VORONEZH STATE UNIVERSITY OF ENGINEERING TECHNOLOGIES
All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy

Portable E-nose for Diagnostic of Inflammation and Diverse Variation in Health Status of Humans and Animals

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Anastasiia Shuba

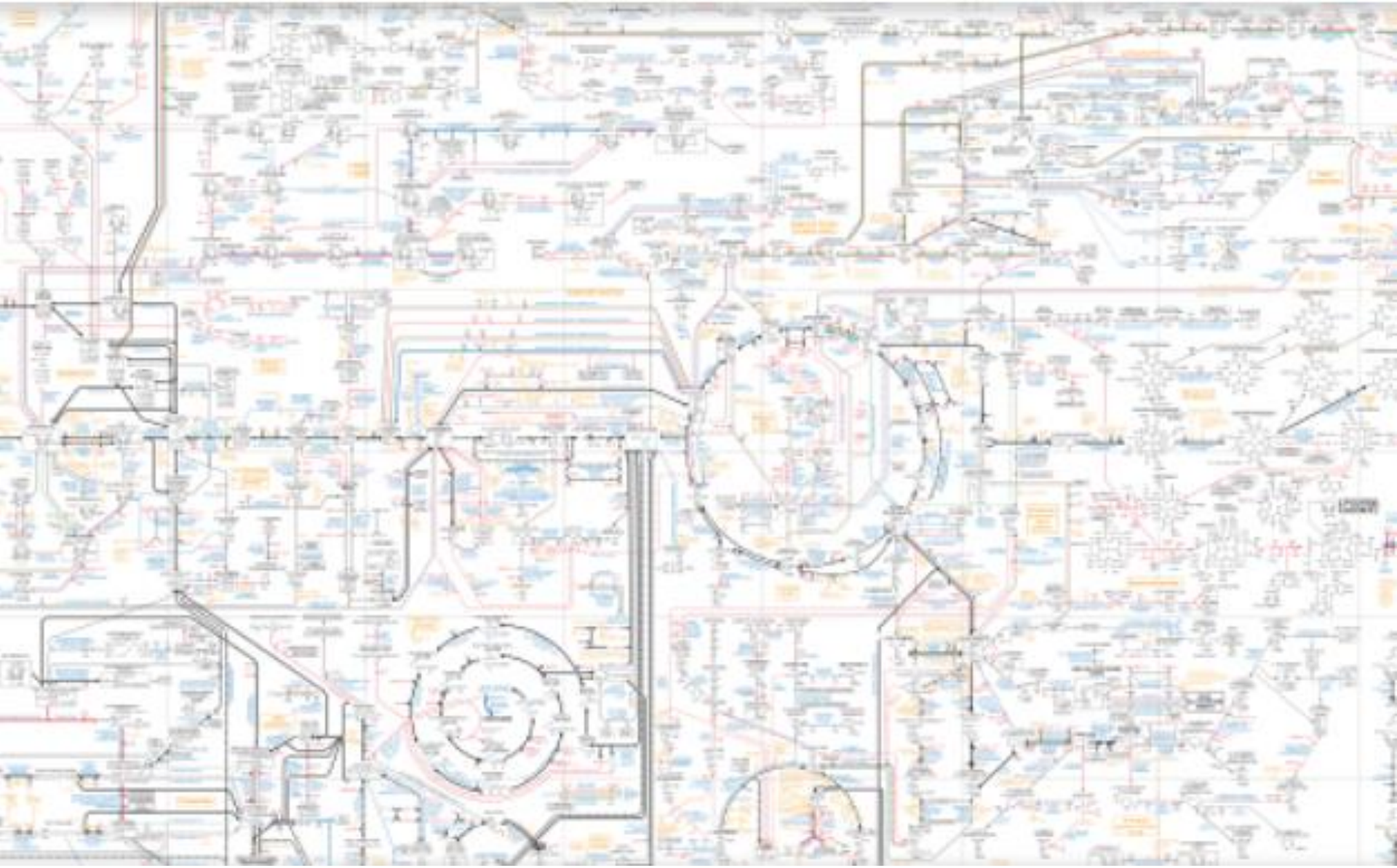
She graduated Voronezh State University in 2009 with Master degree in Chemistry. She continued her education in Voronezh State University of Engineering Technologies and has defended her doctoral thesis in 2013 year devoted to the investigation of living system such as bioassays, food products, using the arrays of selective and non-selective piezoelectric sensors. And proceed her investigation as assistant and docent in that University. She took part in 12 grant research about developing techniques and devices for analysis of gas phase food products, polymers, biosamples (cervical mucus, feces, blood, exhaled breath). Since 2018 year she is a head of grant project supported Russian Science Found about new methods of diagnostic of calves respiratory disease.

Topics of research interest

Developing of the new way and devices for diagnostic using gas phase analysis by sensor array:

- Search and developing new selective phase for sensors covering
- Mathematical treatment of multivariate output data of sensors for excretion of useful information
- Improving precision and accuracy of measurement using sensor system
- Developing of program for sensors devices

Metabolomic maps



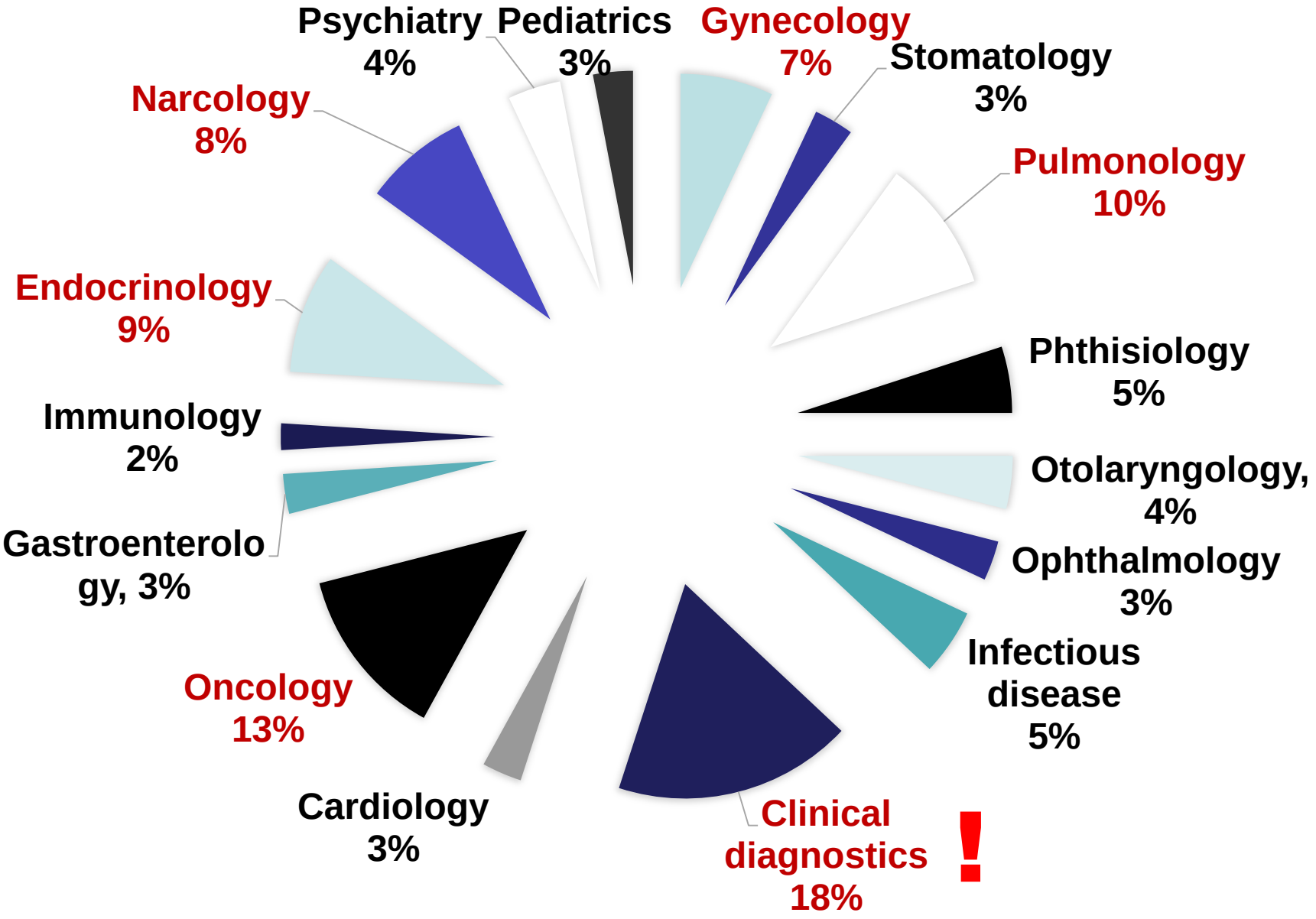
http:// biochemical-pathways.com/#map/1

Why volatile metabolome*

- Volatile metabolome has the most fast changes in the organism
- Instant changes after influence.
- Investigated with gas chromatography, mass spectrometry
- Each biosample has unique volatile metabolome
- Easily, non-traumatic measurement

* Volatile metabolome: problems and prospects- https://www.future-science.com/doi/full/10.4155/bio-2016-0203

The main branches of medicine using electronic nose (more than 150 publ. per year)



Development of new diagnostic tools

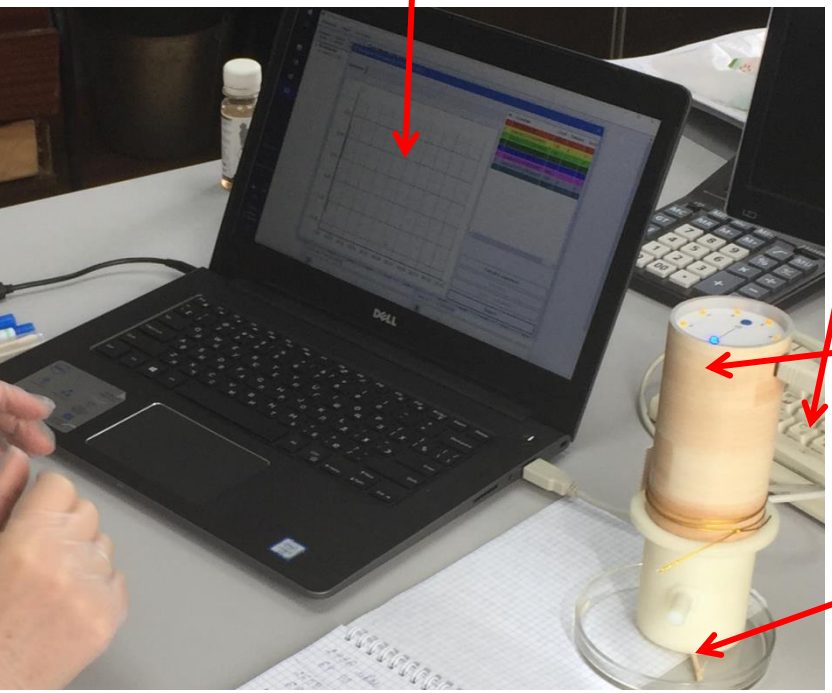
- Biochemical instruments - sensors, chips, PCR, enzyme immunoassay - search for antigens, antibodies, cells, DNA
- Chemical instruments - gas chromatography, mass spectrometry, sensors, chips - search for metabolites (amino acids, organic acids, volatile compounds)
- Physical instruments - conductometry, electrical resistance, temperature, UV-IR spectrometry - search for changes associated with the diagnostic condition

Aim of research

Development and application of a new mobile device based on piezoelectric sensors (portable electronic nose) for assessing the health status of organs and systems of humans and animals by analyzing the volatile metabolome.

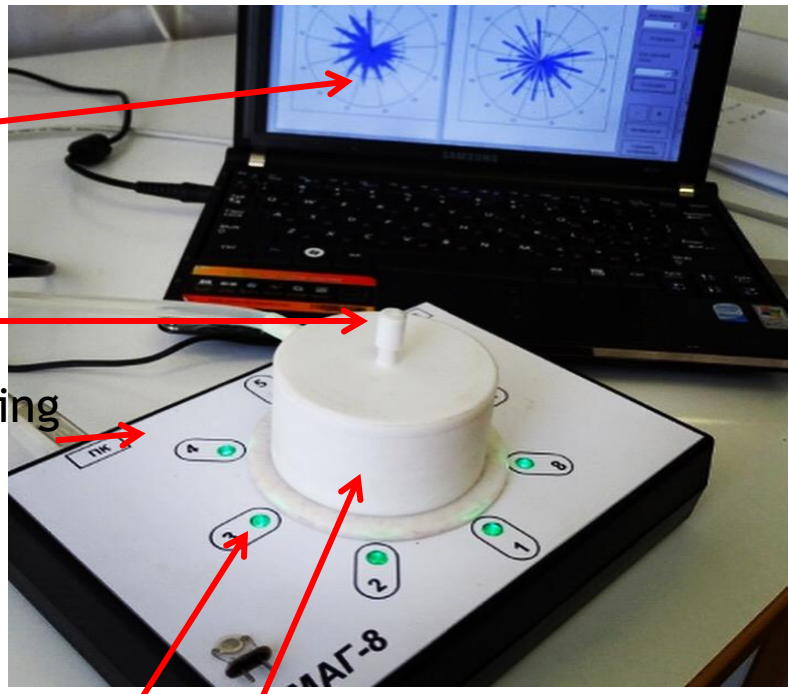
Variants of portable E-nose

Device with software for recording and processing of output data from sensors



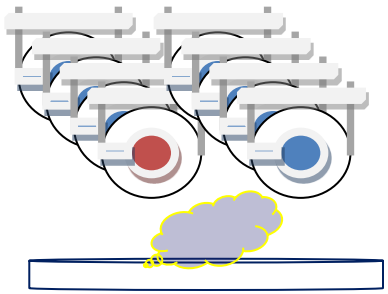
For EGP input

USB-connecting with PC



Indicator of working

Detection cell with piezosensors



Operating conditions and technical specifications of E-nose:

- Ambient temperature from +15 to +35 °C, permissible temperature variation during research is not more than 3 ° C.
- The optimal condition for the investigation of bioassay is stable temperature and humidity in a separate room.
- The device is powered by an alternating current with a voltage of 220 ± 22 V and a frequency of 50 ± 0.5 Hz.
- Frequency range 10 MHz – 20 MHz.
- Overall dimensions – 38x120x170 mm.
- Weight with a cover – 0.25-0.40 kg.

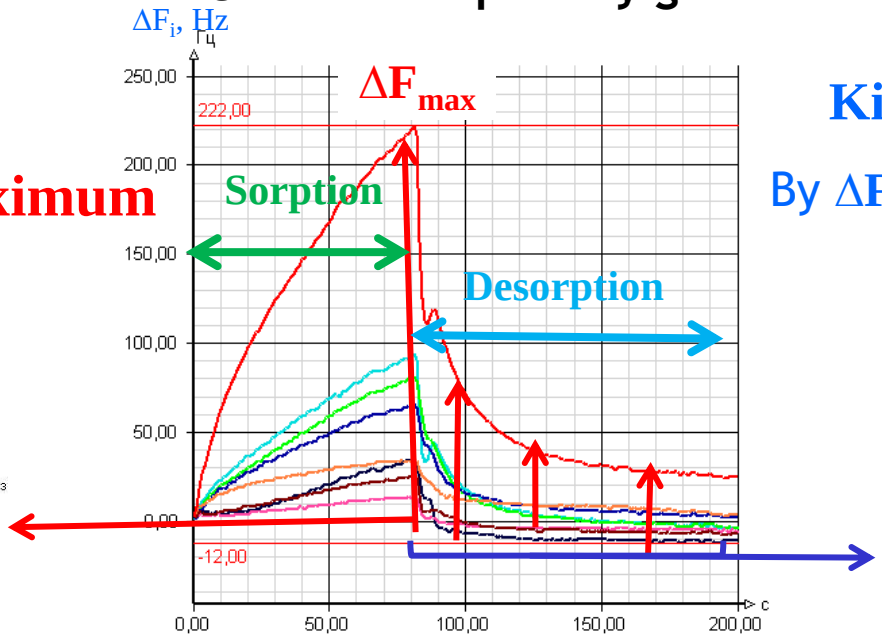
The modifiers using for piezoelectric sensors covering in E-nose

In the investigation, the 14 modifiers are proposed based on polymers and nanostructured solid phase, including:

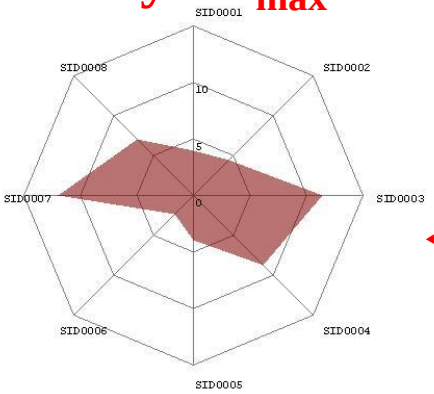
1) Polyethylene glycol 2000 and its esters;
2) Craun ethers;
3) Acid-base indicators;
4) Chromatographic phase (Triton X-100; Tween 40).
5) Multiwalled nanotubes, including with special treatment
6) Nitrate of zirconium oxide
7) Biohydroxyapatite

Output data from sensor array

Choronofrequentcy grams



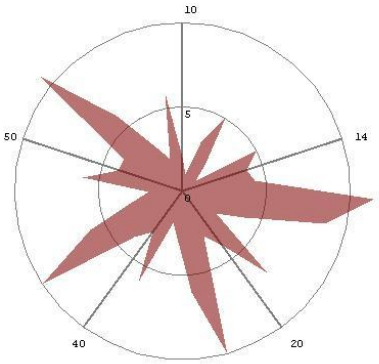
Visual prints of maximum by ΔF_{max}



S_{max}

Kinetic visual prints

By ΔF_i during sorption and desorption



S_{neg}

Square of «visual prints» of sensors signals

Quantitative characteristic

Parameter of sorption efficiency

$$A_{ij}^{max} = \Delta F_{max,i} / \Delta F_{max,j},$$

i, j – number of sensors in an array

Qualitative characteristic

Training of E-nose

by 55 individual substances of normal and pathogenic metabolism, including:

- C_1-C_7 alcohols,
- acetone, ketones, C_5-C_7 cyclic ketones,
- aldehydes, N-, S-containing aldehydes,
- C_1-C_5 carboxylic acids;
- primary, tertiary, cyclic amines, O-containing amines.

Some parameters for identification of substances

For injection of equilibrium gas phase

Parameter	Identification value	Identified substance	Parameter	Identification value	Identified substance
A_{14}^{max}	0.30 ± 0.05	Ammonia, alkylamines	A_{15}^{max}	1.30 ± 0.05	Ethyl acetate, ethanol
	1.6 ± 0.1	Carboxylic acids C_2-C_4		2.5 ± 0.3	Carboxylic acids C_2,C_4
A_{13}^{max}	0.4 ± 0.1	Triethylamine	A_{37}^{max}	0.6 ± 0.1	Carboxylic acids C_2-C_5
	2.4 ± 0.4	Isobutyric acid		4.5 ± 0.6	Piperidine
A_{17}^{max}	0.30 ± 0.05	Aliphatic amines	A_{47}^{max}	0.6 ± 0.1	Ethanol, butanol, acetone
A_{56}^{max}	0.20 ± 0.05	Ammonia	A_{57}^{max}	0.60 ± 0.05	

For frontal input of gas phase

Parameter	Identification value	Identified substance	Parameter	Identification value	Identified substance
A_{14}^{max}	0.65 ± 0.15	Acetone, ketones	A_{15}^{max}	1.10 ± 0.15	Alcohols C_2-C_5
	0.9 ± 0.1	Aliphatic amines		1.4 ± 0.4	Ammonia, amines
A_{17}^{max}	0.50 ± 0.1	Ethylacetate	A_{47}^{max}	1.2 ± 0.2	Ammonia, alkylamines
A_{56}^{max}	1.14 ± 0.02	Acetaldehyde	A_{57}^{max}	0.4 ± 0.1	C_5-C_7 cyclic kenotes

I. Diagnosis of Respiratory Diseases in Calves

Supported by Russian Science Found, grant № 18-76-10015

Calves in age
of 3 to 60 days of life (n=65)

➢Condensate of exhaled breath

➢Nasal mucus

Respiratory diagnosis assessment

WI score
(University of Wisconsin at Madison, USA, 2008)

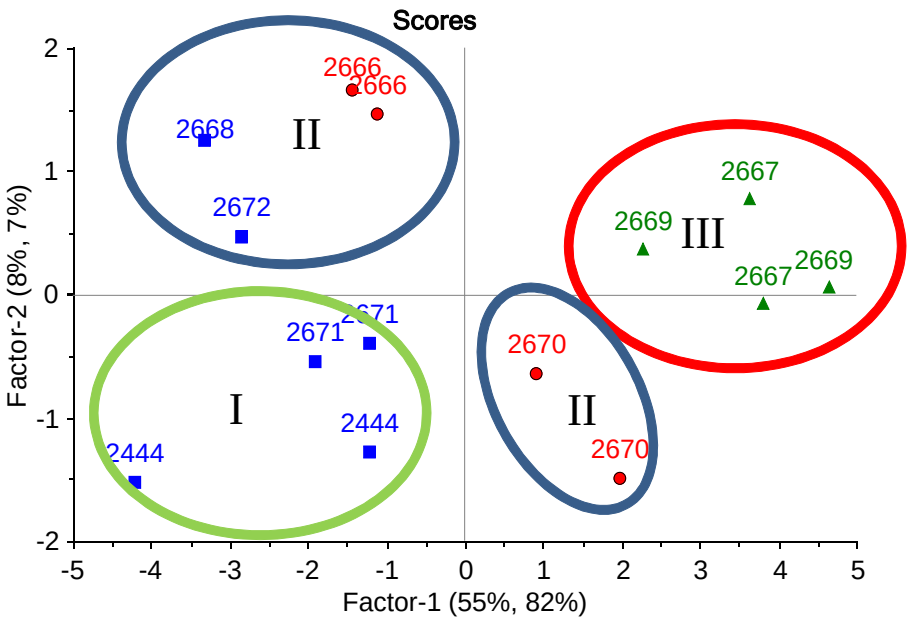
Clinic methods;
Special methods (spirometry, functional probes);
Laboratory methods (hematological, biochemical, bacteriological, molecular-genetic).

Критерии оценки состояния телят при респираторных заболеваниях			
0	1	2	3
Ректальная температура			
37,7-38,3	38,3-38,8	38,9-39,4	≥ 39,5
Кашель			
Отсутствует	Индуцированный единичный кашель	Индуцированный повторяющийся или спонтанный кашель	Самопроизвольный повторяющийся кашель
Выделения из носа			
Нормальные прозрачные выделения	Незначительные односторонние мутные выделения	Двусторонние мутные или обильные слизистые выделения	Обильные двусторонние слизисто-гнойные
			
Оценка состояния глаз			
В норме	Незначительные выделения из глаз	Двустороние выделения из глаз	Обильные выделения из глаз
			
Оценка состояния ушей			
В норме	Потряхивание ухом или головой	Легкое обвисание одного уха	Опущена голова или повисли оба уха
			

Some results from clinical studies of calves

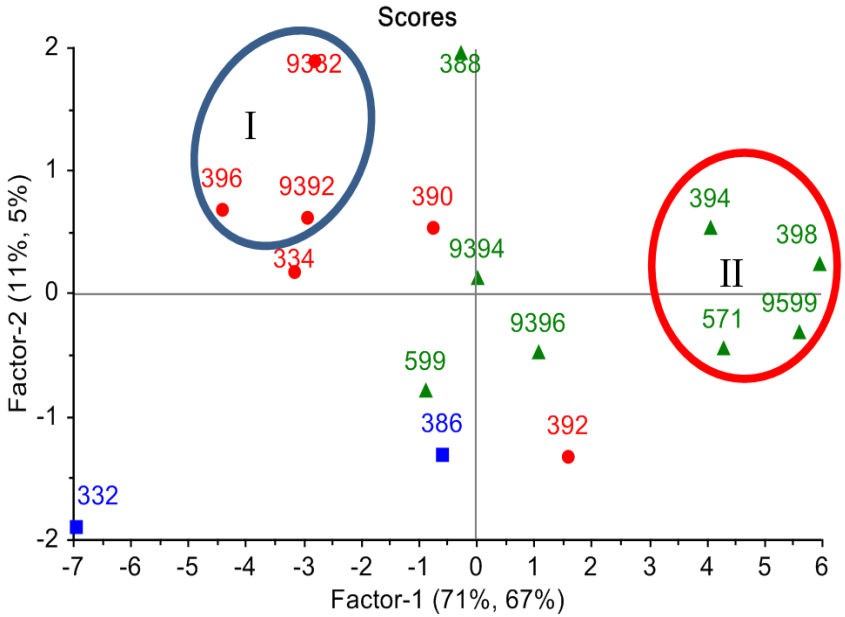
Indicator	Healthy from the respiratory system (n=24)	With subclinical course of respiratory diseases (n=26)	With respiratory symptoms (n=25)
Temperature of the body, °C	39,1 ± 0,2	38,9± 0,4	39,5 ± 0,2
Induced cough	No	Yes	Yes
Spontaneous cough	No	No	Yes
Index respiratory failure	1,42 ± 0,25	1,79 ± 0,49	1,70 ± 0,77
WI score, balls	2,3 ± 0,7	2,8 ± 0,7	5,6 ± 1,6
C(haptoglobin), g/l	4,42 ± 0,30	4,44 ± 0,45	4,48 ± 0,25
C(leukocytes), $*10^9$/l	8,1 ± 1,9	7,8 ± 0,8	7,4 ± 3,2
Segmented neutrophils, %	38 ± 2	36 ± 5	35 ± 7
Lymphocytes, %	55 ± 5	56 ± 7	59 ± 8
Bacterial contamination, CFU/ml	1100 ± 500	4000 ± 2000	9000 ± 5000

Modeling by informative parameters of a sensor array



Error less than 4 %

The diagram of the PLC-model scores for predicting the WI index based on the output data of the array of sensors when analyzing the EGP over EBC samples from newborn calves: I - group "healthy from the respiratory system", II - group "with subclinical course of respiratory diseases", III - group " with symptoms of respiratory tract damage "



Error less than 20 %

The diagram of the PLC model scores for predicting bacterial contamination of tracheal lavage (CFU/ml) according to the output data of the array of sensors in the analysis of EGP over EBC samples from 1-1.5 month old calves: Group I with bacterial contamination of less than 100 CFU/ml and subclinical course diseases, II - group with bacterial contamination of more than 10,000 CFU/ml and damage to the respiratory system

A set of markers of respiratory pathology of calves for different diagnostic groups

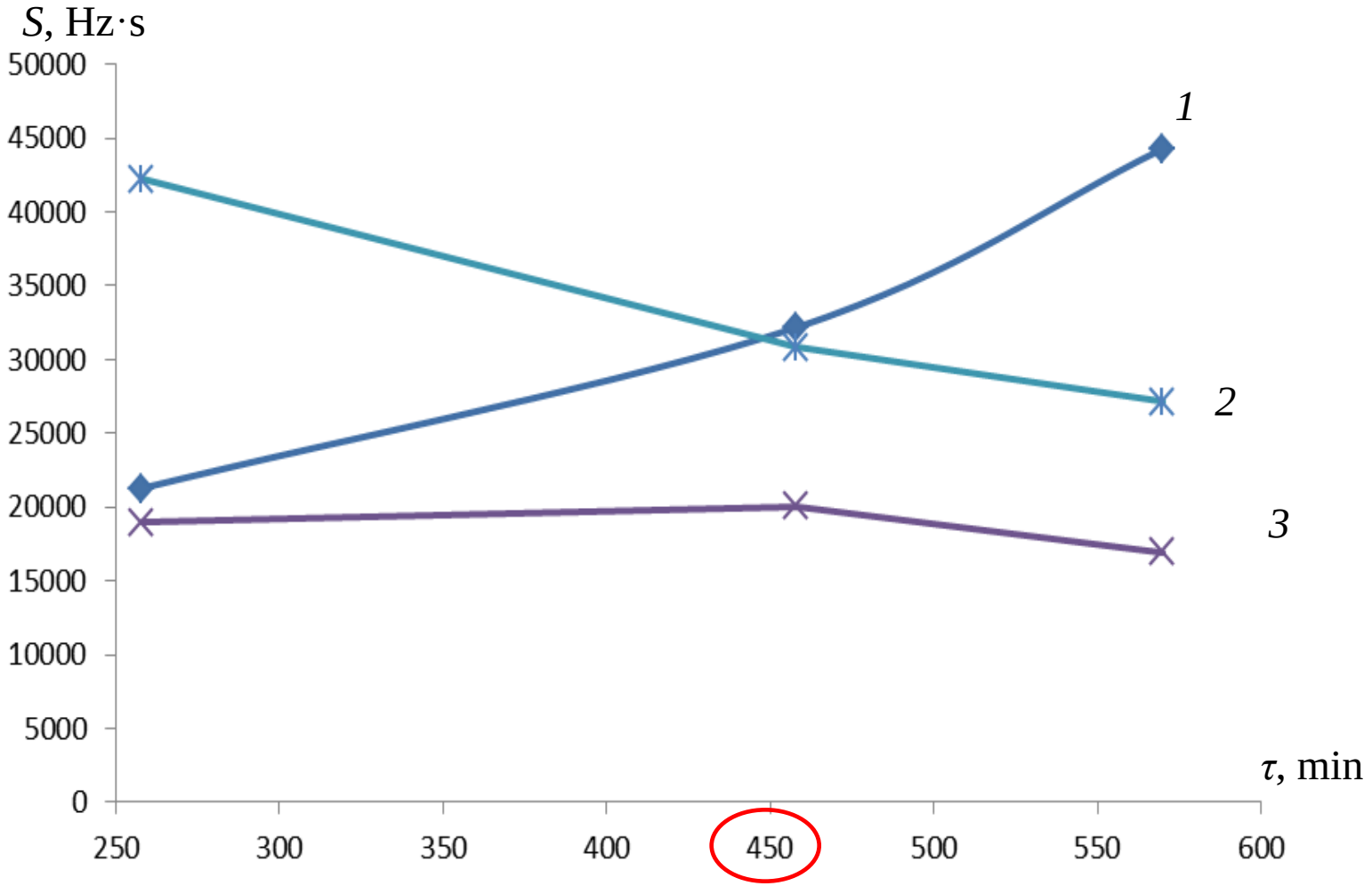
in the EGP over the EBC samples when feeding with milk

Healthy from the respiratory system	With subclinical course of respiratory diseases	With respiratory symptoms
Ethanol, butanol, butyric acid, ethanedial, triethylamine, C_1-C_3 alkylamines		
Acetic acid	Benzaldehyde, methylbenzaldehyde	Hydrogen sulfide, sulfides
Ammonia, alkylamines		
	Isobutyric acid, valeric acid, phenol, ethyl acetate, amino alcohols	

in the GP over the nasal mucus samples

Healthy from the respiratory system	With subclinical course of respiratory diseases	With respiratory symptoms
Methylamine, methylglyoxal, ethanedial, methylpropylamine, aliphatic amines		
Acetone, ethyl acetate	C_1-C_5 acids, methylbenzaldehyde, benzaldehyde, ketones, propanedial	
	3-pyridinecarbaldehyde, propylamine	Alcohols, methyl-O-ethylamine, hexanone, lactic acid, 2-thiophenecarbaldehyde

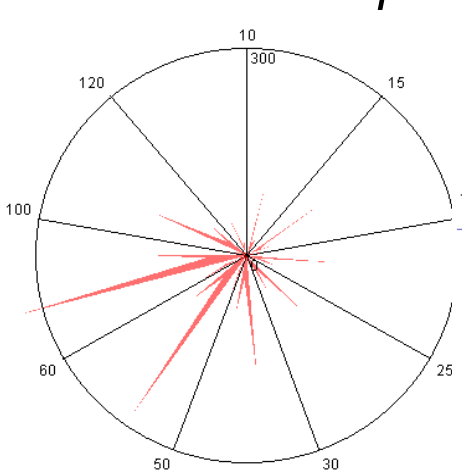
Additional diagnostic information



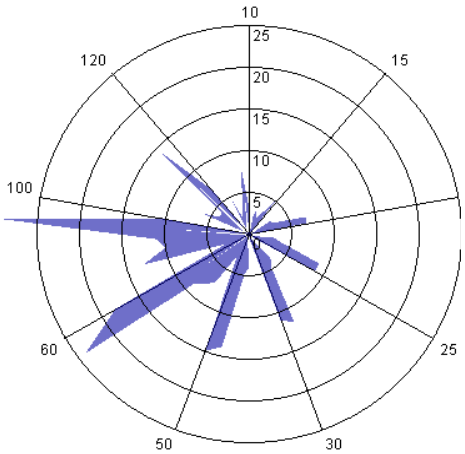
Dependence of the total square of "visual prints" of signals from the array of sensors S, Hz·s in vapors of nasal mucus on the exposure time after sampling for samples from various diagnostic groups: I - group "healthy from the respiratory system", II - group "with subclinical course of respiratory diseases", III - group " with symptoms of respiratory tract damage "

II. Characterizing the Some Deviation from Normal Health Status by Human Skin Odor

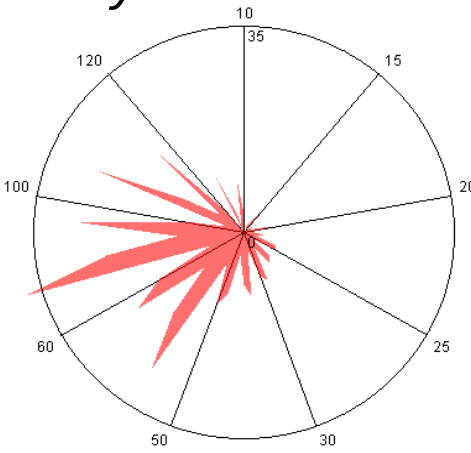
More than 9000 measurements, more than 2.5 thousand people aged from 1.7 to 95 years



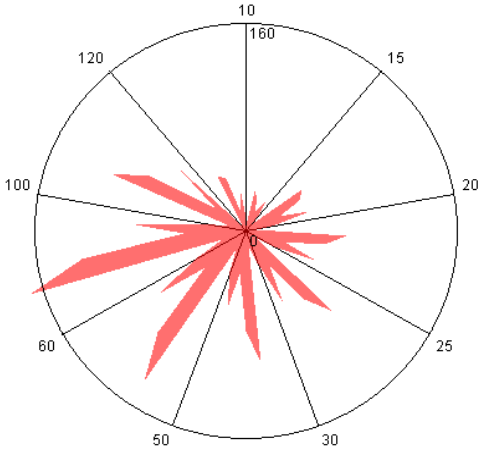
Girl,
5 years old



Girl,
25 years old

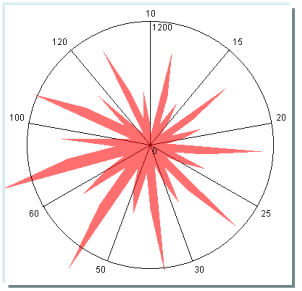


Woman,
50 years old



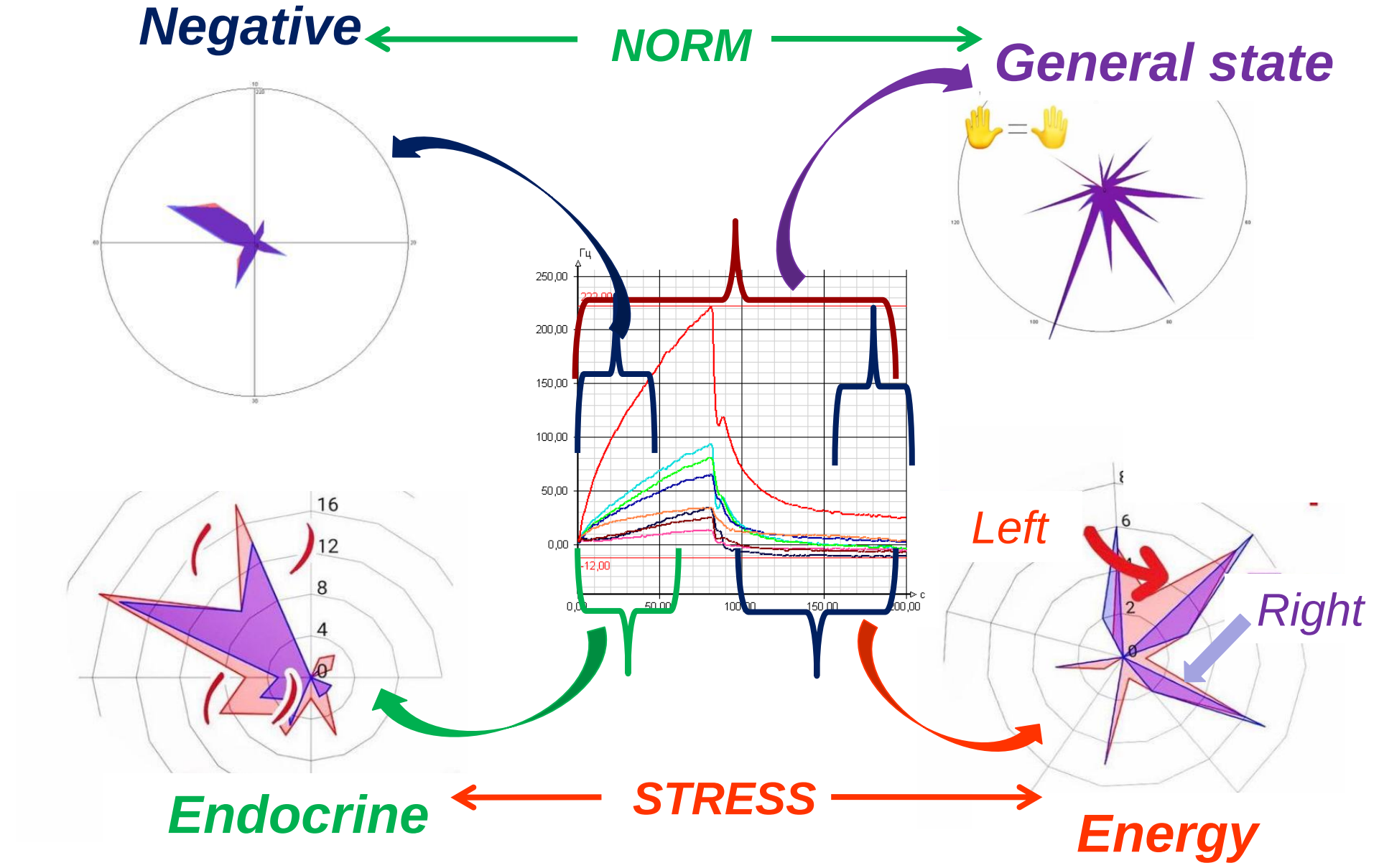
Woman,
75 years old

Measure, save, compare, diagnose changes in the early stages of disease development



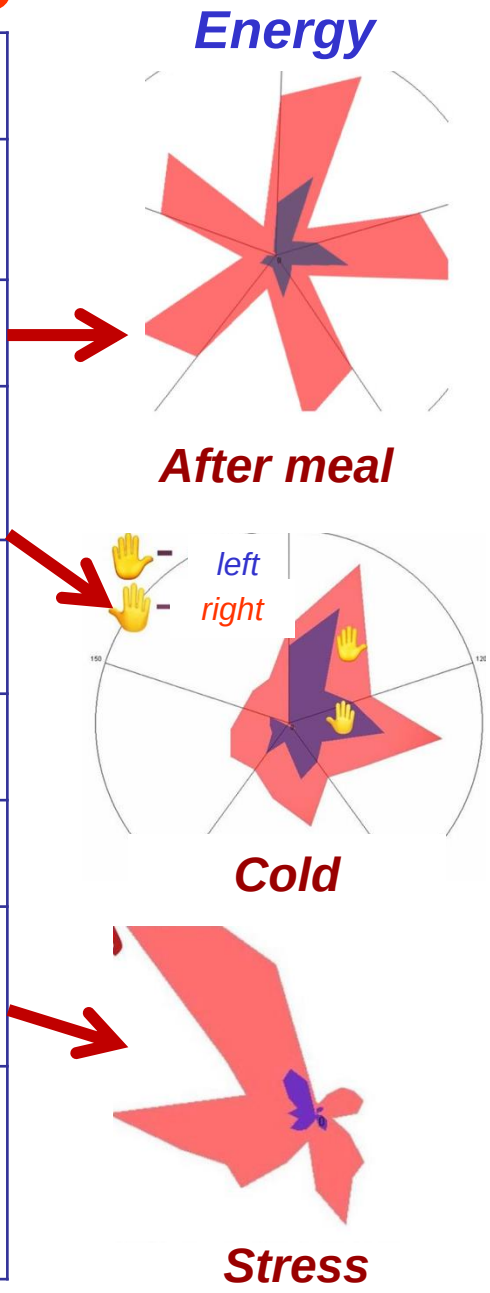
water

Algorithms for «visual prints»



Comparison of left and right hands

Person's state	The trend of changing and the relative difference in the parameter $S_{v.p.}$,%
Norm	Differences between the left and right hands - 5-10%, for the left more than for the right
Norm, after meal	11-30% more for the right hand than for the left one
Cold, temperature for a long time 15-20°C	20% (before meals) – 10% (after meals), the shape of "visual prints" changes
Headache, toothache, other pain, spasm	10-35%, the shape of "visual prints" changes
Virus, malaise without fever	Decrease on the right hand to 38-40%
Menstruation	An increase in the side of the working ovary by 15-25%.
Fatigue, heart failure	The left side is smaller than the right in normal to 12-20%, the shape of "visual prints" changes
Bronchitis, inflammation	The difference between left and right Zakharyin-Ged zone of bronchi for acute bronchitis - 40 %, recovery – up to 15 %, healthy lung and bronchi – up to 5 %



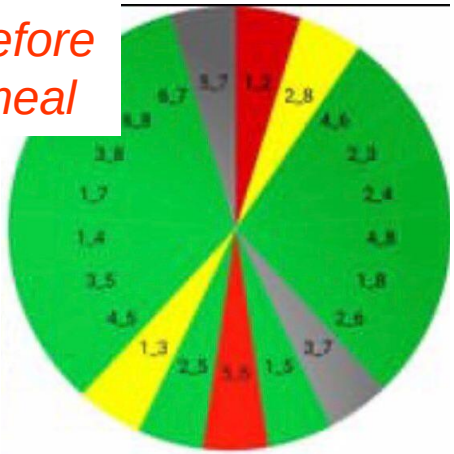
Value of parameters for different health status

Parameter A(i/j)	The numerical range of parameter values			
	Norm	*Description of the deviation state*		
Sector color	green	yellow	red	burgundy
A(1/5)	< 0.75	0.75-0.94 Stress, body weakness	> 0.90 Hormone imbalance	> 0.81 Stress, weakness, severe inflammation
A(1/7)	≤ 1.90	> 1.9	> 2.3 Adrenaline, severe stress	-
A(1/2)	> 1.15	0.90 – 1.14 Inflammation, very hot	< 0.9 Alcohol, Ketones	-
A(2/4)	≤ 0.1	-	0.25-0.30 Ketones, sugar is above normal, hormones are very unbalanced	0.16-0.24 Weakness, exhaustion
A(2/5)	> 0,52	0,48-0,52	<0,48 Ketones, sugar is above normal, hormones are very unbalanced	-
A(5/6)		1,3-1,5 Hungry, weakness	1,8-2,2 Critical condition, hot, stress, wounds	

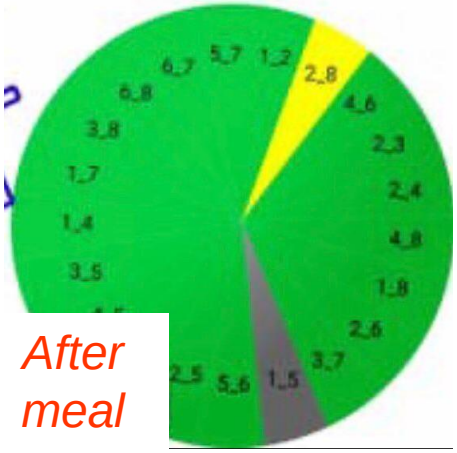
Sphere of health status

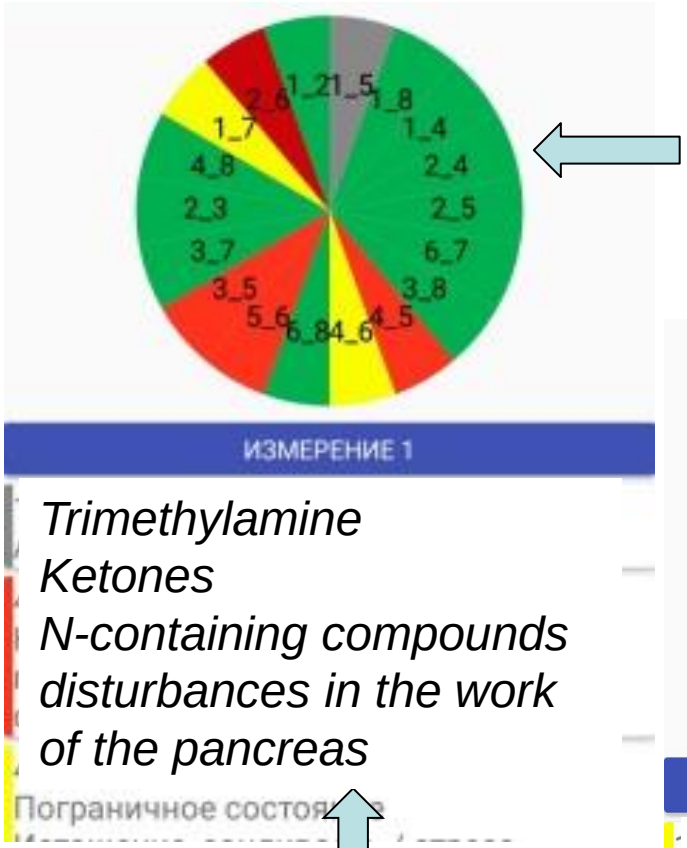
Monitoring influence of different factors

Before meal



After meal

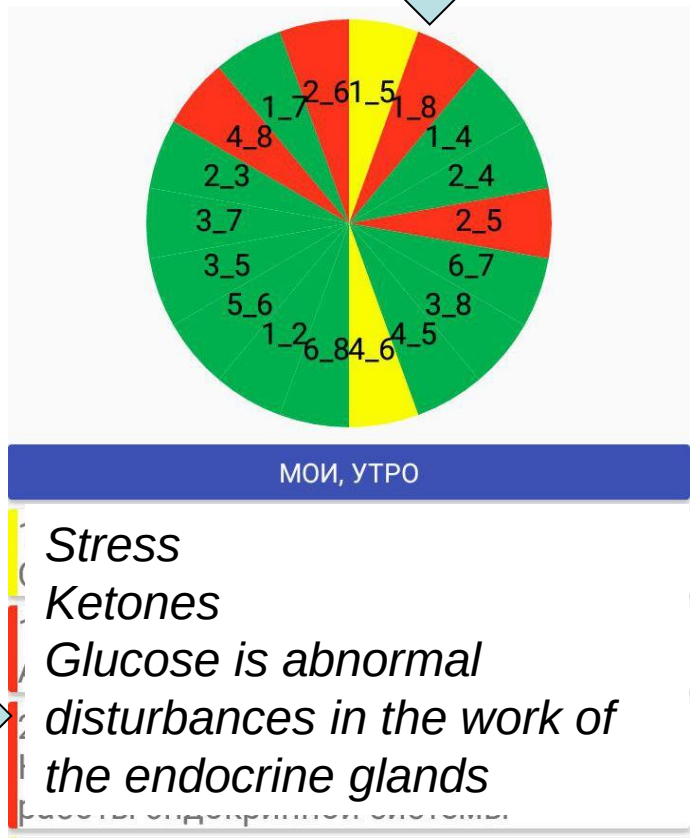




Trimethylamine
Ketones
N-containing compounds
disturbances in the work of the pancreas

The description of health status, including identified substances

The colors in the health status sphere were determined by the value of parameters and depend on the health state



Stress
Ketones
Glucose is abnormal
disturbances in the work of the endocrine glands

Screening of the health status

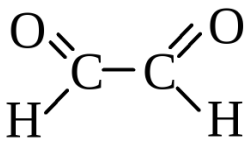
of patients of the children's endocrinology department of the Voronezh children's clinical hospital named after N.N. Burdenko (problems of obesity, prediabetes, diabetes)

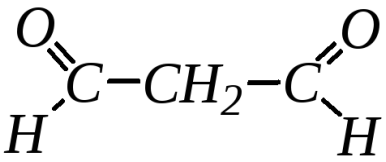
Number of measurements = 330
Number of patients = 20

The prototype nose-diagnostician

N Patients, years old	Diagnosis
1, 10	Obesity grade 4, dyslipidemia, reactive pancreatitis
2, 12	Hyperparathyroidism, grade 3 vitamin D deficiency
3, 13,5	Thyrotoxicosis, juvenile gynecomastia, benign hyperbilirubinemia
4, 17	PEN 2 degree, hypoglycemia, gastroduod
5, 10	Obesity grade 2, reactive pancreatitis, nontox goiter
6,12,5	Obesity 3 degrees, reactive pancreatitis, gastroduodenitis

Matching the identified substances in the bioassay (skin) with the patient's diagnosis





Metabolic disease

Patient N 4, 30 measurements

Group of patients

Isolation of ethandial (glycolysis metabolite)

Isolation of propandial (lipolysis metabolite)

Lack of bile acids

Glyoxal is one of the end-products of carbonyl glycation in the body

Non-enzymatic lipolysis

Lack or inactivation of the lipase enzyme

Suppression of the pancreas

Diagnosis: hypoglycemia - a decrease in the concentration of glucose in the blood.

Diagnosis: obesity / gastroduodenitis / pre-diabetes mellitus.

Violation of the mechanism for the correct breakdown of fats

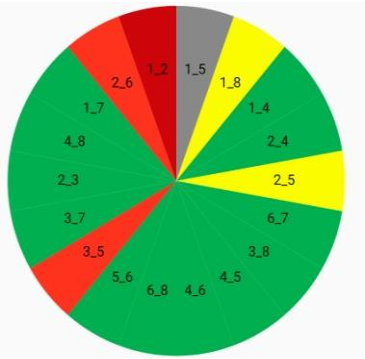
Dynamics of treatment of patients with hypoglycemia

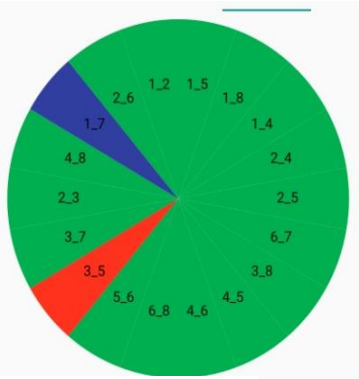
Patient N 4, 17 years old. 17.01.19-24.01.19.

Therapy is the introduction of drugs that normalize metabolism

Date	S «v.p.» left hand, Hz s	S «v.p.» Right hand, Hz s	Number of anomalies parameters (Ai/j)
17.01	**7500**	**7100**	**6**
19.01 fasting	**8000**	**7800**	**4**
19.01 After meal	**7500**	**7800**	**3/4**
23.01 After the injection	**8600**	**6700**	**2**
24.01 After meal	**10200**	**8200**	**2**
Cyclic, aliphatic amines, alcohols, acids, stress hormones			**Methylamine, water**

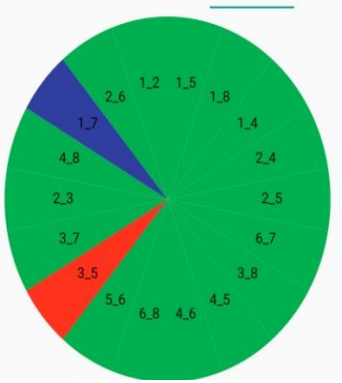
17.01





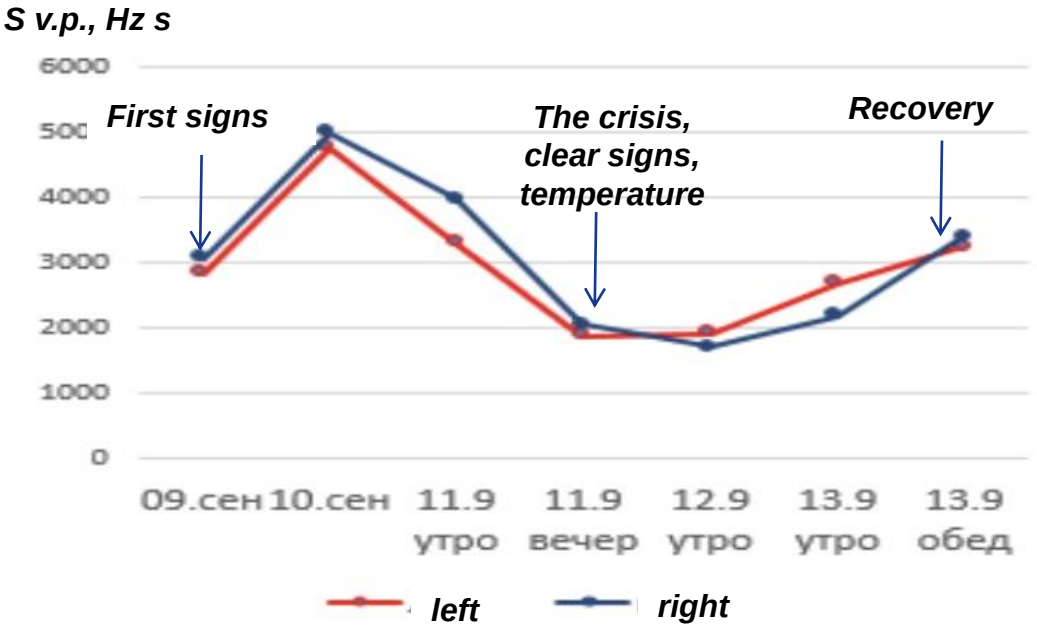
23.01 after the injection

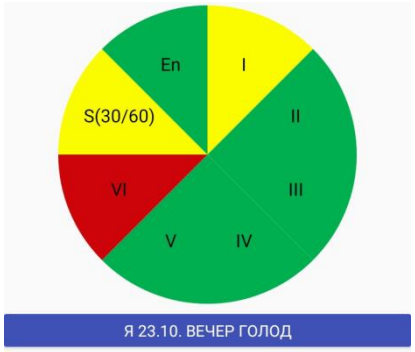
24.01 after meal (extract)



Ethanol, acids - hunger

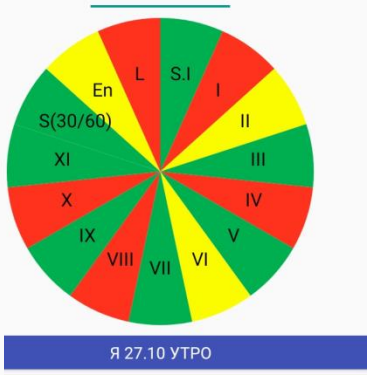
Dynamics of COVID-19 treatment





Before clinical symptoms

the height of the disease



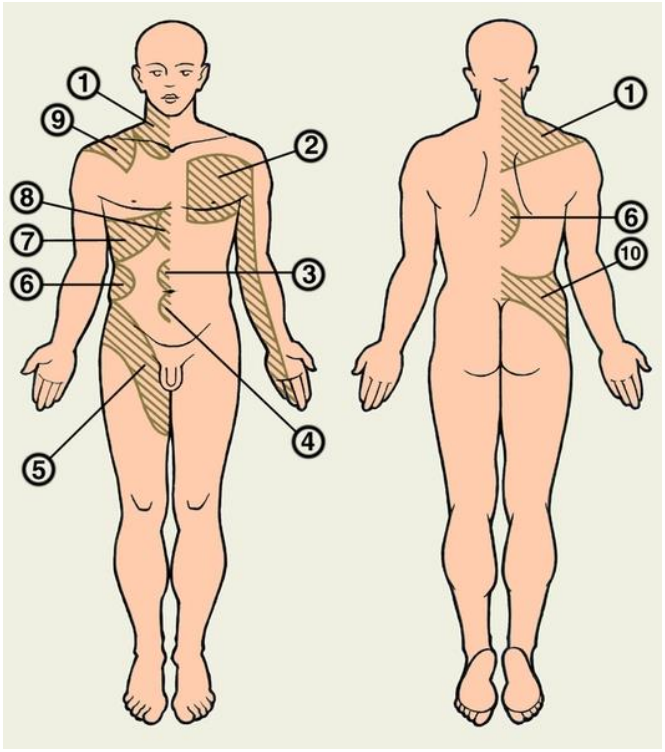
Recovery

The reaction of the left hand is most informative. The right one is more related to digestion.

The acute phase, the onset of the disease, the general response of the sensor is displayed on the screen. Additionally, a description of the signs on the spheres of state.

The scheme of the Zakharyin-Ged zones

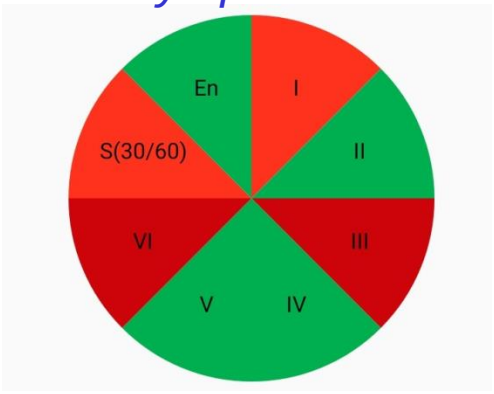
lungs and bronchi (1), heart (2), intestines (3), bladder (4), ureters (5), kidneys (6), liver (7 and 9), stomach and pancreas glands (8), genitourinary system (10).



Dynamics of COVID-19 treatment with pleuritis

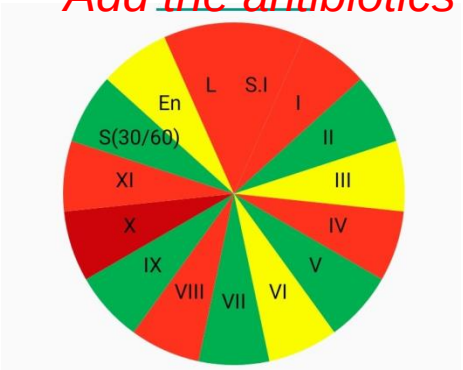
By zone of lungs and bronchi

Before clear clinical symptoms



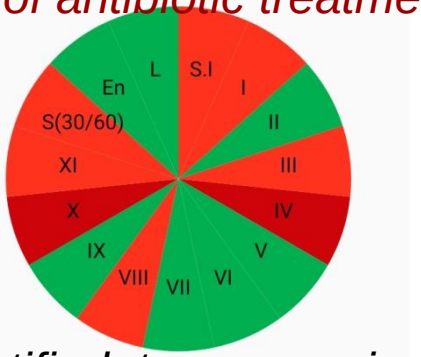
Identified S-containing compounds, butyric acid , ethyl acetate, chloroform

The height of the disease
Add the antibiotics



Identified ternary amines, aromatic amines, ketones, C_4-C_5 alcohols, S-containing compounds, benzene, butyric, lactic acids

The end of antibiotic treatment



Identified ternary amines, iso-alcohols, S-containing compounds, benzene

Diagnosed health status

1.Diseases of the skin. Control of exacerbations of chronic diseases (eg, atopic dermatitis).
2. At the points of projections - the state and dysfunctions of organs.
3. The vegetative-vascular system. Condition of large vessels.
4. Nerve conduction.
5. Spasms and pain. The condition of the digestive tract: bile ducts, bile ducts, liver, pancreas.
6. Prediabetic diabetic conditions, ketoacidosis.
7. The state of the hormonal background, the work of the ovaries, adrenal glands.
8. Excitement, depression, stress. The type of nervous activity.
9. Energy level.
10. Negative metabolic processes, inflammation.
11. Fatigue, heart failure, loss of energy.
12.Bronchitis, pneumonia.
13. Lymphostasis. Hyperhidrosis.
14. Closeness and heredity of metabolism in relatives (predisposition to diseases).
15. Dynamics of changes during the day, week, month, year. When taking medication.
16. Establishment of the chemical composition of the volatile metabolome, the corresponding process of the abnormal processes.
17. Clinical diagnostics by the smell of other bioassays (blood, urine, sweat, etc.).

By skin smell

Sensitivity and specificity of other proposed solutions using portable E-nose

Name of approach	Type of biosamples	Methods of clinical analysis	Sensitivity	Specificity
Diagnosis of STIs	Cervical mucus	PCR, ELISA, smear microscopy	87,5 %	100 %
Diagnosis of neoplasms	Whole blood	Ultrasound, hysteroscopy	81 %	87 %
Diagnosis of HbPylori and stomach inflammation	Exhaled air	FGS, histology	90 %	95 %
Diagnosis of inflammation	Urine	CUA, CBA, Bacterial culture	77	85
Diagnosis of dysbiosis	Feces	Bacterial culture	100 %	78 %
Diagnosis of pneumonia	Exhaled breath condensate	Microscopy of tracheal lavages, PCR	100 %	67 %

Thank you for your attention!

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