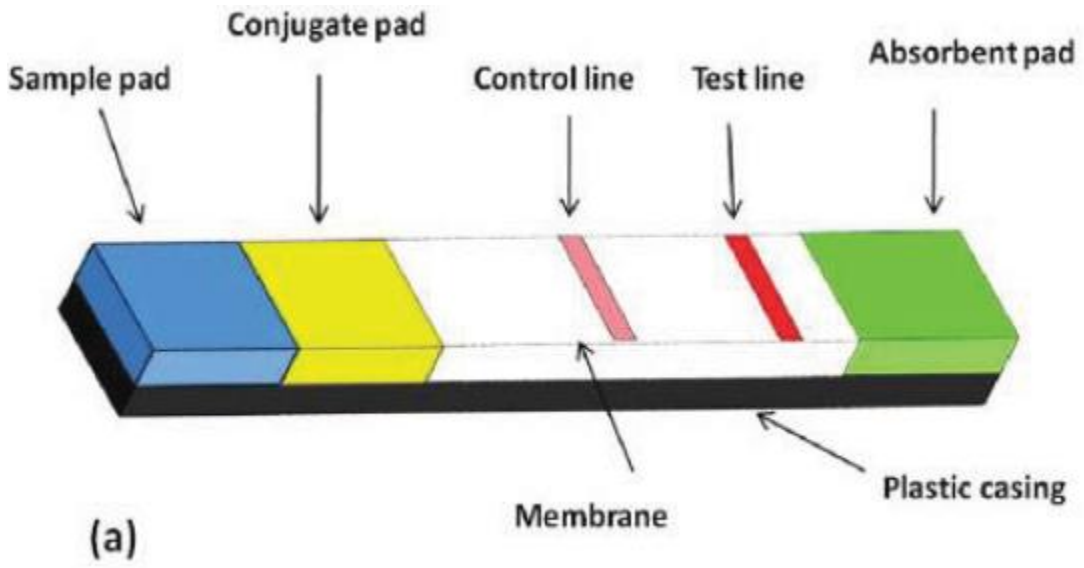
Best if performned in the third week of suspected pregnancy.

The blood test can detect already 2 mIU/ml.

LATERAL FLOW ASSAYS (LFA)

Series of membranes, overlapping

Material: cellulose, nitrocellulose, cellulose acetate, glass fibres, etc.



LATERAL FLOW ASSAYS (LFA)

SAMPLE PAD

– sample introduction (pipette)
– alternative blood plasma separation (to remove the red blood cells)
– uniform flow of the sample

CONJUGATE PAD

– contains antibodies specific for the analyte
– antibodies are bound to particles that can be detected

– blue color – latex particles (nm size)modrá barva – latexové částice (velikost n

– red color - Au particles

MEMBRANE

– different material, usually hydrophobic nitrocellulose

– two zones on the membrane : control and test

– test zone contains immobilized antigens that can capture the analyte with the bound color particle

– control zone – to test the functionality of the LFA

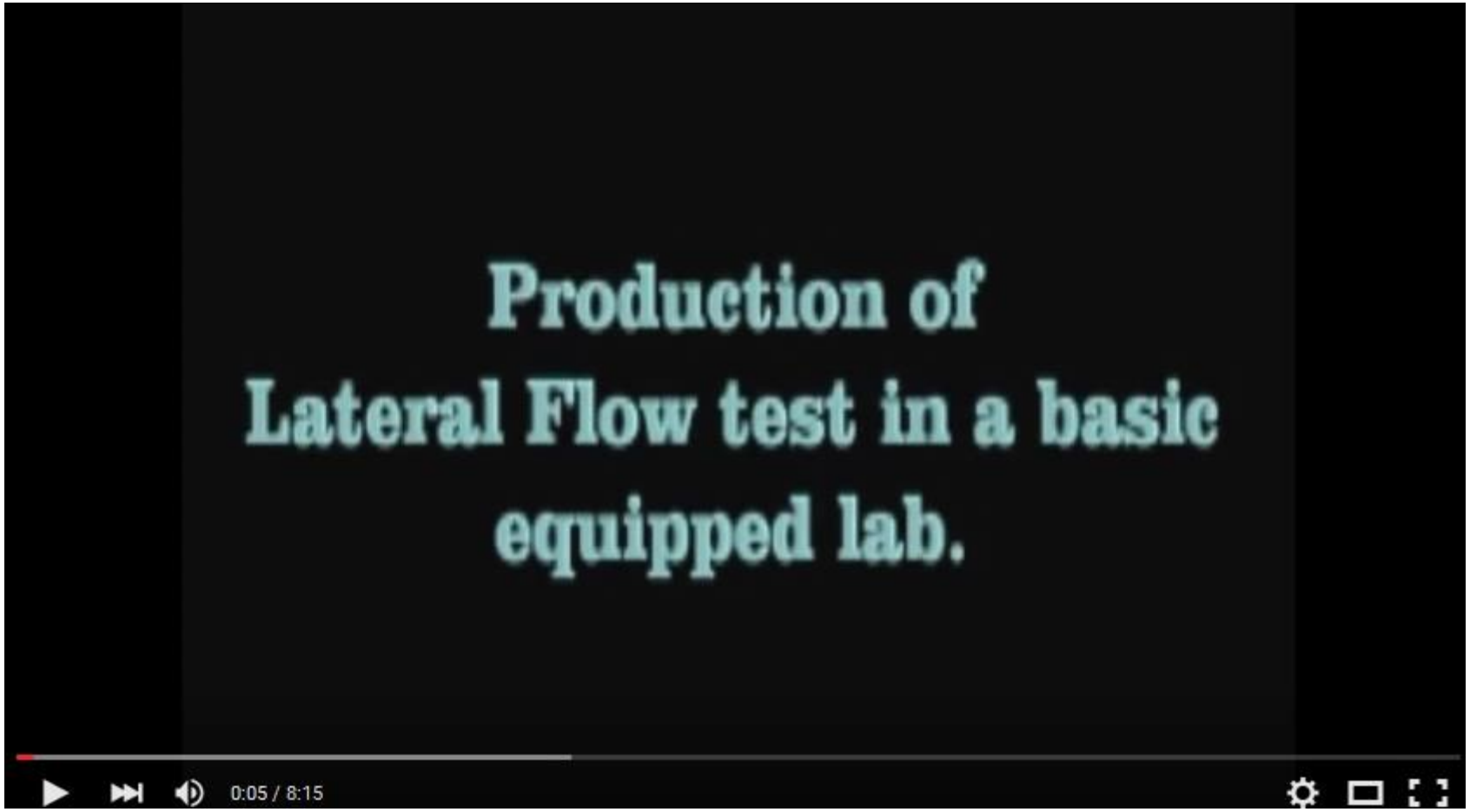
ABSORBENT PAD

– usually cellulose

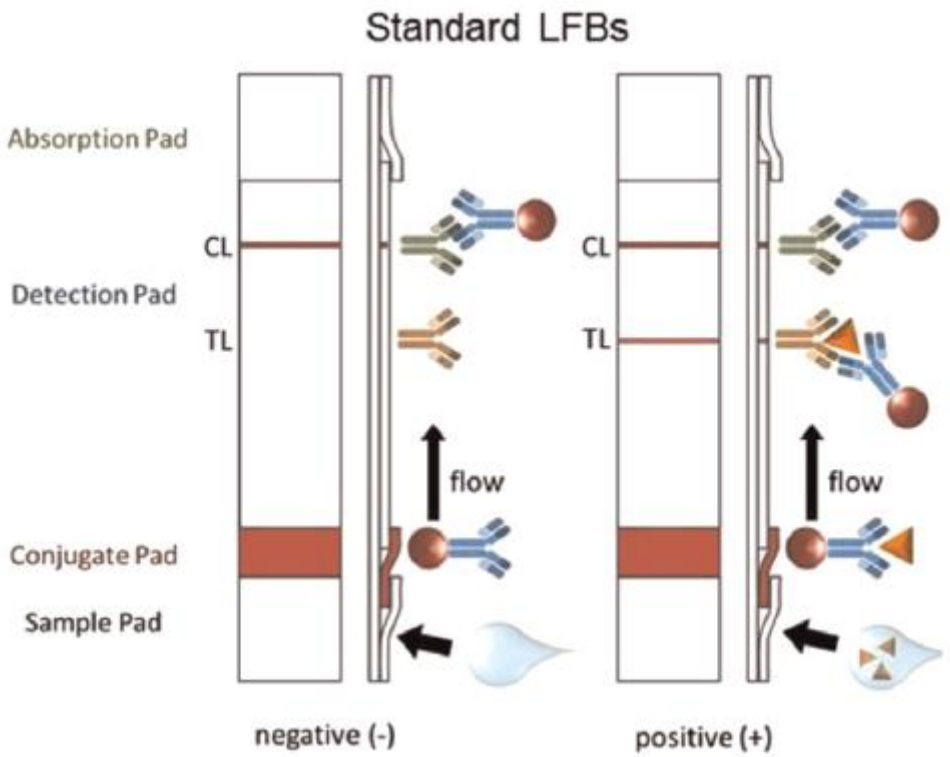
– keeps constant flow of reagents and sample

– collection of waste

PREPARATION OF LFA



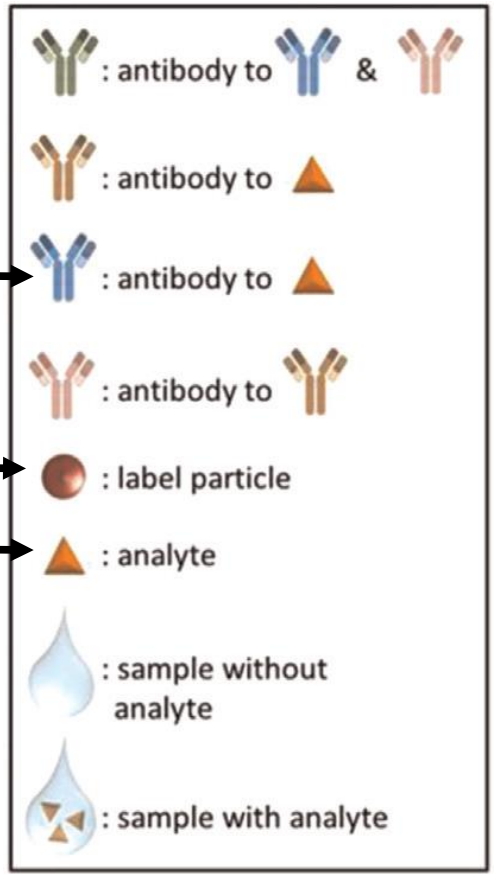
STANDARD LFA



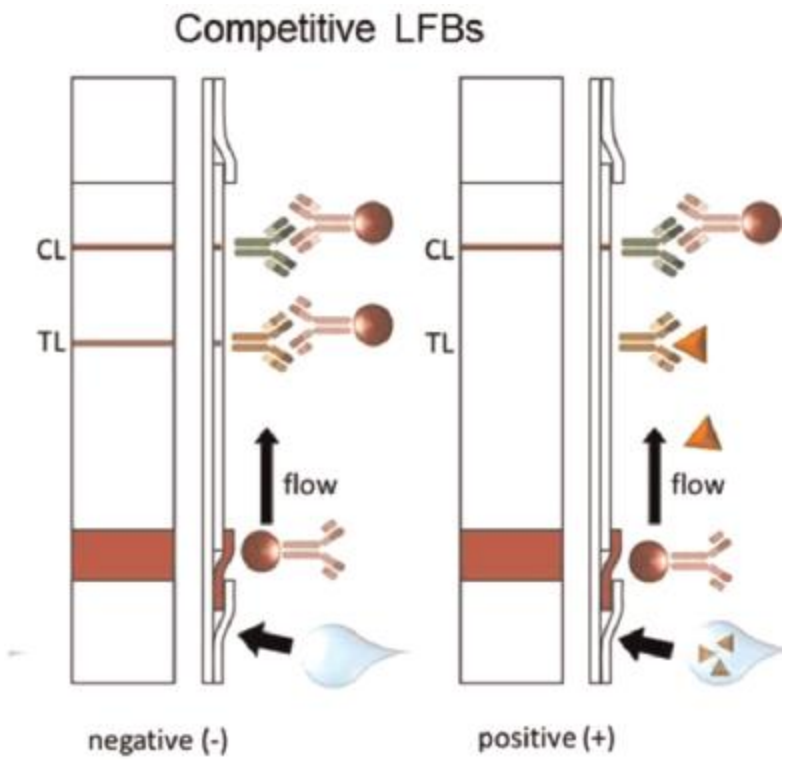
Protilátka

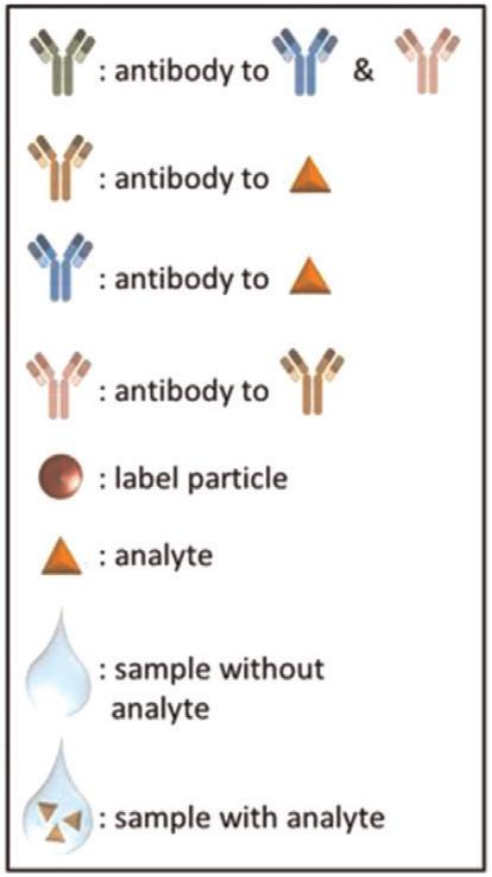
Au nanočástice

Stanovovaná látka



COMPETITIVE LFA





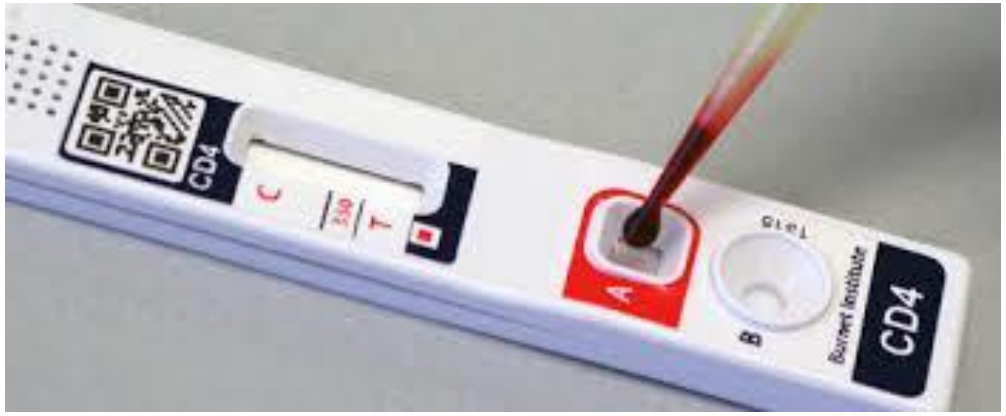
AIDS TEST (2012)

http://www.omegadiagnostics.com/products/by-area/hiv/

VISITECT HIV 1/2

VISITECT® HIV 1/2 is a qualitative test for the detection of antibodies to HIV 1/2 virus in human serum, plasma and whole blood specimens. The test is easy-to-use, offers high sensitivity and specificity and produces accurate results within 15 minutes.

AIDS TEST (2012)



Omega Diagnostics Group has announced the launch of a ground-breaking new point-of-care disposable test for the detection of HIV.
VISITECT CD4 detects CD4 T-cell levels and was developed by the Burnet Institute in Melbourne, Australia.
An easy-to-use semi-quantitative lateral flow test, it uses a finger-prick blood sample to produce a visual result in 40 minutes, enabling patients to receive life-saving antiretroviral treatment before leaving the clinic.

EBOLA, DENGUE FEVER, YELLOW FEVER TESTS

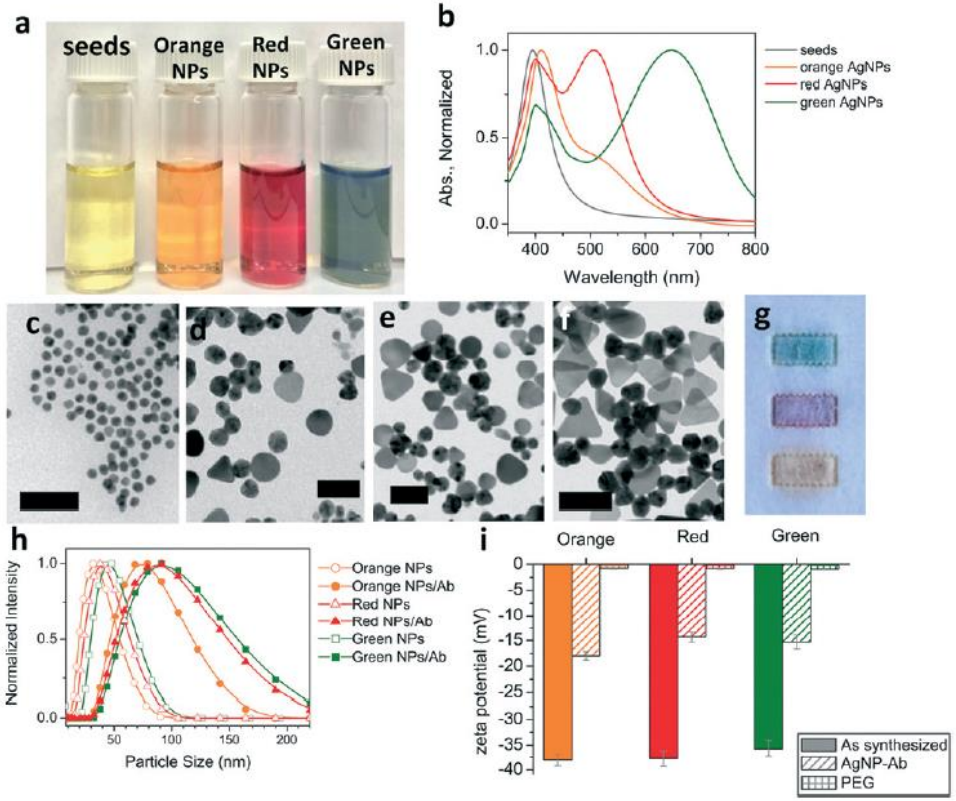
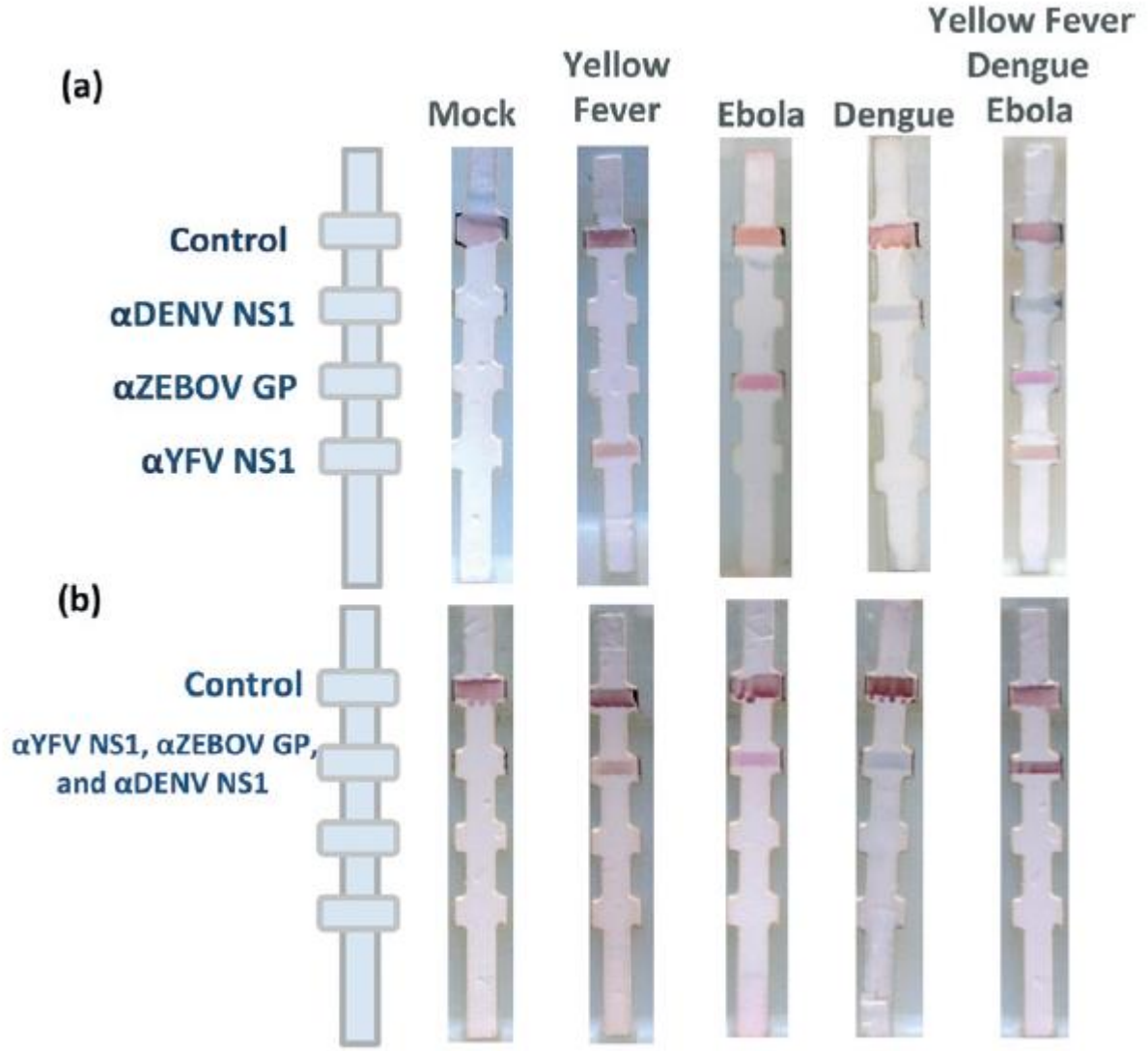


Fig. 1 AgNPs for multiplexed detection. a) Vials of AgNPs during stepwise growth and b) their corresponding absorption spectra. TEM images of c) Ag seeds, d) orange AgNPs, e) red AgNPs, and f) green AgNPs. Scale bars: 50 nm. g) Green, red, and orange (top to bottom) AgNPs on nitrocellulose paper. h) DLS and i) zeta-potential of AgNPs before and after mAb conjugation.



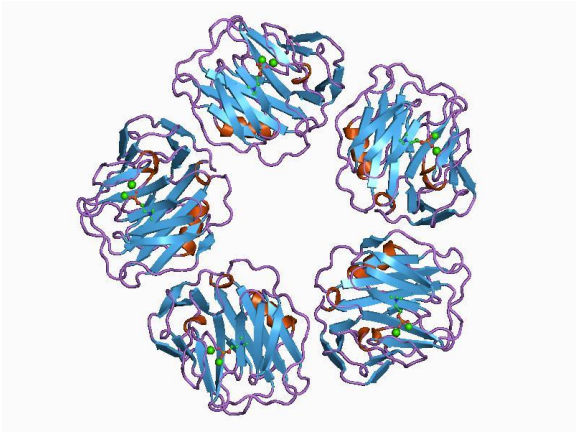
Yen et al. Lab Chip, 2015, 15, 1638

CRP test

C-reactive protein (CRP) is a protein that increases in the blood with inflammation and infection as well as following a heart atack, surgery, or trauma.

Normal concentration in healthy human serum is between 5 and 10 mg/L, increasing with aging. Higher levels are found in late pregnant women, mild inflammation and viral infections (10–40 mg/L), active inflammation, bacterial infection (40–200 mg/L), severe bacterial infections and burns (>200 mg/L).

The CRP level in blood increases with bacterial infections already after 6-12 hours from infection.



CRP pentamer
v krevní plasmě


iDNES.cz
CRPtest
test pro rozlišení
virové a bakteriální
infekce
2 testy
rychlý
výsledek
5min.
CRPtest
0:00 / 1:00
HD

GLUCOMETERS

Device for measurement of glycemia at home (self monitoring)

Depending on the level of glycemia and previous experience one can estimate the amount of insulin that is necessary

The goal is to achieve the glycemic curve of a non-diabetic person

Glucometers can often calculate weekly and monthly averages from the measured values.

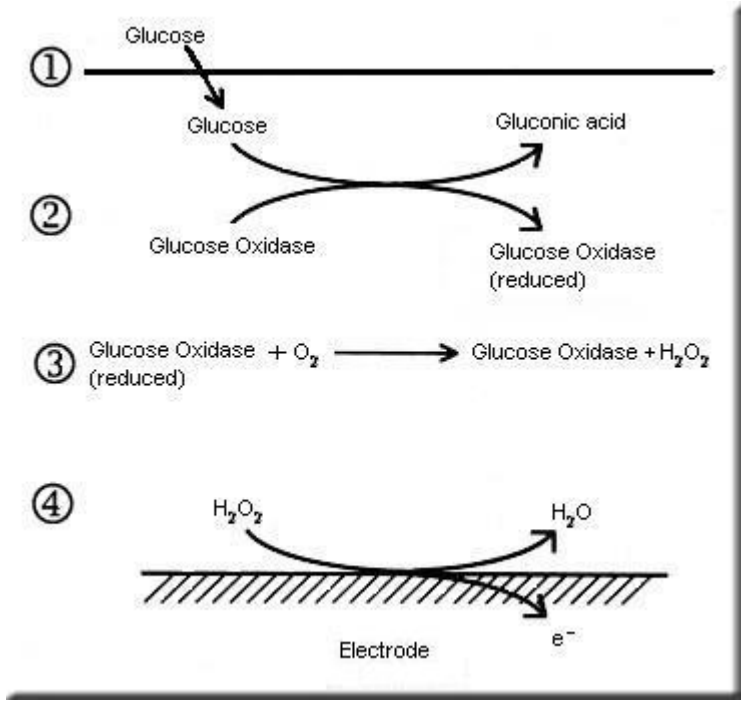
Typically electrochemical methods

The test strip contains narrow capillary that sucks the blood inside.

The glucose is oxidized and H_2O_2 is released.

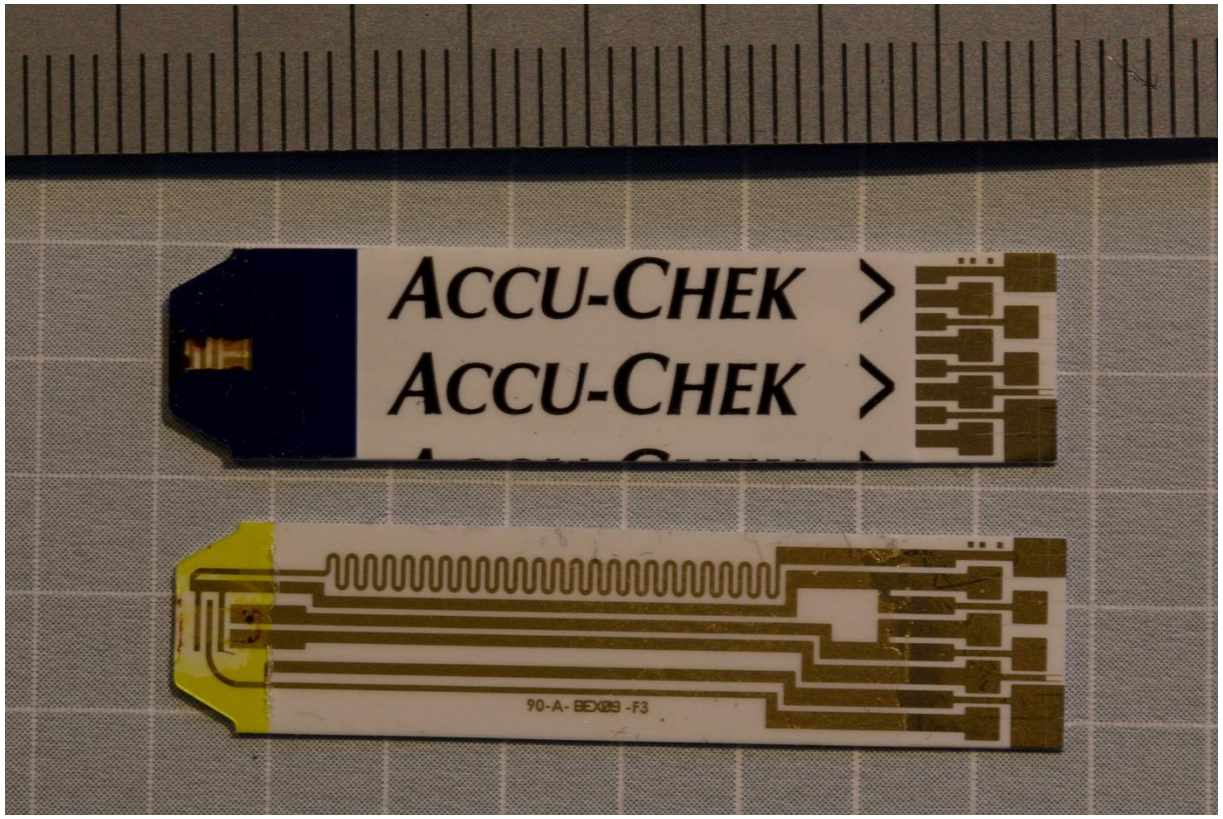
The more glucose the more H_2O_2

H_2O_2 is electrolytically decomposed, electrons are measured as electric current and recalculated to the glucose content.

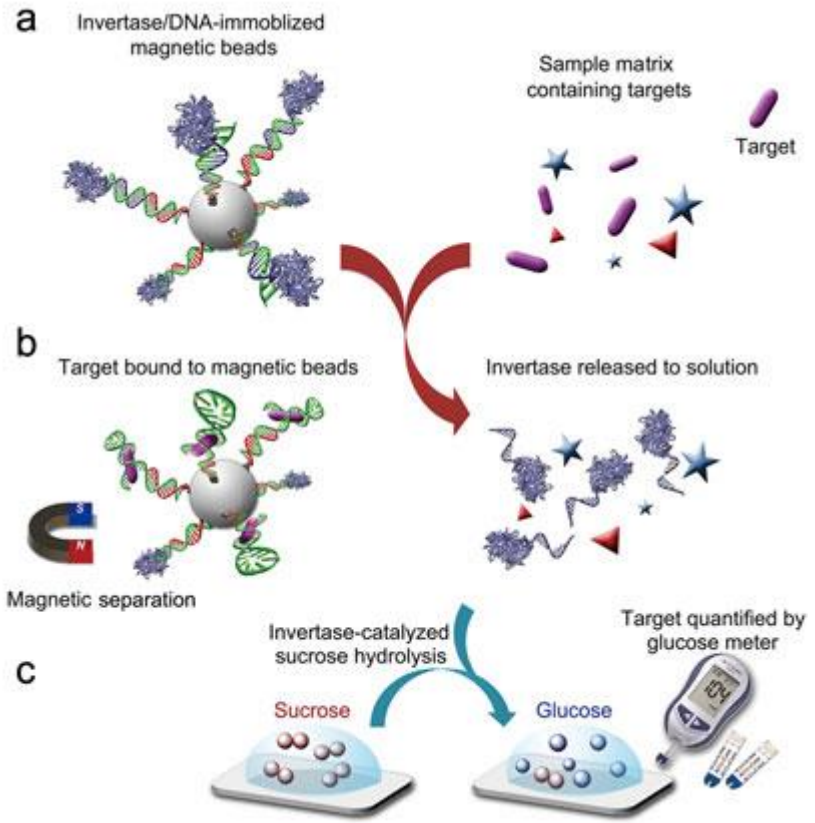


ACCU CHECK (Roche)

https://www.accu-chek.cz/



EXAMPLE – USE OF GLUCOMETER FOR OTHER PURPOSE



Analysis of kokainu, interferon gamma, ions

The method is based on the target-induced release of invertase from a functional-DNA–invertase conjugate. The released invertase converts sucrose into glucose, which is detectable using the meter.

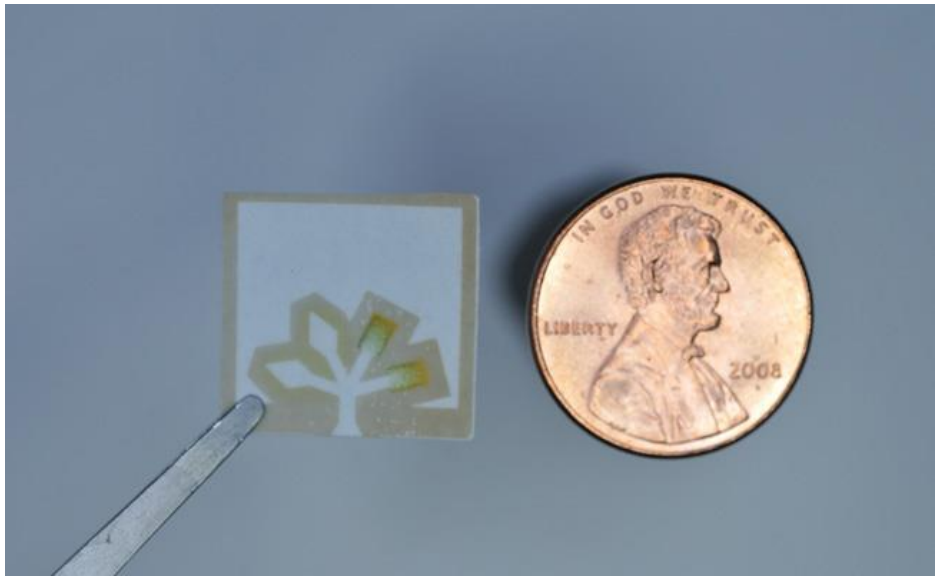
Xiang, Lu, Nature chemistry, 3,2011, 697-703

PAPER MICROFLUIDICS

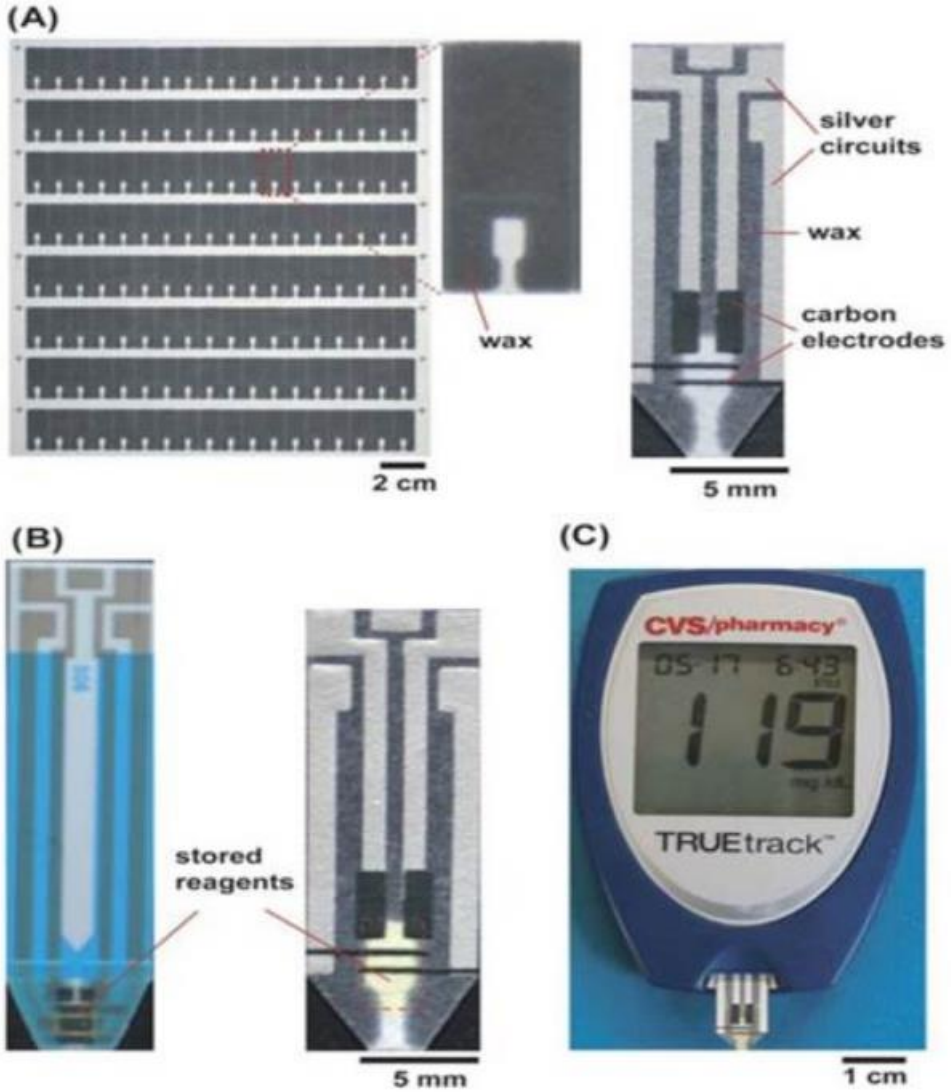
http://gmwgroup.harvard.edu/

George M. Whitesides

George M. Whitesides was born August 3, 1939 in Louisville, KY. He received an A.B. degree from Harvard University in 1960 and a Ph.D. from the California Institute of Technology (with J.D. Roberts) in 1964. He was a member of the faculty of the Massachusetts Institute of Technology from 1963 to 1982. He joined the Department of Chemistry of Harvard University in 1982, and was Department Chairman 1986-89, and Mallinckrodt Professor of Chemistry from 1982-2004. He is now the Woodford L. and Ann A. Flowers University Professor.

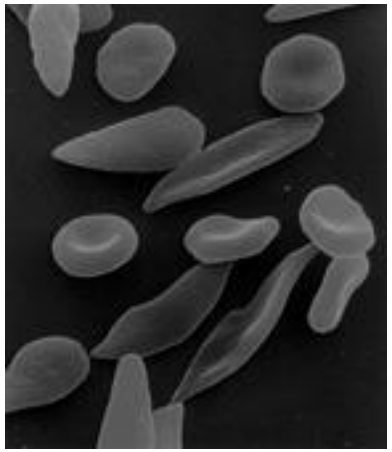


GLUCOMETER WITH PAPER SENSOR

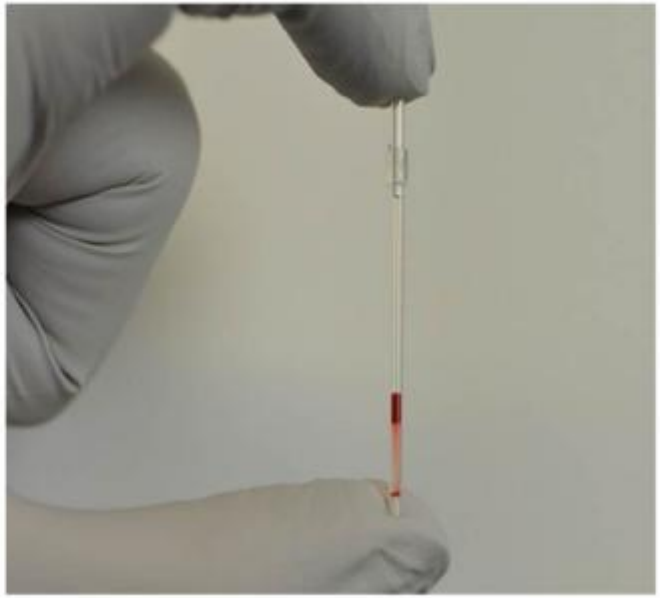


A paper-based electrochemical sensor that can be read with a commercial glucose meter. (A) Wax printing allows the high-throughput production of devices with hydrophobic regions and screen printing is used to add electrodes on top of the printed devices. (B) Comparison of a commercial test strip for glucose with a paper-based test strip. (C) The paper-based test strip fits into a commercial glucose meter.

Sickle cell anemia



The change of shapes of RBC

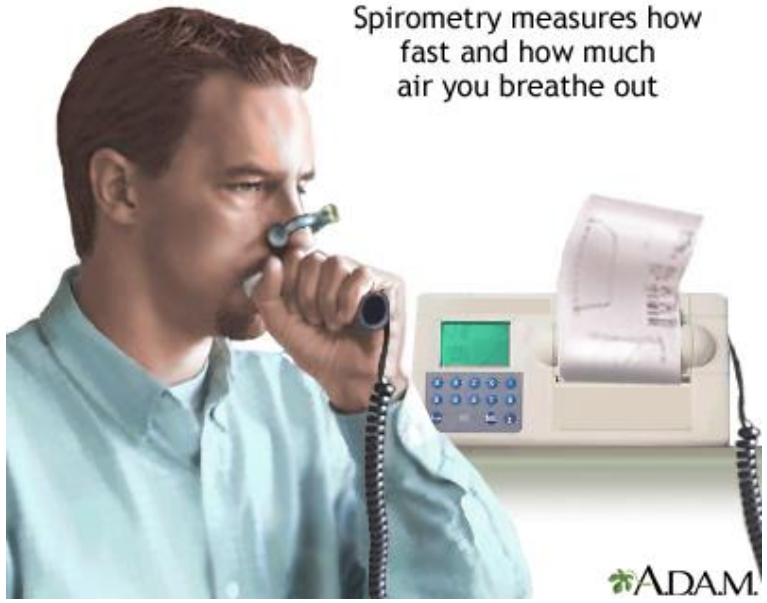


The average life span of people with sickle cell anemia is 42 for men and 48 for women

A low-cost, rapid test for sickle cell disease. After centrifugation over an aqueous multiphase system, blood cells from a donor with sickle cell disease separate by density. Dense cells characteristic of sickle cell disease form a red layer at the bottom of the test.

A universal mobile electrochemical detector (uMED). The uMED device (left) can perform a variety of electrochemical analyses on commercial and custom electrodes. Results can be transferred through the audio jack of any phone to enable connectivity (right).

BREATH MONITORING



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

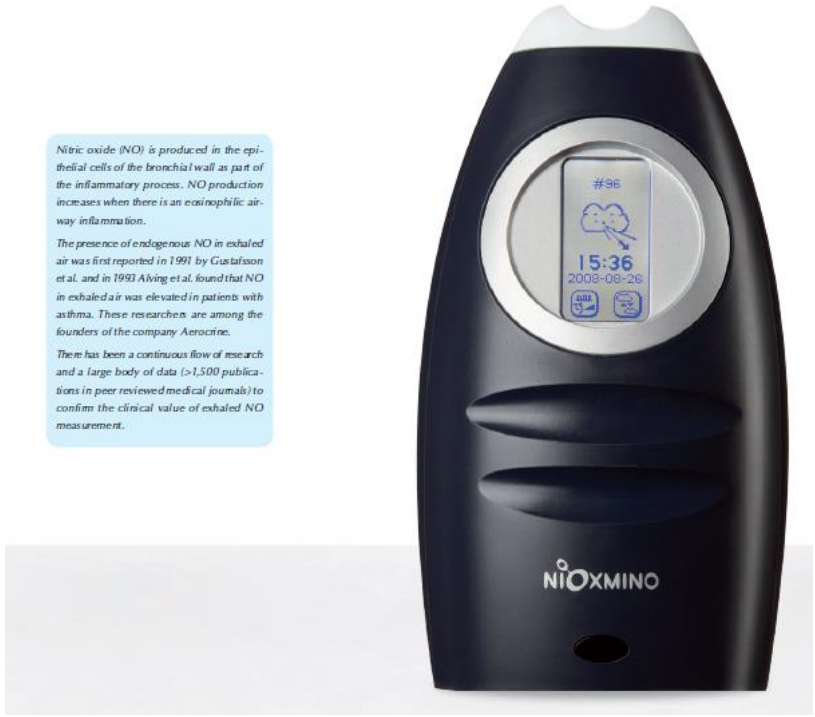
The New NIOX MINO®
Asthma Inflammation Monitor

From the company that is revolutionizing the way asthma is managed

Nitric oxide (NO) is produced in the epithelial cells of the bronchial wall as part of the inflammatory process. NO production increases when there is an eosinophilic airway inflammation.

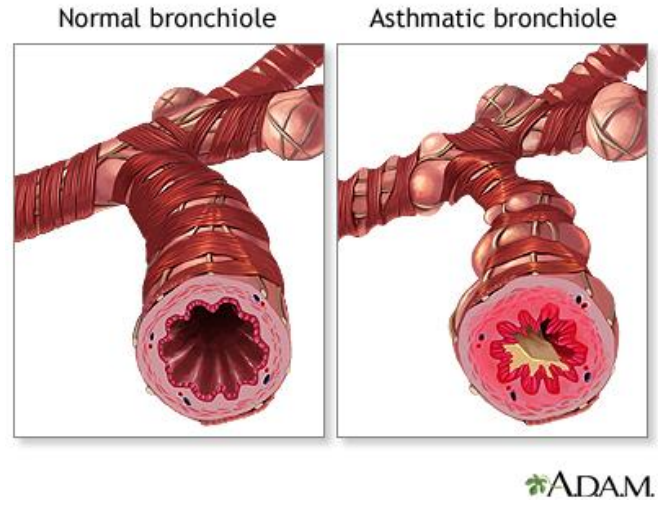
The presence of endogenous NO in exhaled air was first reported in 1991 by Gustafsson et al. and in 1993 Alving et al. found that NO in exhaled air was elevated in patients with asthma. These researchers are among the founders of the company Aerocrine.

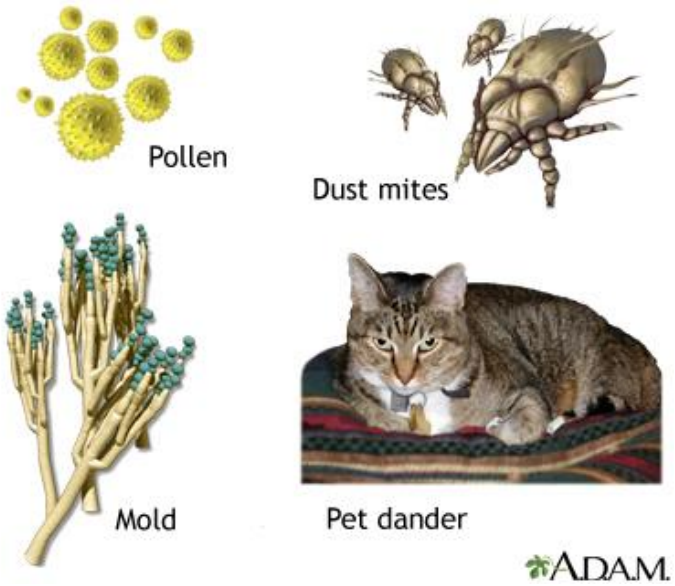
There has been a continuous flow of research and a large body of data (>1,500 publications in peer reviewed medical journals) to confirm the clinical value of exhaled NO measurement.

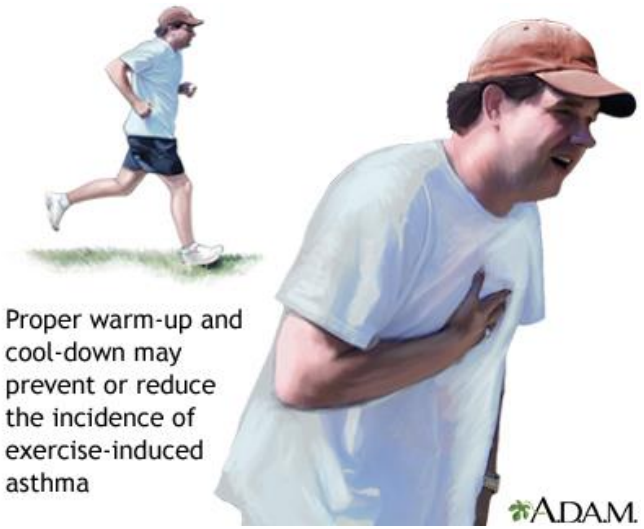


ASTHMA

Astma – a chronic disease that affect the lungs and the airways by narrowing the bronchioles.
It is characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasms.
There is no cure for asthma. Symptoms can be prevented by avoiding triggers, such as allergens and irritants, and by the use of inhaled corticosteroids.
In 2015, 358 million people globally had asthma, up from 183 million in 1990.







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

LUNG CANCER

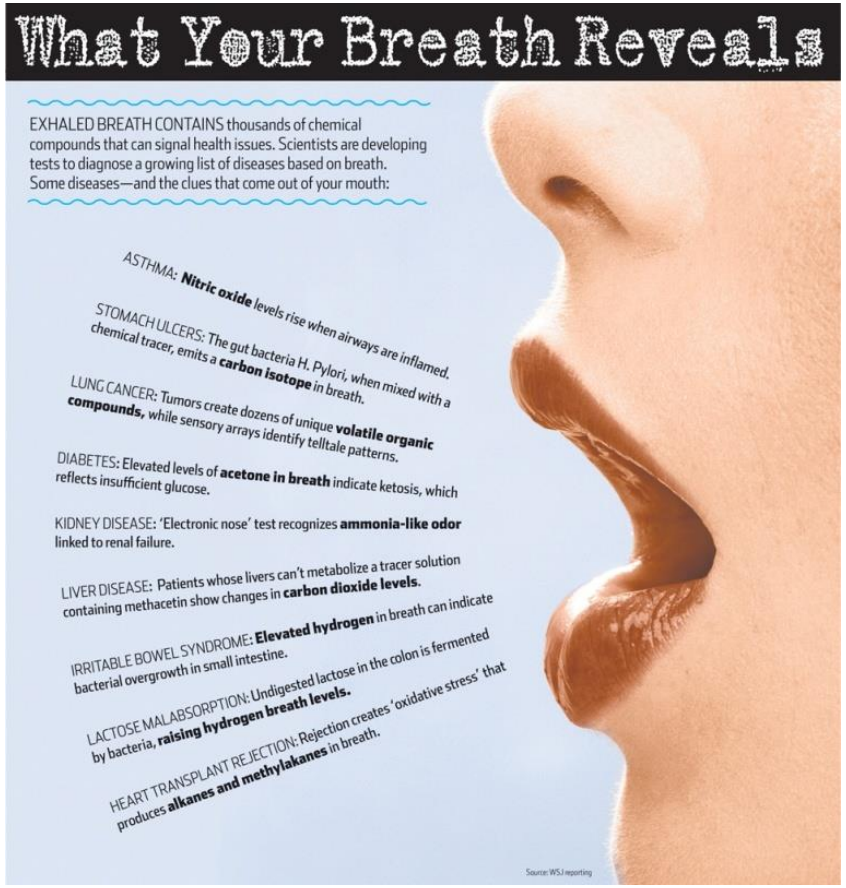
The most common cancer in the world

Non-small-cell lung carcinoma (NSCLC) and Small-cell lung carcinoma (SCLC)

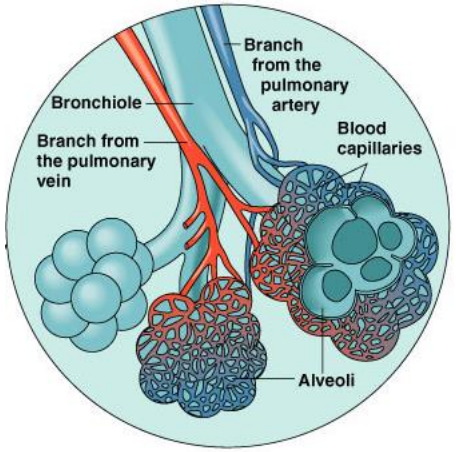
NSCLC typically surgery

SCLC chemotherapy

BREATH ANALYSIS

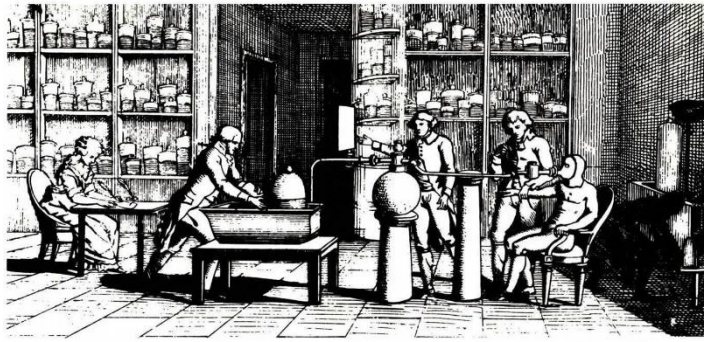


Average person exhales between 10-90.000 L of air per day



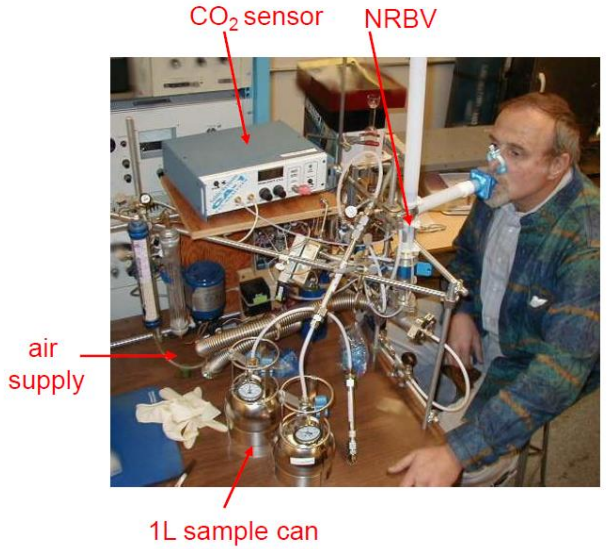
Some analytes can be analyzed by CE-C4D, some by CE-LIF

BREATH ANALYSIS



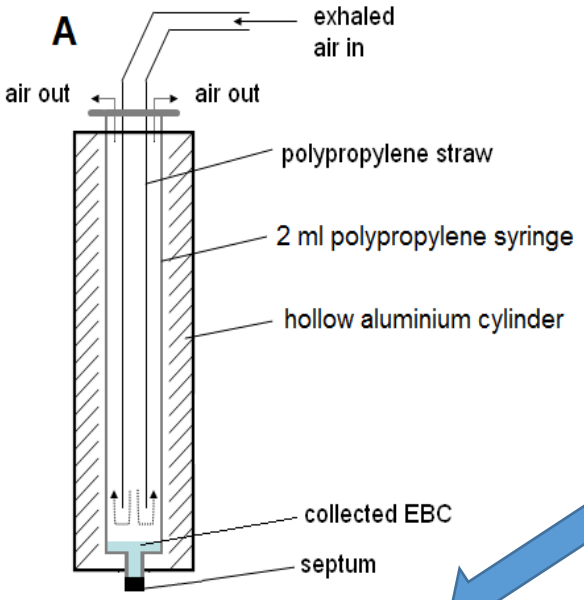
Antoine Lavoisiser 1784

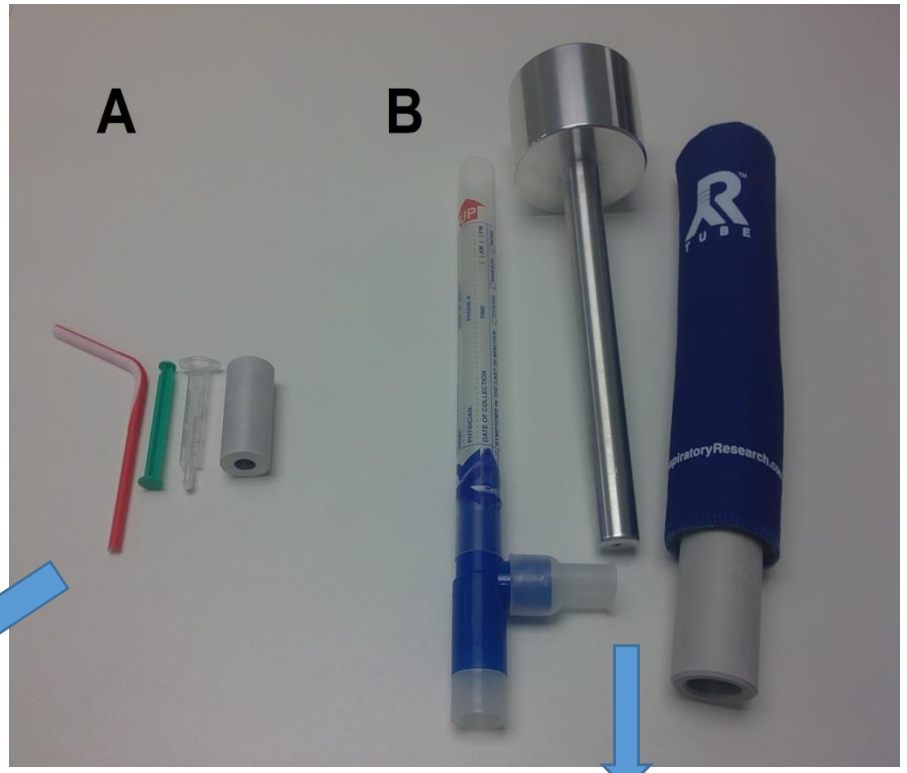
Breath Research Institute of the Austrian Academy of Sciences




Technion
Israel Institute of
Technology
SEVENTH FRAMEWORK
PROGRAMME
LCaos
A Nanoscale Artificial Nose
To Detect Lung Cancer
erc
0:01 / 1:58
CC

EXHALED BREATH CONDENSATE(EBC)– analysis of single exhalation

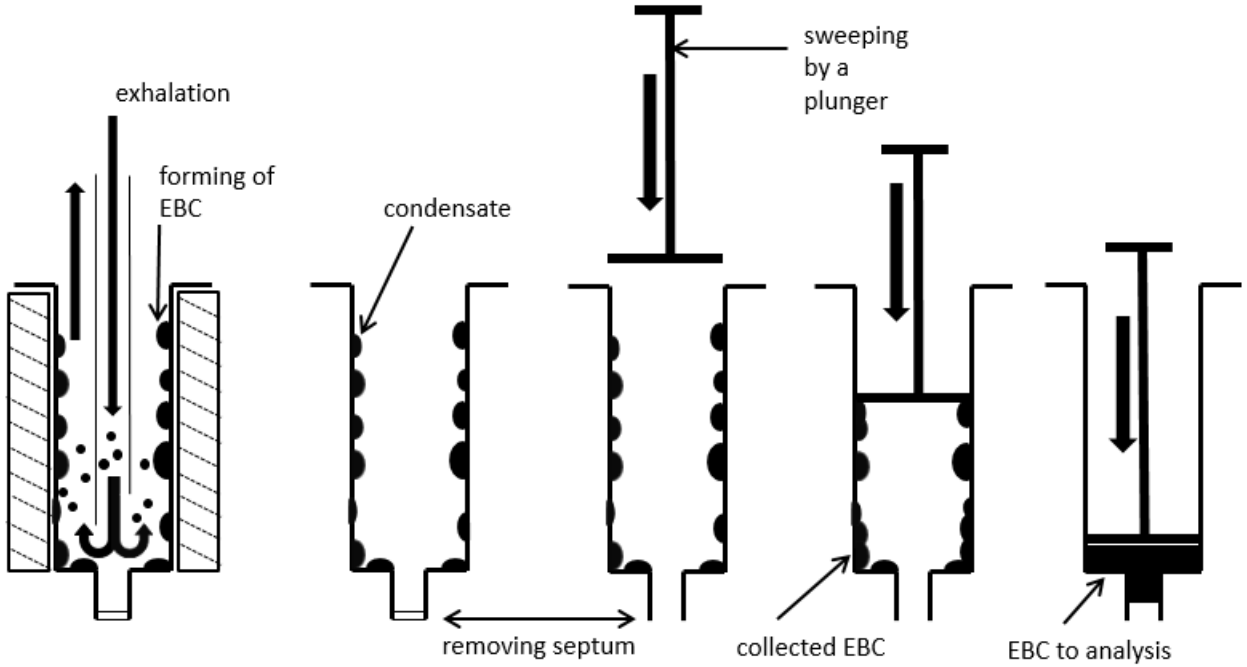




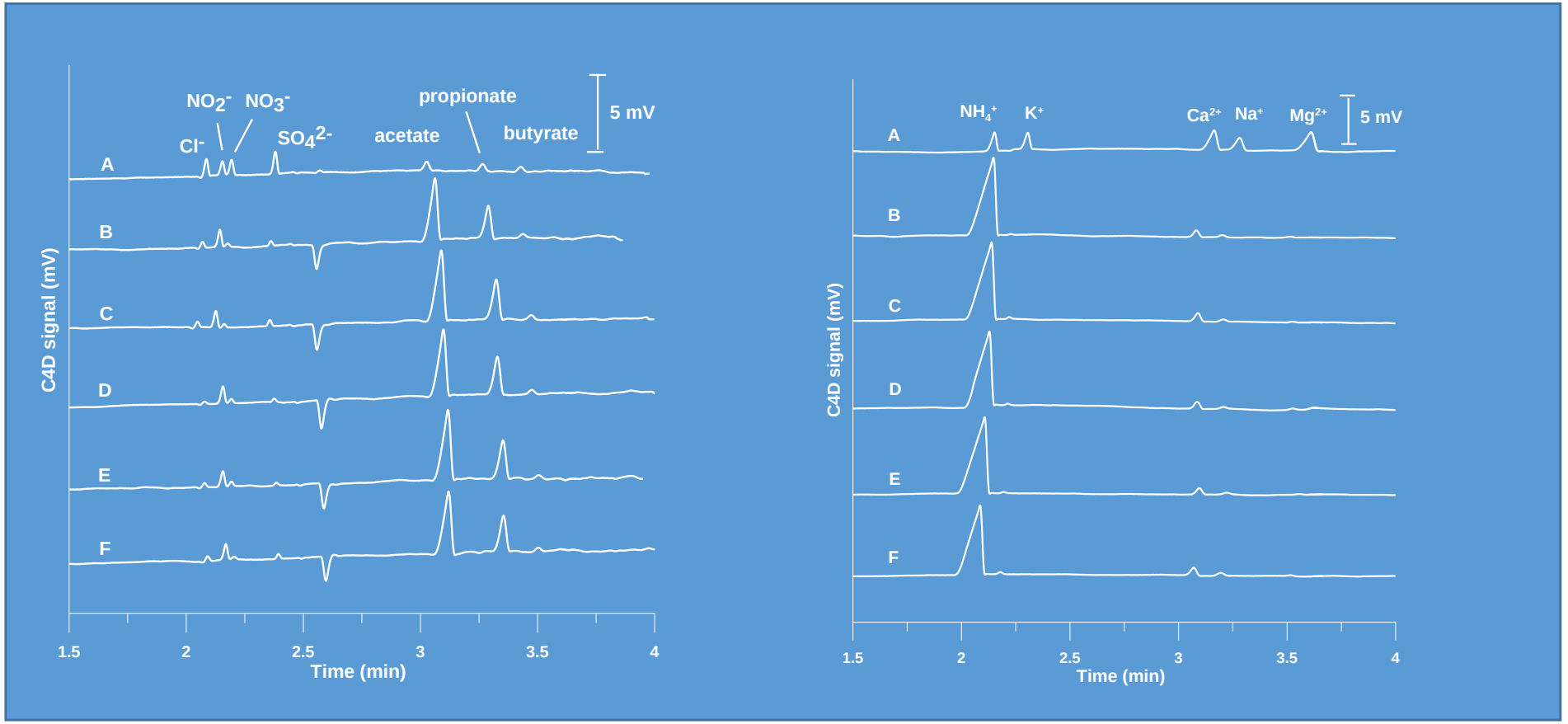
Price: 0,05 EUR
Volume of EBC/1 breath:
20 µL

Price: 20 EUR
Volume of EBC/1 breath :
25 µL

EXHALED BREATH CONDENSATE (EBC)

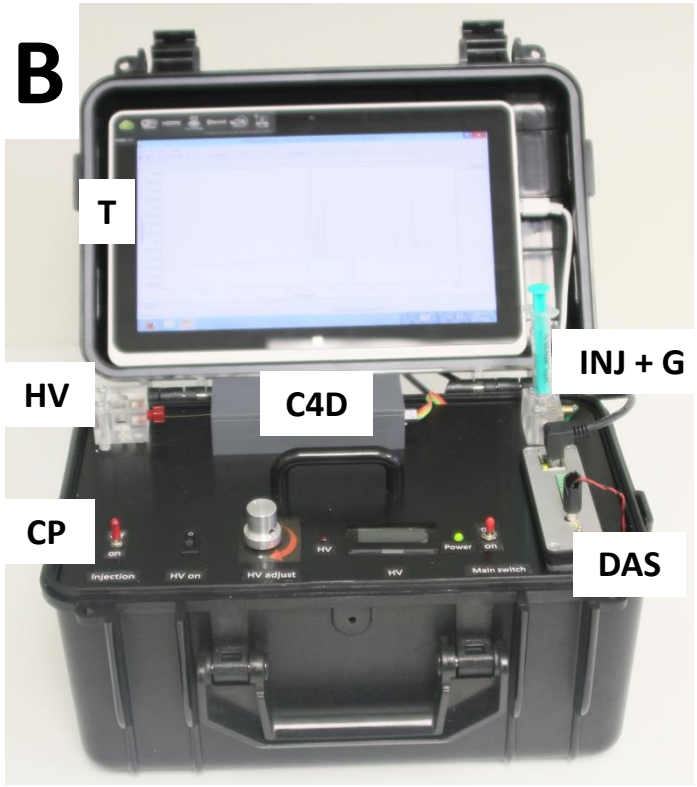


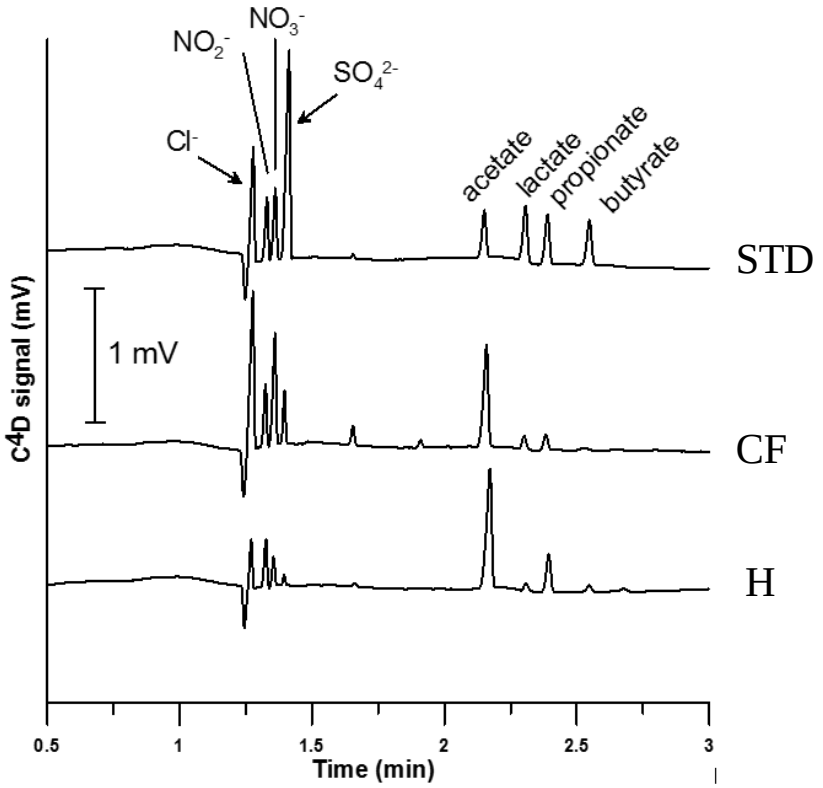
5 consecutive exhalations, analysis by CE



PORTABLE CE INSTRUMENT

EBC ANALYSIS





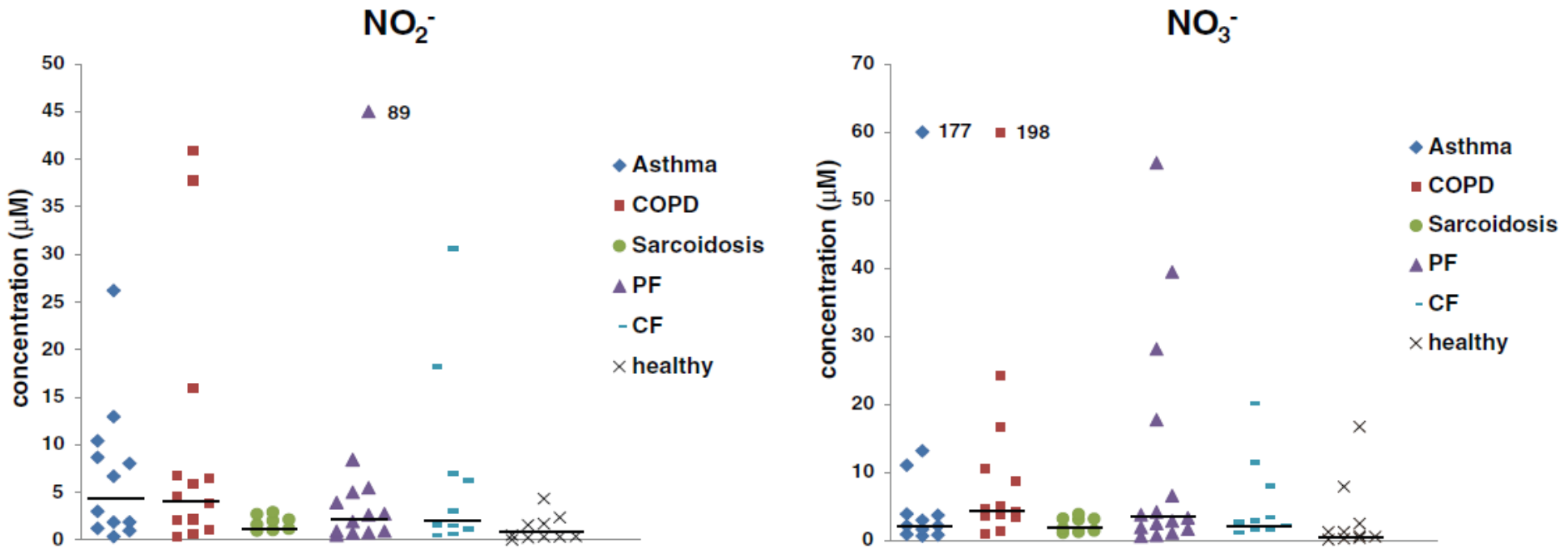
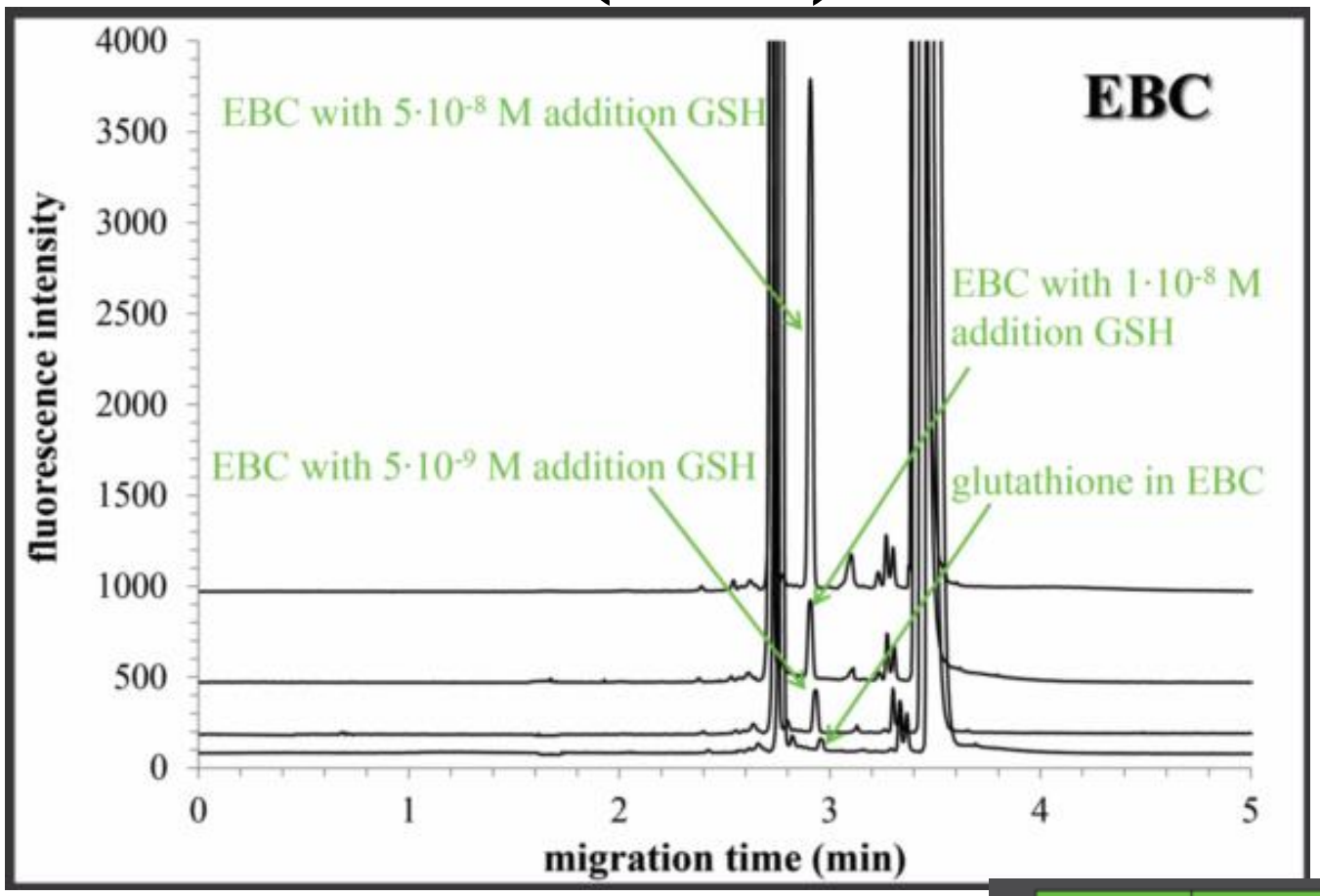


Figure 3. Scatterograms of the concentration distribution of selected ions (nitrite and nitrate) in EBC of patients with various respiratory diseases and healthy controls. The horizontal lines mark the median concentration. When out of scale on the graph, the number next to the respective symbol shows the measured concentration in μM.

Gregus et al, Journal of Breath research, 2015.

Glutathione (GSH) in EBC

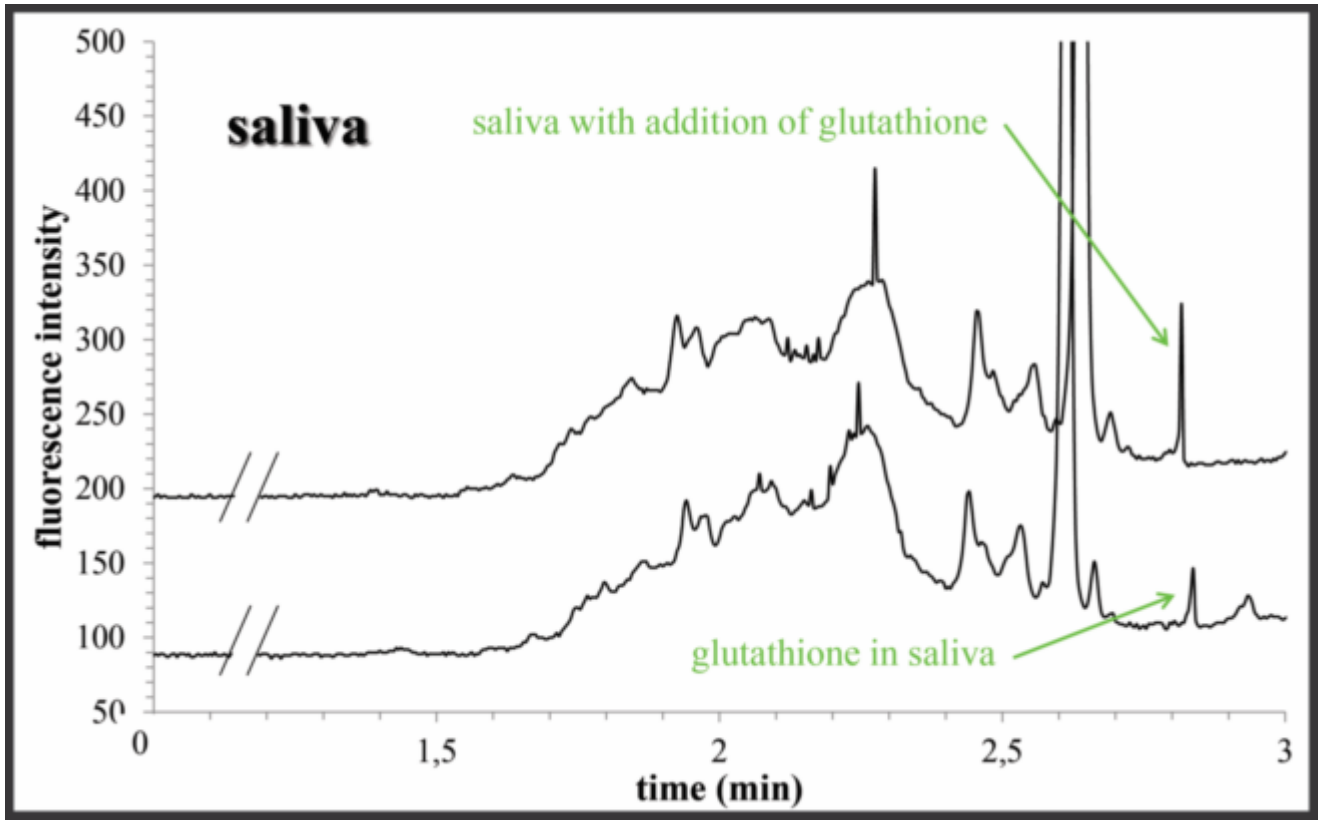


LOD [nM]	LOQ [nM]	RSD	
		migration time	peak area
0.18	0.59	0.40 %	2.76 %

Tab.1 : Analytical parameters od the developed method in real sample EBC

1000x lower LOD than C4D !!

Glutathione (GSH) in saliva



CF – cystic fibrosis

Cystic fibrosis (CF) is a genetic disorder that affects mostly the lungs, but also the pancreas, liver, kidneys and intestine

It is caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein

There is no known cure for cystic fibrosis. Lung infections are treated with antibiotics, which may be given intravenously, inhaled, or by mouth. Sometimes, the antibiotic azithromycin is used long term. Inhaled hypertonic saline and salbutamol may also be useful

Half of the patients in the Czech Republic has a life span of 32 let.
It is estimated that every 25th person is a carrier.

CF DIAGNOSTICS

genetic disorder

impaired ion transport

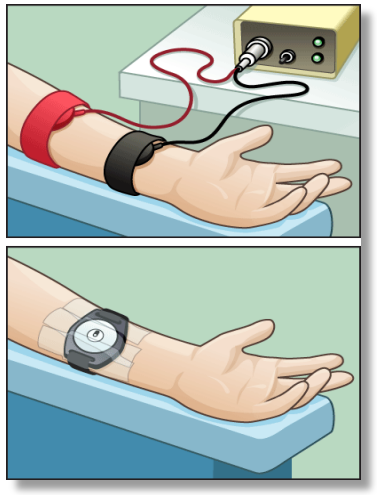
breathing problems, lung infections, GI problems

DIAGNOSIS:

ELEVATED CHLORIDE

IN SWEAT

SWEAT TEST



PAINFULL

USE OF CHEMICALS ON THE SKIN (pilocarpine)

TEST DURATION ABOUT 30 MINUTES

ALTERNATIVE SKIN WIPE

PAINLESS

NO CHEMICALS (DI WATER)

TEST DURATION ABOUT 10 SECONDS

CE-C4D SIMULTANEOUS SEPARATION OF ANIONS AND CATIONS

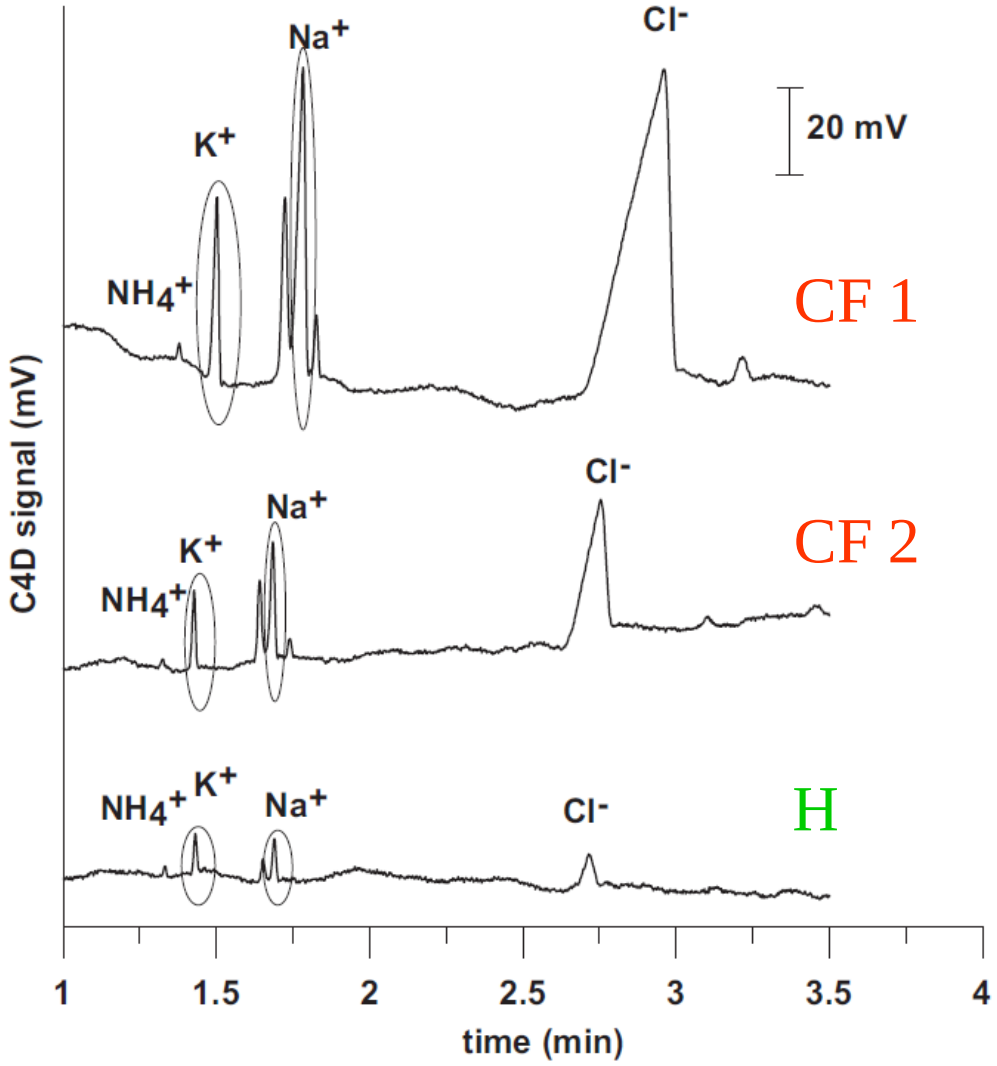


Fig. 2. DOEI-CE separation of skin wipe of a person with dF508 and G551D mutation before (A) and 14 days after (B) the treatment with Kalydeco. Trace (C) is a comparison with skin wipe from a healthy volunteer. CE conditions: the same as in Fig. 1.

USE OF ION RATIOS vs. CHLORIDE CONCETRATION

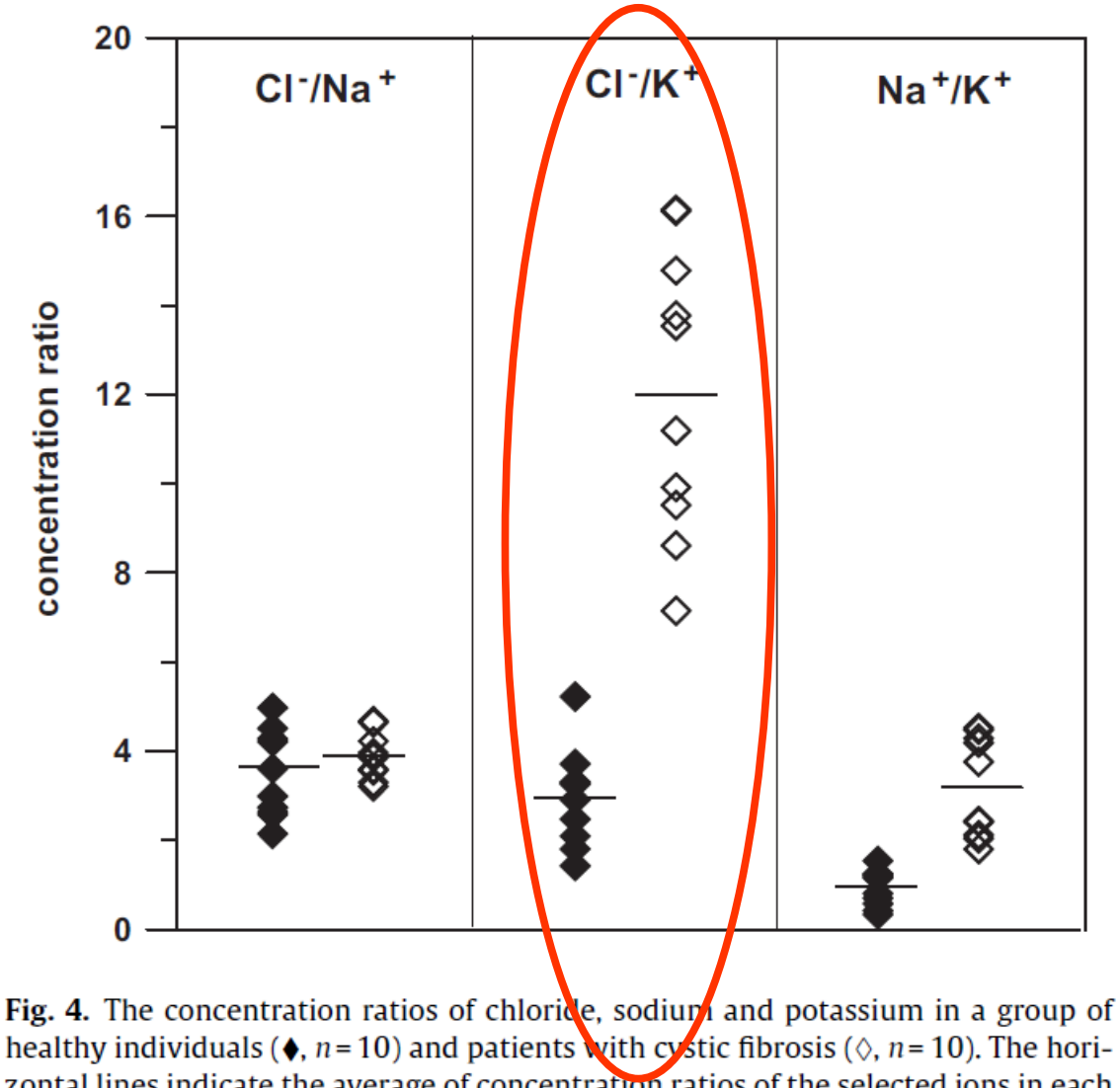
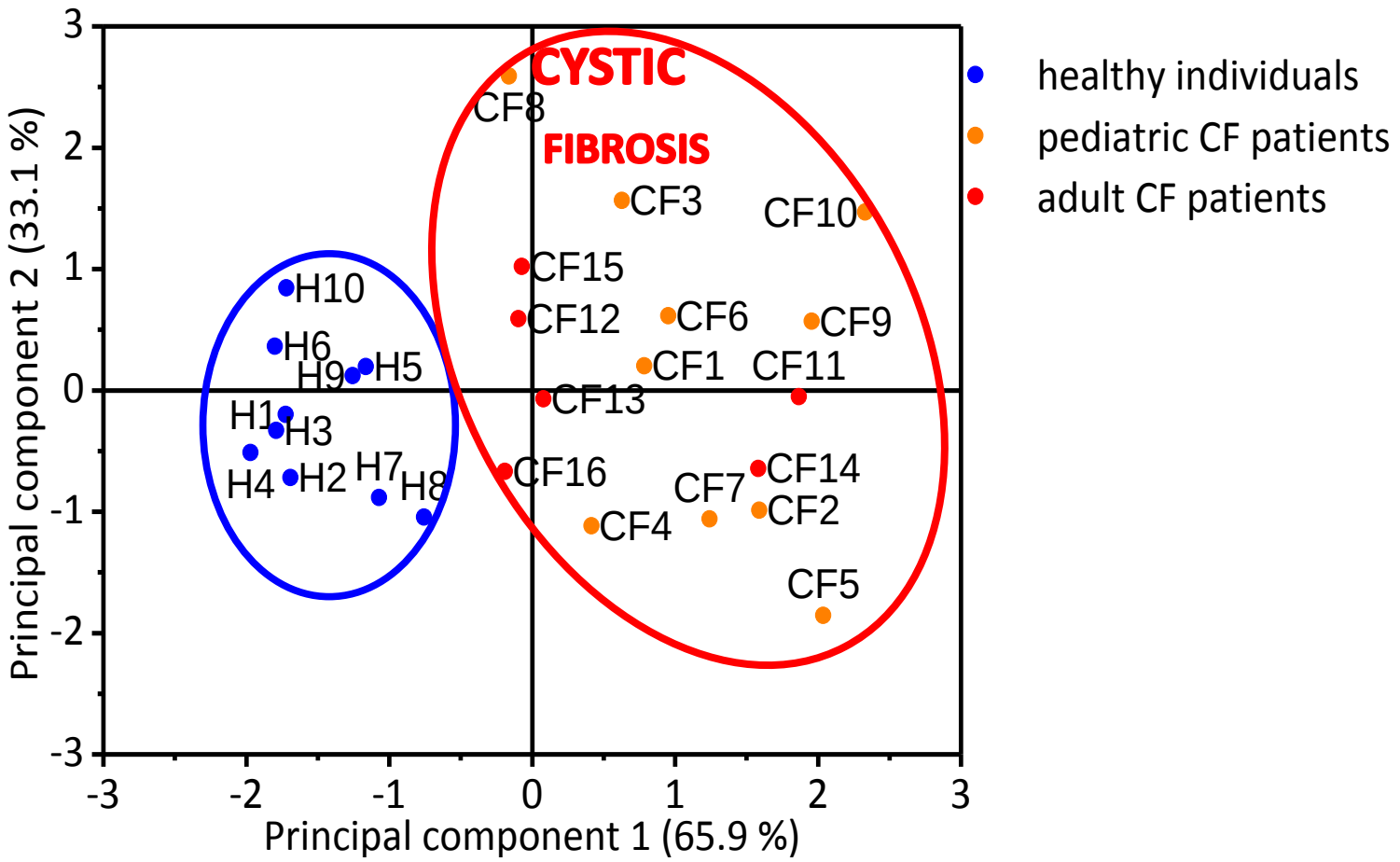


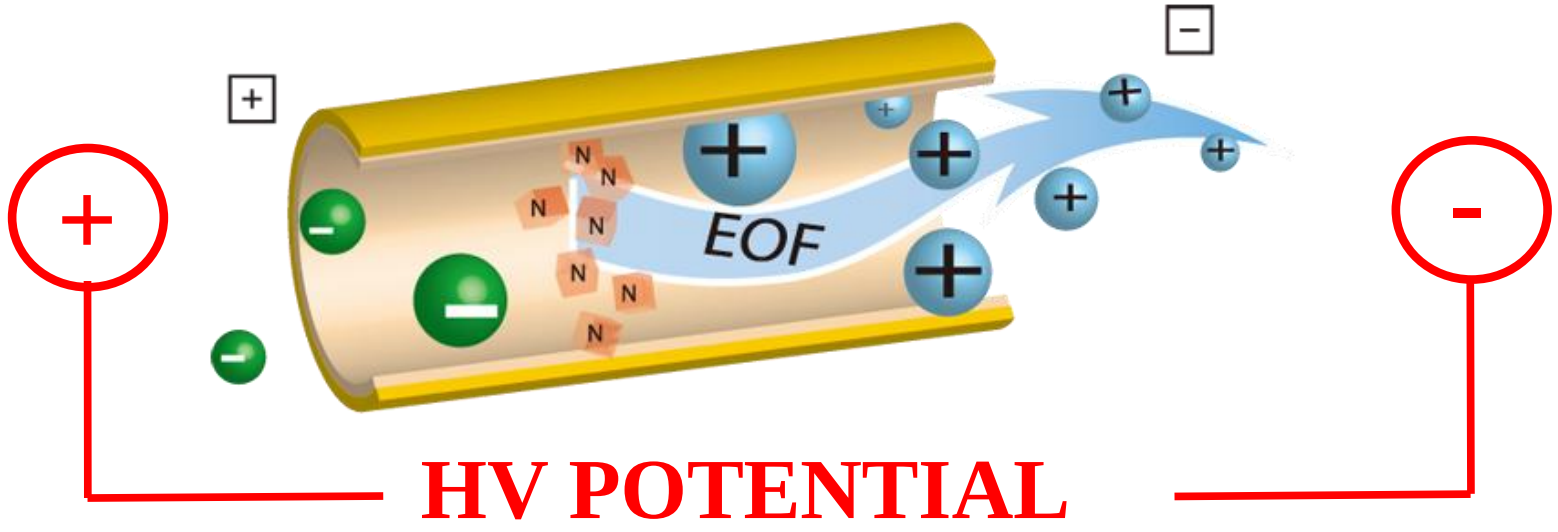
Fig. 4. The concentration ratios of chloride, sodium and potassium in a group of healthy individuals (♦, $n = 10$) and patients with cystic fibrosis (◊, $n = 10$). The horizontal lines indicate the average of concentration ratios of the selected ions in each group Samples analyzed by DOEI-CE, CE conditions: the same as in Fig. 1.

PCA IN CF DIGNOSTICS

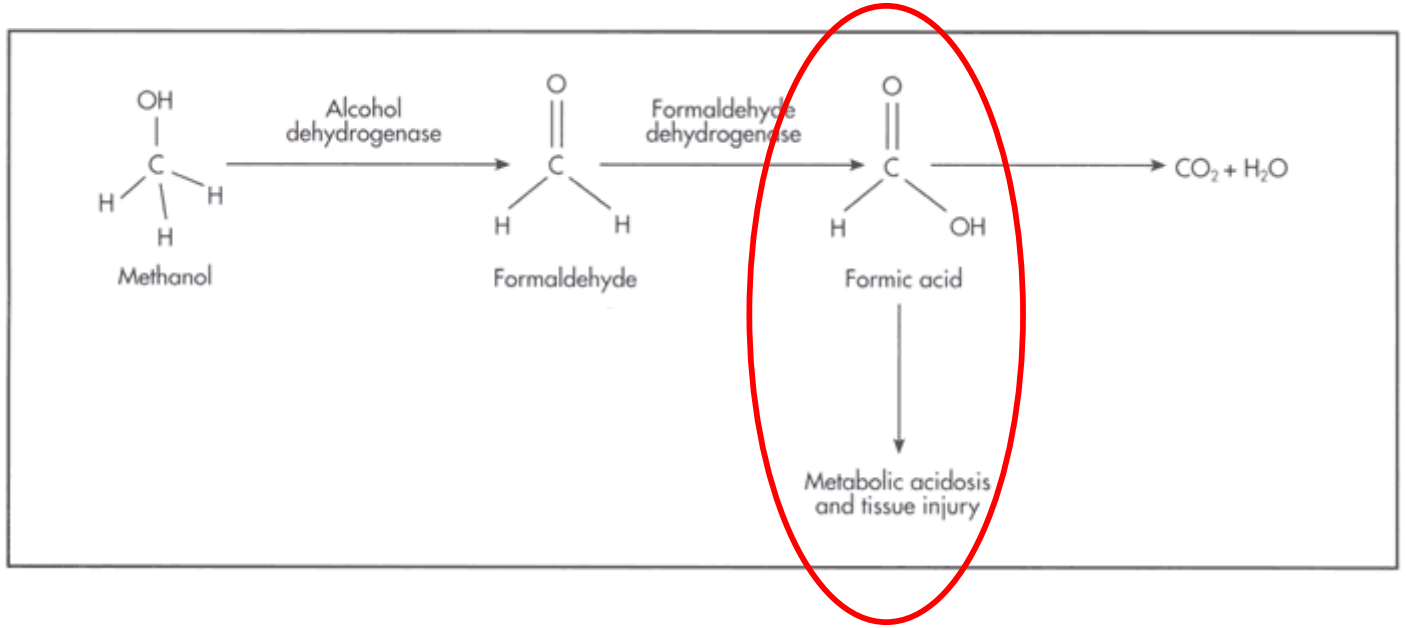


CAPILLARY ELECTROPHORESIS (CE) IN DIAGNOSIS OF INTOXICATIONS

FAST
SMAL SAMPLE VOLUMES
HIGH RESOLUTION



METABOLISM OF METHANOL



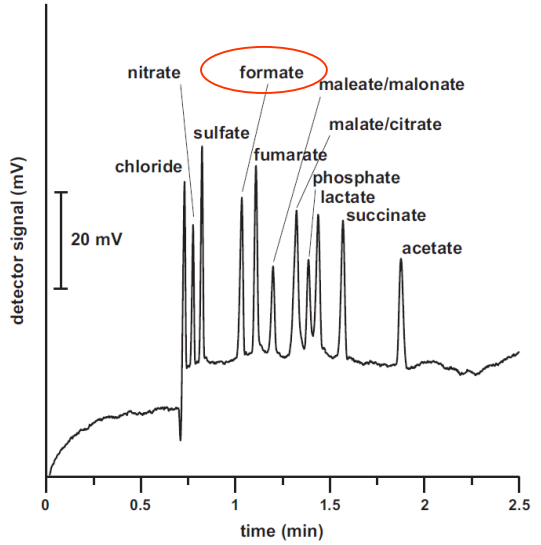
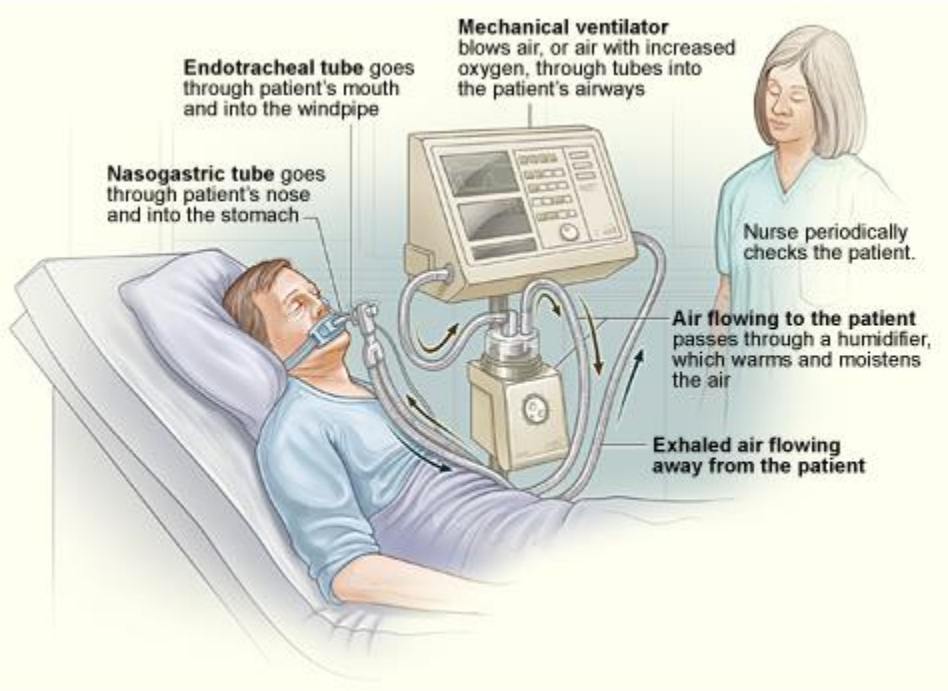


Fig. 3. Separation of model mixture of 13 anions and organic acids. CE conditions: separation electrolyte: 10 mM HIS/15 mM GLU, 30 μM CTAB, pH 4.68. Separation voltage: −18 kV. Hydrodynamic injection: 10 cm/20 s. Anion concentrations: 50–200 μM. C4D detection.

CE

POSSIBLE TO DETECT METHANOL WITH ALCOTESTER?

PATIENT INTOXICATED WITH METHANOL UNDER VENTILATION



NO FORMATE IN BREATH CONDENSATE

NEED FOR INVASIVE SAMPLING – BLOOD/SERUM

CE SEPARATION OF BLOOD PLASMA OF METHANOL INTOXICATED PATIENT

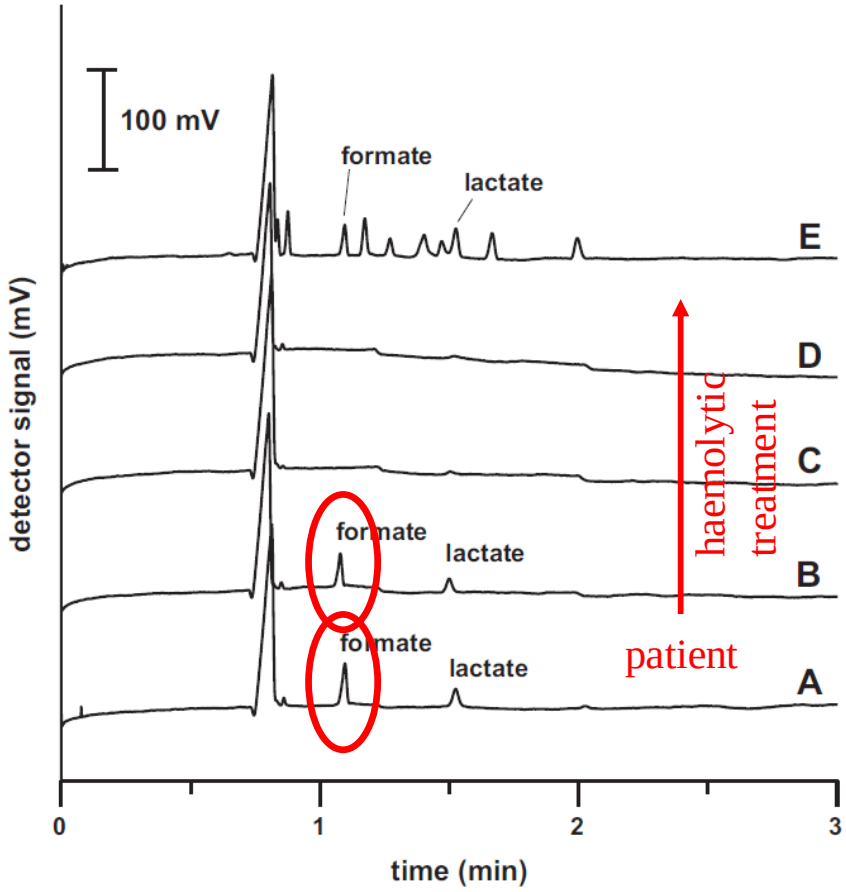
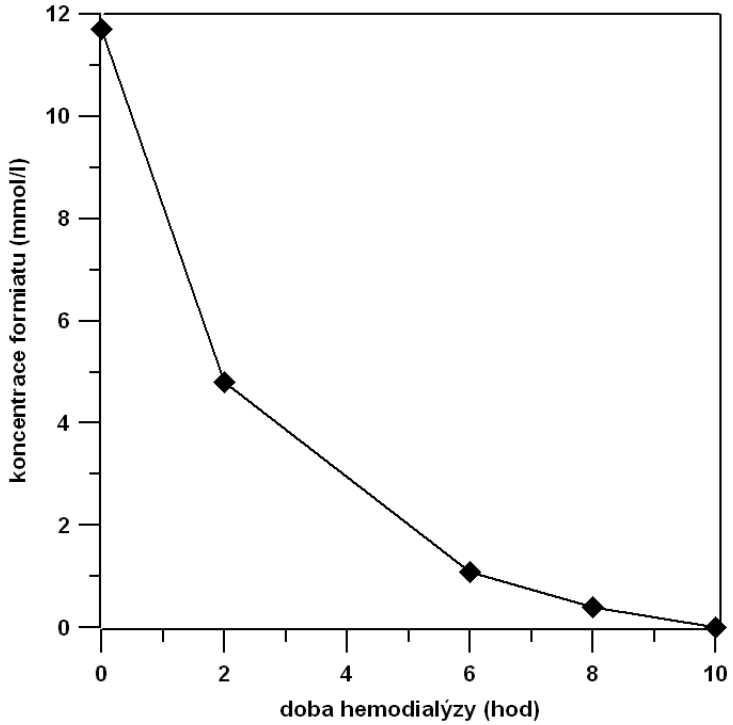


Fig. 6. CE analysis of diluted serum samples and a standard solution. Diluted serum sample of a patient in the beginning phase of hemodialytical treatment (trace A), 1 h after the after the first sample (trace B), 25 h thereafter (trace C) and 1 week after (trace D). Trace E is a model solution containing 1 mM chloride and 0.1 mM concentrations of other analytes. CE conditions as in Fig. 3.

MONITORING OF HAEMOLYTIC TREATMENT



PORTABLE METHANOL ANALYZER

Able to determine the concentration of methanol and HCOOH in 5-10 min

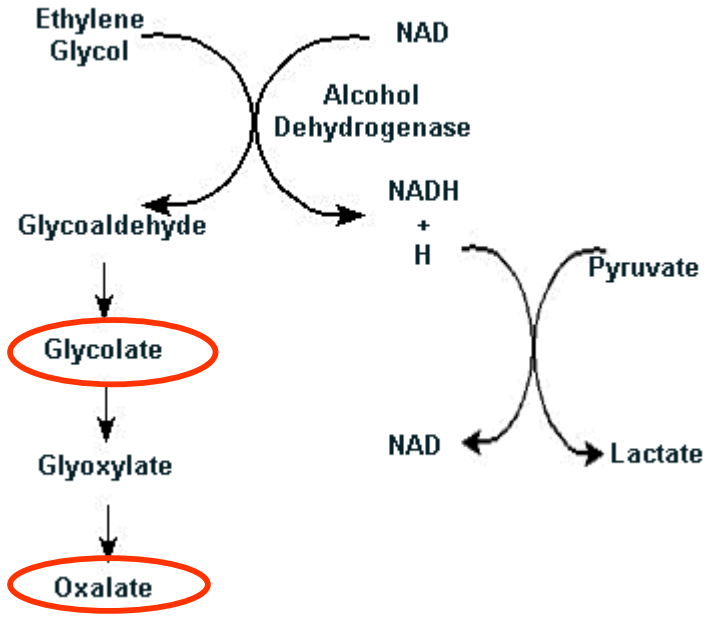
Analysis from one drop of blood serum(1-10 uL)

Simple use, for instance in the emergency vehicles, at doctors office.

ETHYLENGLYCOL INTOXICATIONS

occurs usually as unitentional ingestion of antifreeze (small children, pets)

Symptoms not easy to distinguish from other intoxications



ETHYLENE GLYCOL INTOXICATION

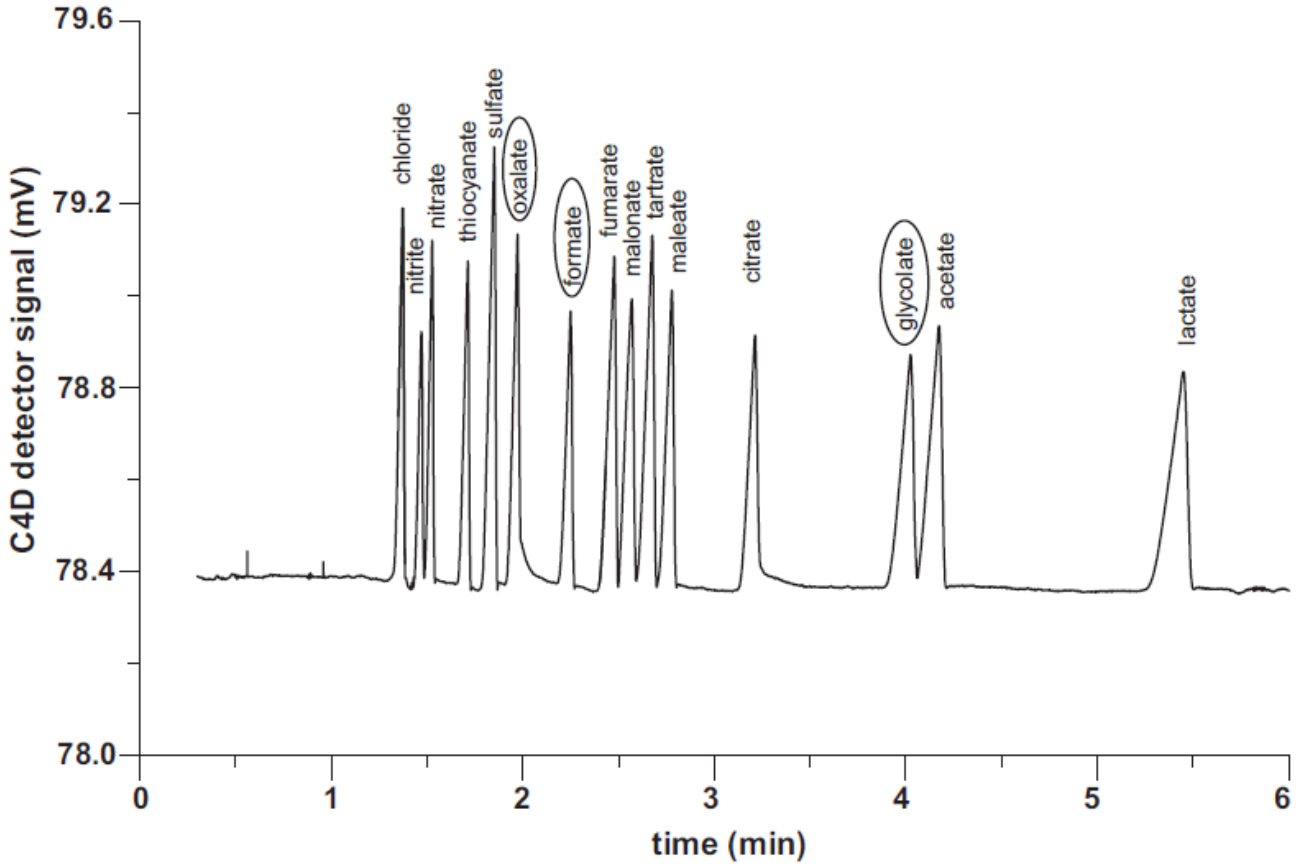
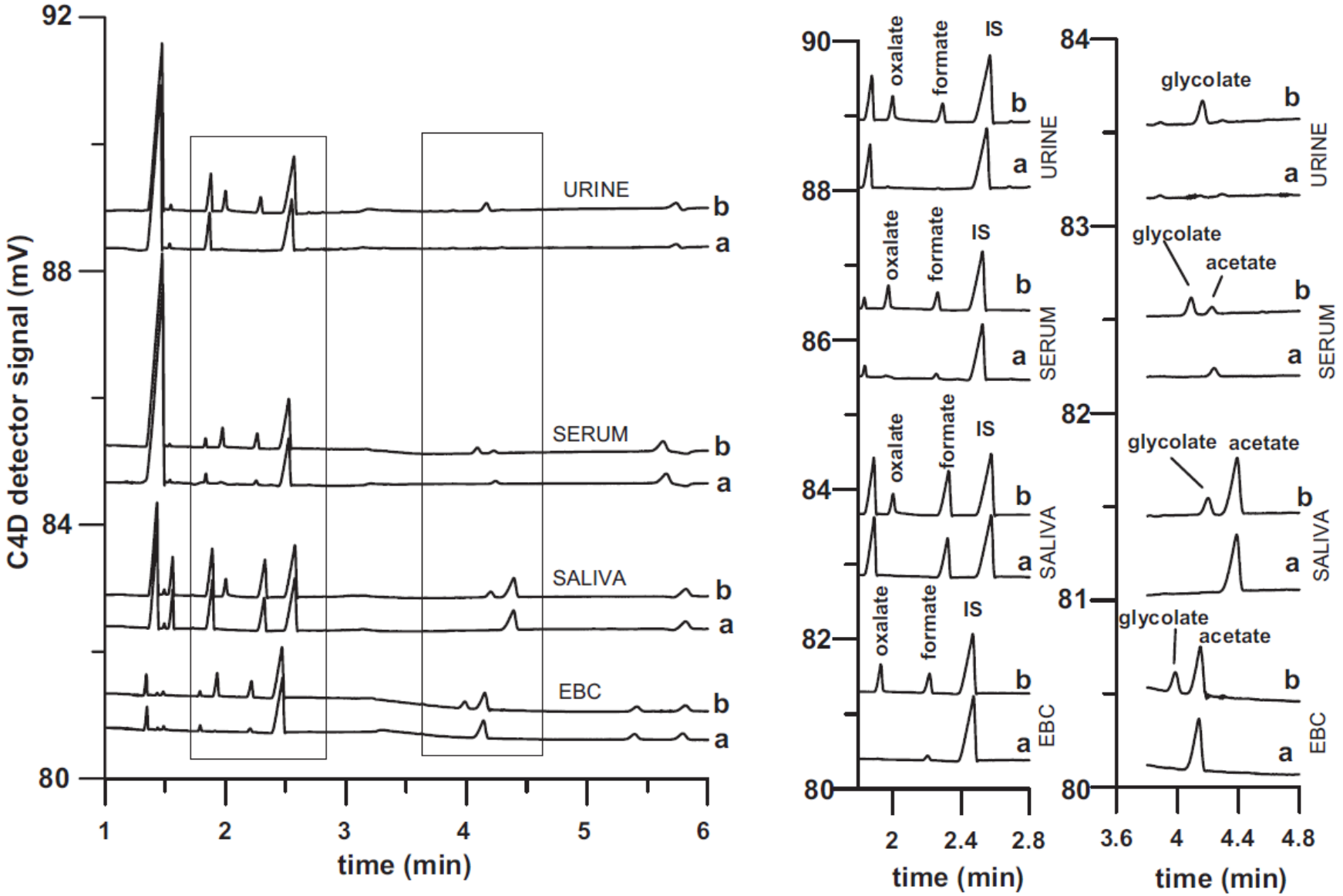


Fig. 2. Separation of model mixture of 15 inorganic and organic anions. CE conditions: separation voltage −15 kV, detection C4D, analyte concentrations: 50 μM, except glycolate, acetate and lactate (100 μM).

CE ANALYSIS OF BIOLOGICAL SAMPLES



CONCLUSIONS

ANALYTICAL CHEMSITRY FINDS WIDE APPLICATION IN POC DIAGNOSTICS

SIMPLICITY, SPEED

USE NEAR PATIENT, PORTABLE DEVICES

JE STÁLE CO OBJEVOVAT ☺