New Way and Sensor Device for Investigation of Biosamples and Health Status of Humans and Animals

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Abstract—**This paper discusses an application of a portable electronic nose based on an array consisting of 8 piezoelectric sensors with nanostructured solid-state coatings to detect volatile biomolecules secreted by nasal mucus and skin. A fundamentally new approach is proposed for a quick assessment of the status of the human body as a whole (normal, stress, inflammation) and the work of individual systems (reproductive, endocrine, digestive). This approach includes processing the output curves of sensors to assess of the qualitative and quantitative composition of the gas mixture of biomolecules secreted by the skin in the Zakharyin-Ged zone. Two algorithms for visualizing signals from an array of sensors are proposed. The first algorithm allows constructing the four "visual prints", reflecting the state of the human body (general, endocrine, energy, negative). The second algorithm constructs the health status sphere, which specifies the possible causes of deviation in health. Also, a portable electronic nose was applied in the veterinary field to assess the health status of calves' respiratory system. Unlike the traditional approach in diagnostics using a sensor array, one sample of nasal mucus was monitored for 5-9 hours with an interval of 2-3 hours. The new parameter is proposed to separate the diagnostic group of calves according to respiratory tract health. The changes in the composition of the gaseous phase over nasal mucus samples were considered. Using an electronic nose with nanostructured piezoelectric sensors, the speed and simplicity of measurement allow painlessly scanning the body for metabolic disturbances and estimating certain pathologies' presence and treatment effectiveness. The proposed algorithms and device for analysis of biosamples from humans and animals could be implemented to rapidly diagnose health status on farms, hospitals, and at home.**

Keywords- sensor; electronic nose; visualization; volatile compounds; metabolism; noninvasive diagnostic; skin; biofluid.

I. INTRODUCTION

Biosamples are complex objects to analyze. The problem of their analysis is connected with the absence of constant composition and in its almost instantaneous change when substances are excreted from the sample. Despite the emergence of new methods for analyzing and studying biostructures at the level of individual cells, the scheme remains traditional: a selection of biomaterial - sample preparation - detection of target components. The use of highly selective and effective methods of analysis (gas chromatography-mass spectrometry, high-pressure liquid chromatography.) suggests a special sample preparation, which can change the natural profile of the biosamples. The purpose of the analysis determines the method of sample preparation, but the integrity of the biological object is lost.

Furthermore, the results obtained by advanced analysis methods do not reflect a biological object's complex structure and behavior. Therefore, recently, in analyzing living objects (food, environmental objects, human and animal biosamples), complex methods with a multivariate analytical signal have been used more often. Such methods by methodology include artificial tongues, noses, eyes, and their portable variants [1][2]. The undoubted advantage of highly sensitive sensor systems with a rapid response is the ability to monitor the state of small volumes and masses of biological samples in a reasonably short time (from 2 to 9 hours). Given the lack of their contact with the environment (in vitro), primarily with oxygen, small volumes of biosamples, which means fast processes of changing their properties, open up a unique opportunity to obtain information about the status of the studied object, even if the specific methods for determining individual substances or

laboratory indicators (primarily microbiological) are unavailable.

A possible approach for assessing the body's status in the absence of biomaterial selection is to analyze the chemical composition of the gas, sweat of the skin in the zones of Zakharyin-Ged. Earlier, the presence of redness, peeling, rash, temperature changes in these areas was widely used as an additional parameter to confirm the malfunction of organs corresponding to these zones. The detection limits of modern methods of analysis, the complexity of the instrumentation of the most sensitive methods do not allow noninvasive scanning and determining the chemical composition of the gas phase of secretions from the skin. Therefore, creating an integrated system for scanning a volatile metabolome using a device with a sensitive detector to biomolecules of normal and disordered metabolism, inflammation, and microbial metabolites is actual for now.

The purpose of this paper is the 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.

We will demonstrate our approach in two ways: 1) analysis of nasal mucus samples of calves for the diagnosis of respiratory diseases and 2) characterizing the health status of humans by skin odor in the Zakharyin-Ged zones.

Further, in Section II, the related works and state-of-the-art technologies for health status assessment are described. In Section III, the features of the experiment, description of biosamples, and methods of analysis are presented. Section IV contains technical characteristics of the proposed device, characteristics of used sensors and their coatings, a description of the procedure for obtaining and recording output data of the sensor array. Section V shows the results of applying the proposed portable electronic nose (e-nose) for solving diagnostic problems according to the purpose of the work. Section VI is devoted to conclusions and perspectives of development.

II. Related work

There are some state-of-the-art approaches, including portable devices, for assessing the state of the body using electronic noses and various data processing methods: for exhaled breath air [3][4][5], for the analysis of biomaterials (blood, urine, secrets of the endocrine glands and others) [6][7][8]. The different approaches for assessing health status by skin based on electrochemical or optical methods are proposed [9][10][11].

At the same time, several devices have been developed to detect body odor with different types of sensors to discriminate volunteers and estimate changes in their body odor during physical activities [12][13]. There are examples of developing unique construction with sensors to diagnose tuberculosis [14], renal dysfunction [15], heart failure [16] by volatile compounds from the skin.

The mentioned researches are based on training the sensor array by volatile compounds, measuring the body odor, and calculating the relative of normalized signals used for processing by different multivariate data analysis methods for classification or discrimination. However, the investigation of healthy people in different conditions is missed. Also, the application of such complex multivariate algorithms is not understandable for users and difficult to use in a hospital.

We want to propose a sensor device with the simple measurement, calculation, and visualization of sensors signals with the verbal characteristics of state understandable by any user. The new technique of estimating respiratory organ state in animals and differentiating the disease on farms based on a simple parameter of the sensor array was one of the investigation tasks.

III. Materials and Methods

This section represents the additional methods used to diagnose the health state of animals and humans.

A. Diagnosis of Respiratory Diseases in Calves

The 17 samples of nasal mucus from calves (10-20 days of life), both with signs of respiratory system damage and conditionally healthy, were analyzed. A sampling of nasal mucus was carried out with sterile cotton swabs in individual sterile containers. The time from sampling to analyzing on e-nose was taken into account.

The calves were clinically studied in detail using a point system (WI score) developed at the University of Wisconsin, Madison (USA) [17]. Samples of nasal secretions were investigated in the laboratory at the All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy using bacteriological and molecular genetic (PCR) analysis for infectious rhinotracheitis, parainfluenza-3, viral diarrhea-disease cattle mucous membranes, rotavirus, adenovirus, chlamydia, pathogenic mycoplasmas (*M. bovis, M. bovirhinis*). Also, the hematological indicators in the blood (leukogram, haptoglobin concentration) were determined to confirm inflammation.

For the isolation of cultures and typing of microorganisms from the nasal mucus samples, meat and peptone broth, milk salt, enterococcal agar, Endo medium, blood agar, glucose-serum broth and agar produced by Research Center for Pharmacotherapy (St. Petersburg, Russia) were used. The isolated Escherichia coli were typed in an agglutination reaction using O-serums.

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

The forearm area of human skin was chosen to analyze the volatile metabolome by a portable electronic nose. Over 100 volunteers aged 3 to 80 years took part in the investigation for two years (72 female, 35 men). The volunteers periodically were clinically (visits to physicians) and laboratory (general analysis of blood, urine, biochemical analysis of blood (glucose, cholesterol, some hormones: thyroid-stimulating hormone, free thyroxine, progesterone, follicle-stimulating hormone, estradiol, lutein hormone, adrenaline, cortisol)) tested to control health status.

Clinical monitoring of health status and measurement of volatile metabolome were performed on average once a month in the absence of pathology. When volunteers had

diseases (acute respiratory viral infections, bronchitis) or exacerbation of chronic pathologies (diabetes, heart failure), monitoring of the volatile metabolome of the skin and the main clinical and laboratory parameters (glucose level, heart rate, blood pressure) was carried out 2-3 times a day until the health state stabilized due to the therapy. The state "inflammation" corresponds to both an increase in laboratory parameters (leukocyte count, erythrocyte sedimentation rate) and some cases, a change in biochemical parameters indicating the inflammation in organs - lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, C-reactive protein. During the experiment, we monitored inflammation with different diagnoses: arthritis (n=2), gastritis (n=5), sinusitis, pyelonephritis, pneumonia (n=2), adnexitis (uni- and bilateral, n=3), endometritis (n=3), osteochondrosis (n=2), diabetes (n=2), inflammation of soft tissue and tendon, systemic lupus erythematosus.

For conditionally healthy volunteers, the results of laboratory tests corresponded to the typical reference values, and the symptoms did not match with clinically significant ones for illness. Clinically not diagnosed conditions, so-called descriptive states (tiredness, excitement, agitation, stress, lack of sleep, spasm, pain), were recorded from the volunteers' words.

In total, by March 2020, we performed 964 measurements.

IV. Description of Portable E-nose and Measurement Technique

The characteristic of used piezoelectric sensors and coatings, hardware and software of a portable e-nose, as well as technique of measurement, are presented below.

A. Making of Piezoelectric Sensors

We used piezoelectric quartz resonators (PQR) with a natural frequency of 14 MHz with an established linear response with a film mass on its electrodes up to 20 μg/cm^2. The array contained eight piezoelectric sensors with electrodes covered by films of carbon nanomaterial, hydroxyapatite, zirconium salts of different mass (1-5 μg) (NANO-BIO array).

1) *Characteristics of the Used Sorbents*

Hydroxyapatite (HA) $Ca_5(PO_4)_3OH$ was obtained by the sol-gel method developed at Nizhny Novgorod State University, named after N. I. Lobachevsky [18] and optimized by us to obtain nanostructured coatings with good sorption properties.

The reaction was carried out according to the following equation:

$5Ca(NO_3)_2 \cdot 4H_2O + 3H_3PO_4 + 10NaOH \rightarrow Ca_5(PO_4)_3OH + 10NaNO_3 + 29H_2O$

To a solution of calcium nitrate (2 mol/dm^3) prepared from $Ca(NO_3)_2 \cdot 4H_2O$ in bidistilled water has added a solution of H_3PO_4 in the amount necessary to maintain the ratio Ca/P = 5/3. The resulting solution was thermostated for one hour at 37 °C; then its pH was adjusted to 7-8 using a NaOH solution with a concentration of 2 mol/dm^3. A hydroxyapatite sol began to form at pH = 4. The reaction mixture was kept at 37 °C for 1 hour. Then, the resulting gel was centrifuged and dried in air. The obtained sorbent can be stored for at least 0.5 years in airtight conditions. Multi-walled carbon nanotubes (CNT) were obtained in the Institute for Extra Pure Materials of the Russian Academy of Sciences (Chernogolovka, Russia) by gas-phase chemical deposition during ethanol pyrolysis. Nickel was used as a catalyst; deposition temperature was 450-500 °C. Then nanotubes were washed with HNO_3 concentrated (Reachem, Russia). The solvent for the suspension of HA and CNTs was chloroform. Zirconium nitrate (ZrO) ($ZrO(NO_3)_2 \cdot 2H_2O$) (chemically pure) was obtained from aqueous solutions containing zirconium and nitrate ions (Reachem, Russia). The solvent for the suspension of ZrO was acetone. According to the preliminary experiment results, these sorbents are selective and sensitive to volatile metabolites of bacteria and inflammation [19]. Also, the variation of sorbent mass affects the selectivity and sensitivity of volatile organic compounds (VOCs) micro weighting [20].

Therefore, the main array of sensors consists of:

Sensors 1, 8 – CNT phases of different masses.

Sensors 2, 7 – phases of ZrO of different masses.

Sensor 3 – dicyclohexane-18-Crown-6, DCH-18C6.

Sensors 4, 5 – HA phases of different masses.

Sensor 6 – polyethylene glycol succinate, PEGS.

Additionally, for the analysis of the gas phase over the nasal mucus samples, we used an array with polymer polar films (additional sensor array), which we previously used in our work with other biological samples [21][22], namely polyethylene glycol 2000 (PEG-2000), polyoxyethylene sorbitan monopalmitate (Tween), t-octylphenoxypolyethoxyethanol (TX-100), dicyclohexane-18-crown-6 (DCH-18C6), polyethylene glycol sebacinate (PEGSb), bromocresol blue (BCB). These polymeric compounds were dissolved in organic solvents according to their polarity: PEG-2000, Tween, TX-100 – in acetone, DCH-18C6, PEGSb - in toluene, BCB – in ethanol. The solutions of polymeric sorbents were immediately used to form films on the piezoelectric resonator electrodes without their pretreatment with ultrasound (step 3).

2) *The Method of Forming Films on the Surface of the Piezoelectric Quartz Resonator*

The films were uniformly deposited to the electrodes of PQRs, fat-free with acetone or chloroform, by immersion in solutions of sorbents.

The forming of films on the electrodes of resonators was performed using the following procedure:

Step 1 – the measurement of the initial oscillation frequency of the piezoelectric resonator (10 or 14 MHz) F_0, Hz with an accurate record, for example, 9999280 Hz;

Step 2 – suspension was prepared in the beaker as dissolution of sorbent (0.5 g) in 10 ml of solvent;

Step 3 – processing in an ultrasonic bath for 15 minutes at a power of 90 W;

Step 4 – exposure of the quartz piezoelectric resonator in suspension for 15 s;

Step 5 – drying the coating in an oven (40 minutes at a temperature from 50 ° C) in the holder vertically;

Step 6 – the measurement of the oscillation frequency of the sensor, calculation of the coating mass (Δm) according to the Sauerbrey equation [23]:

$$\Delta m = \frac{\Delta F \cdot 0.2}{2.27 \cdot 10^{-6} \cdot F_0^2} \quad (1)$$

where ΔF is the change in the oscillation frequency of the quartz plate of the resonator after film deposition and removal of an unbound solvent, MHz;
$2.27 \cdot 10^{-6}$ – calibration constant of PQR at normal condition, cm^2/g;
F_0 — base oscillation frequency of the PQR, MHz;
0.2 – the area of electrodes of PQR, cm^2.

B. Characteristic of Portable E-nose

The portable device for diagnosing the status of humans and animals is a miniature case, consisting of two functional parts (Fig. 1): head 1 and the protective part of the body 6. A microprocessor 2 with terminals for sensor mount sockets, a block for fixing and transmitting information 3 to the recording device (laptop, tablet, personal computer). The sockets are located in cover 4, into which removable sensors 5 are mounted on the outside, separated from the environment by the protective part of the body 6, which is tightly attached to the head 1. Optionally e-nose is supplemented by an internal gas-permeable gasket 7, which separates the body's sensor and free air region of the body 6.

The protective nozzles of various types 8 from inert materials (fluoroplastic) are used depending on the nature of the analyzed sample to reduce interfering factors (external fluctuation in airflow, temperature, air composition in the near-sensor space).

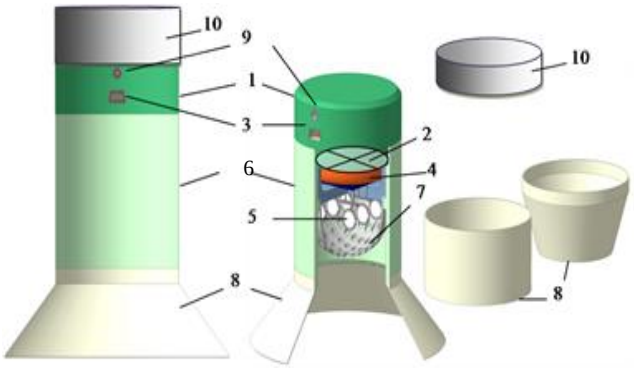


Figure 1. General view of the 3D model of the portable e-nose for diagnostics: 1 – head; 2 – microprocessor with terminals for sensor mount sockets; 3 – block recording and transmitting information to a recording device of any type; 4 – cover; 5 – removable sensors; 6 – protective part of the body; 7 – internal gas-permeable gasket; 8 – nozzles; 9 – power supply from the electricity; 10 – removable battery.

The e-nose is powered by either an electronic device via a USB cable, either from electricity 9 or a removable battery 10. The developed portable device is an electronically counting frequency meter with 8 channels to measure the oscillation frequency of BAW-type PQRs with a base oscillation frequency of 5 to 20 MHz with a resolution of 1 Hz a time interval (step) of 1 second. The electronic counting frequency meter is switched on in the network (220 V); it warms up for 10-15 minutes. In this case, the sensors should be in the device to reduce measurement errors. Nevertheless, their subsequent inclusion is also possible. It takes about 5-10 minutes to stabilize the baseline of the oscillation frequency of the quartz plate.

To simultaneously record (read) the oscillation frequency of each sensor every second for a specific time interval (from 1 s to a maximum of 6000 s), the device is connected to a computer via USB cable, and other connection options are possible (via Wi-Fi, Bluetooth).

Operating conditions and technical specifications of E-nose are presented below:

- Ambient temperature from +15 to +35 ° C.
- Humidity is up to 98% at temperature +35 °C.
- 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 of using PQR 4 MHz – 20 MHz.
- The reference frequency oscillator is 4 MHz.
- Overall dimensions – 38x120x170 mm.
- Weight with a cover – 0.40 kg.

C. Specification of Software

The responses are recorded in the instrument software, which saves the measurement and converts it into analytical information – a change in the oscillation frequency of each resonator individually at each measurement moment relative to the starting point of measurement (-ΔF, Hz). The total output curve is displayed in a set of chronograms for all resonators installed in the e-nose (Fig. 2).

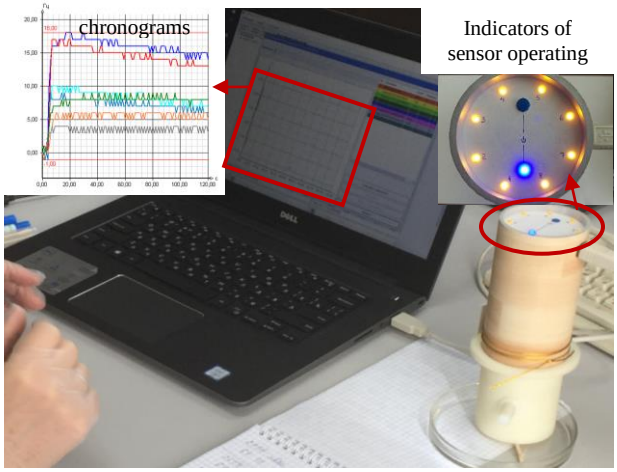


Figure 2. E-nose connected with the laptop when measuring the sample of nasal mucus.

During the interaction of vapors with the surface of the piezoelectric sensors, sorption occurs on the films or electrodes, resulting in frequency changes. Individual colors reflect a change of the base oscillation frequency in time for each of the eight piezoelectric sensors (Fig. 2).

In the developed software based on chronograms, the "visual print" is constructed using different algorithms depending on the purpose of analysis. The quantitative characteristic of "visual prints", therefore, the total amount of volatile substances excreted by samples and sorbed by piezoelectric sensors, is the area of "visual print" ($S_{v.p.}$, Hz·s). The "visual print" area is calculated in software as a sum of definite integrals of time dependence the signals of sensors during measurement (chronograms).

Additionally, in software, the parameters of sorption (*A(i/j)*) are calculated by equation (2), which can be used for the identification of volatile substances in the gas phase over samples [24][25] or to describe additional analytical information about sample characteristics.

$$A(i/j) = \Delta F_i / \Delta F_j , \quad (2)$$

where $\Delta F_{i(j)}$ are the maximum responses of piezoelectric sensors during the sorption of the gaseous phase over the biosamples or skin.

D. Technique of Measurement

The gas phases over nasal mucus samples and skin were studied with the front input method into the detection cell. The open detection cell of the device contacted the forearm area (20 cm^2) of the skin or Petri plate with mucus sample to analyze the volatile substances excreted by the biosamples. The registration time of the sorption of volatile substances excreted by biosamples was 80 s, the registration of desorption was 120 s. Thus, the total time of one measurement was 200 s. The frontal analyte input method in detail is described in [26][27].

Preliminary, the electronic nose was trained in the same technique of measurement by 45 individual substances of normal and pathogenic metabolism: C_1-C_5 alcohols, 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.

Analysis of the gas phase above the nasal mucus samples using an additional array of sensors with films of polymer sorbents was carried out immediately after measurements with the main array of sensors with solid-state nanostructured films by the same technique. A detailed description of the measurement technique of nasal secretion samples is provided elsewhere [27].

V. Results and Discussion

Examples of using developed sensor device for medical applications are provided in this section, including sensor data processing algorithms to extract information concerning health status.

A. Diagnosis of Respiratory Diseases in Calves

We selected three diagnostic groups of calves based on the results of clinical studies, the determination of hematological and biochemical markers of inflammation (leukocytosis, an increase in the concentration of haptoglobin in the blood serum), pathogens of viral and bacterial infections accompanied by damage to the respiratory system: 1 - "healthy respiratory system" (n=4), 2 - "with signs of respiratory disease" (n=7), 3 - "with the subclinical course of respiratory diseases" (n=6).

A natural change in the mucus composition taken at a weekly interval can reflect only significant changes in the condition – for example, a vivid manifestation of the inflammatory process. Unlike state-of-the-art approaches to diagnosing respiratory diseases by one measurement of biosamples [6-8], for the first time, it has been proposed to monitor one sample for 5-9 hours with an interval of 2-3 hours. It allows recording changes in the state at the micro-level associated with microbiological contamination of the sample or its absence. The areas of "visual prints" were calculated for all samples of nasal mucus. Early it was shown that the values of the "visual prints" area correlate with biochemical indicators of inflammation characterizing the disease of respiratory organs in calves [28].

The results of one-day monitoring of nasal mucus samples can be divided into three groups (Fig. 3).

1) Positive (increasing) dynamics of changes in the value of the integral analytical signal of the sensor array (area of "visual print") - indicates the destruction of nasal mucus and production of a large number of volatile compounds, including microorganisms metabolites.

2) The negative (decreasing) dynamics of the change in the value of the analytical signal of the sensor array indicates the decreasing of volatile substances excreted from nasal mucus due to increase of its viscosity by the high level of proteins, mucin, which is observed in the acute phase of respiratory disease [29].

3) The almost constant value of the integral signal from the array of sensors, which is observed at the first sign of respiratory disease (subclinical course), indicates that the excretion of substances at the destruction of nasal mucus and the production of metabolites by microorganisms are not so active.

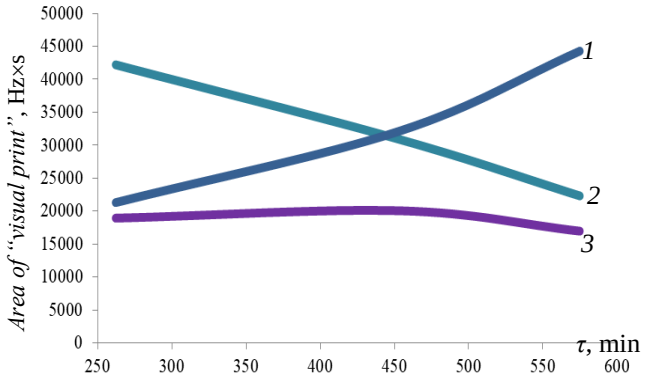


Figure 3. Total "visual prints" area of signals of the sensor array in vapors of nasal mucus of calves with different diagnoses: 1 –healthy respiratory system, 2- with signs of respiratory disease, 3 - with the subclinical course of respiratory disease.

When a sample of nasal mucus from an animal with the subclinical course of the respiratory disease is taken within 5 minutes after the first selection, then the time dependence of area of "visual print" for this sample will be like for conditionally healthy (curve 1, Fig. 3). These facts are in good agreement with the data on the formation of local protection of the respiratory tract of calves [29].

The maximum exposure time of nasal mucus samples from the moment of sampling to analysis by the array of sensors should not exceed 7 hours; with a longer exposure time, the differences in the concentration of volatile substances between diagnostic groups are disappeared.

The study of the sorption of the gas phase over nasal mucus samples on an additional array of sensors with polymer hydrophilic films coatings showed the same trend with more significant differences between groups, since sensors with polymer films are more sensitive than solid-state sensors [30], although with a shorter operation time [31]. Consequently, during the delayed analysis of nasal mucus samples, it was proposed to calculate the ratio of the sum of sensor signals ($\Sigma F_{ref.}$) with hydrophilic polymer coatings of the first measurement (1mtesurement) to the one carried out after 2 hours for each sample using the equation (3):

$$\sum F_{ref.} = \frac{\sum_{i=1}^{6} \Delta F_{i(1measurement)}}{\sum_{i=1}^{6} \Delta F_{i(after\,2hous)}} \quad (3)$$

The deviation in sensor array responses due to internal factors (microelectronic scheme, reproducibility of sensors surface) is no more than 5 % [16]. Therefore, if indicator ΣF_{ref} deviates by more than 15%, the differences in gas phases over biosamples are statistically significant. Consequently, based on the established trend by one-day monitoring of nasal mucus samples, the group "healthy respiratory system" – $\Sigma F_{ref.} \leq 0.85$, and for the group "early signs of respiratory disease" – $\Sigma F_{ref.} \geq 1.15$, the values $F_{ref.}$ 1.00 ± 0.15 is assigned to the "with the subclinical course of respiratory diseases" group.

Table I shows the proposed classification based on the value of indicator $\Sigma F_{ref.}$ and actual diagnostic group for the 17 studied samples. The proposed indicator was used to rank additional 4 samples of nasal mucus of calves to check the implementation of such classification. These calves were also clinically and laboratory tested to determine the diagnostic group (in italics at the bottom of Table I). The proposed ranking for 4 samples also correlates with the established diagnostic group. Hence, samples of nasal mucus from calves with or without infections of the upper respiratory tract (nasal cavity, trachea, bronchi) can be differentiated using the $\Sigma F_{ref.}$.

Thus, several measurements of one biosample during the day and simple indicator $\Sigma F_{ref.}$ allow clarifying the degree of damage respiratory tract in calves associated with the contamination sample.

TABLE I. RELATIVE CHANGE $\Sigma F_{REF.}$. IN THE TOTAL AMOUNT OF VOLATILE SUBSTANCES IN THE GAS PHASE OVER NASAL MUCUS SAMPLES FROM CALVES.

Sample	**ΣF_{ref}**	**WI**	**Results of bacteriological, mycological, PCR investigations of nasal mucus samples**	**CFU /ml[a]**	**Diagnostic group**
2675	0.78	3	*E. coli, Ent. faecium, yeast-like fungi*	800	1 – healthy respiratory system
2664	0.85	2		200	
2444	0.82	2		350	
2442	0.85	3		150	
2	1.26	8	*E. coli O142, Ent. faecium, Ent. faecalis, Pseudom. aeruginosa, yeast-like fungi, Adenovirus, Mycoplasma bovis*	3500	2 – with signs of respiratory disease
3	1.29	8		2400	
2679	1.25	5		600	
2667	1.31	5		1500	
2669	1.40	7		4500	
2670	1.47	8		6000	
2671	1.44	8		5600	
2677	0.96	4	*E. coli O142, Ent. faecium, Ent. faecalis, yeast-like fungi, Adenovirus*	700	3 – with the subclinical course of respiratory disease
1	0.98	5		540	
4	1.09	4		200	
5	1.13	4		450	
2666	0.99	4		850	
2668	0.92	4		750	
2672	0.99	4	*E. coli, Ent. faecalis*	1000	3
2665	1.37	6	*E. coli, Ent. faecium, Adenovirus*	3400	2
2663	1.15	4		2300	3
2669	1.44	8	*E. coli, adenovirus, yeast-like fungi*	4200	2

a - colony forming units (CFU) of mesophilic aerobic and facultative anaerobic microorganisms.

Trends in changes in the composition of the gas phase of biosamples during a day were established based on assessing changes in proportions of VOC classes in the gas phase over samples $w_{i,ref.}$ calculated by the formula (4):

$$w_{i,ref.} = \frac{w_{i,(followingmeasurement)}}{w_{i,(previousmeasurement)}}, \quad (4)$$

where w is a proportion of VOCs calculated via equation (5) [32]:

$$w_i = \frac{\Delta F_i}{\sum_{i=1}^{8} \Delta F_i} \quad (5)$$

where $\sum_{i=1}^{8} \Delta F_i$ is sum of sensors signals in an array (main or additional).

For samples of nasal mucus from calves from the group "healthy respiratory system" in the first 4 hours, changes in the proportions of different classes of VOCs in the gas phase were observed within 25%. In the next 3-4 hours, the proportion of volatile aromatic, cyclic amines increases several times and the proportion of aldehydes, ketones, organic acids decreases by 30-60% (Table II).

TABLE II. RELATIVE CHANGE IN THE PROPORTION ($W_{i,REF.} \pm 0.08$) OF INDIVIDUAL CLASSES OF VOCS IN THE GAS PHASE OVER SAMPLES OF NASAL MUCUS DURING ONE DAY MONITORING

τ, min	Diagnostic group (samples)	$W_{i,ref}$; *i* – sensor coating					
		HA[a]	*CNT*	*ZrO*	*BCB*	*DCH-18C6*	*PEGS*
124	1 (2675, 2444, 2442, 2664)	1.30	1.27	1.37	0.74	1.41	1.27
246		1.19	1.14	1.22	0.79	1.22	1.16
377		1.01	0.88	1.00	1.14	0.99	1.05
453		0.53	0.76	2.86	1.39	0.75	1.79
517		0.69	0.67	0.63	0.91	0.56	0.64
126	2 - (2679, 2, 3, 2667, 2669, 2670, 2671)	1.19	1.03	1.06	0.69	1.08	1.10
136		1.19	1.09	1.22	0.80	1.22	1.25
258		1.37	1.39	1.45	0.89	1.45	1.37
380		1.59	1.49	1.52	0.91	1.45	1.61
453		1.14	1.08	1.11	2.44	1.09	1.19
557		0.93	0.98	0.88	0.92	1.02	0.88
126	3 - (2677, 1, 4, 5, 2666, 2668	0.63	0.57	0.53	0.60	0.63	0.60
256		1.35	1.15	1.33	0.80	1.41	1.33
377		1.39	1.25	1.32	1.15	1.25	1.32
498		1.27	1.15	1.19	1.69	1.23	1.25
503		0.91	1.03	1.01	0.91	0.85	1.01

a - Predominantly adsorbed VOC classes on sensor coatings: DCH-18C6 – hydroxyl, carboxylic acids; BCB – aromatic, cyclic amines, nitro compounds; PEGS – aromatic compounds, oxy-amines; CNT – C_1-C_5 primary, secondary alkylamines, ammonia; HA – C_2-C_5 alcohols, acetals, branched and cyclic ketones, amines; ZrO – alkylamines, cyclic amines, oxy-amines.

For samples of nasal mucus from calves from the group "with signs of respiratory disease", conversely, in the first 2-3 hours after sampling in its gas phase, the proportion of volatile aromatic, cyclic amines, nitro compounds is significantly decreased and the proportion of oxy- and hydroxyacids is increased, with an increase in the proportion of ketones, oxy-compounds in the next 4 hours. For samples of nasal mucus from calves from the group "with the subclinical course of respiratory diseases", a decrease in the proportions of all VOC classes in the gas phase over samples is observed in the first 2 hours after sampling. In the next 6 hours in the gas phase, the proportion of VOCs increases up to 35% with the predominance of ketones, acids, amines (Table II). Thereby, parameters $\Sigma F_{ref.}$ and $w_{i,ref.}$ can be applied for estimating respiratory organ state in animals and differentiating the disease on farms.

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

A forearm zone of skin was chosen to scan the whole organism's health status according to information about the diagnostic significance of the Zakharyin-Ged zone. The primary measurement database contained the responses of 8 sensors in 200 seconds (1590-1600 signals).

Based on the analysis of the skin odor of 100 volunteers in various states according to their words and the results of laboratory tests, a primary algorithm of “visual print” construction has been developed for linking the features of the forms of “visual prints” with the human condition and possible causes of deviation (Table III, Fig. 4).

"Visual print" is a circular diagram of the sensor signals at specific points in the process of sorption and desorption of volatile substances from the gas phase over biological samples.

TABLE III. ALGORITHM FOR CONSTRUCTING “VISUAL PRINTS” OF SIGNALS FROM AN ARRAY OF 8 SENSORS FOR ASSESSING THE HEALTH STATUS OF PEOPLE

Name of an algorithm of “visual print” construction (characteristic)	Time of recording the sensor responses, s / number of sensors used to build a “visual print”
"General state" (the most complete information about reproductive, digestive systems)	30, 45, 60, 80, 100, 120, 180 / 8
"Energy" (reflects the strength and intensity of the metabolome part, which shows the ability of the body to act)	110, 120, 130, 140, 150 / 3
"Endocrine system" (reflects malfunctions of the endocrine glands, primarily the pancreas)	10 20 30 60 / 4 Additionally, the parameter is calculated: $\gamma = \Delta F_4(60\ s)/\Delta F_4(20\ s)$. At $\gamma \leq 2$ pathology occurs
"Negative" (the severity of destructive processes in the body)	20, 30, 170, 180 / 8 Additionally, parameters are calclulated: $\beta_1 = \Delta F_4(20\ s)/\Delta F_4(170\ s)$. $= \Delta F_4(30\ s)/\Delta F_4(180\ s)$. At $\beta_1, \beta_2 \leq 2.5$ pathology occurs

Integral analytical signals of the sensor array when measuring the volatile metabolome of the forearm skin are visualized in the form of 4 “visual prints” (general state, energy, endocrine, negative, Table III). These multidimensional responses represent a particular, differentiated part of the chronograms of the most informative sensors, reflecting the sorption features of volatile organic substances established in the preliminary training experiment. "Visual prints" differ in the set of time points of recording the sensor responses (mask) and the number of sensors.

The largest number of points on the "visual print" corresponds to the "General state" algorithm - all 8 sensors and 7 time points on the chronograms of complete measurement (Table III). It reflects the complete information about the nature and concentration of volatile substances in the gas phase above the skin.

The "Energy" algorithm includes the desorption time on the most sensitive sensors. It estimates the rate of desorption of the volatile metabolome of the skin, presumably associated with the production of adenosine triphosphoric acids and, as a result, production of the energy by the cell.

The "Endocrine system" algorithm uses only the beginning of the sorption of volatile substances, where the difference between volatile products of hormone metabolism and other substances is maximally manifested.

The "Negative" algorithm takes into account the initial sorption time and the final desorption time of substances, allowing the maximum differentiation of heavy compounds (cyclic and aromatic amines, ketones), which are associated with the development of negative processes (oncology, inflammation, tissue and organ damage).

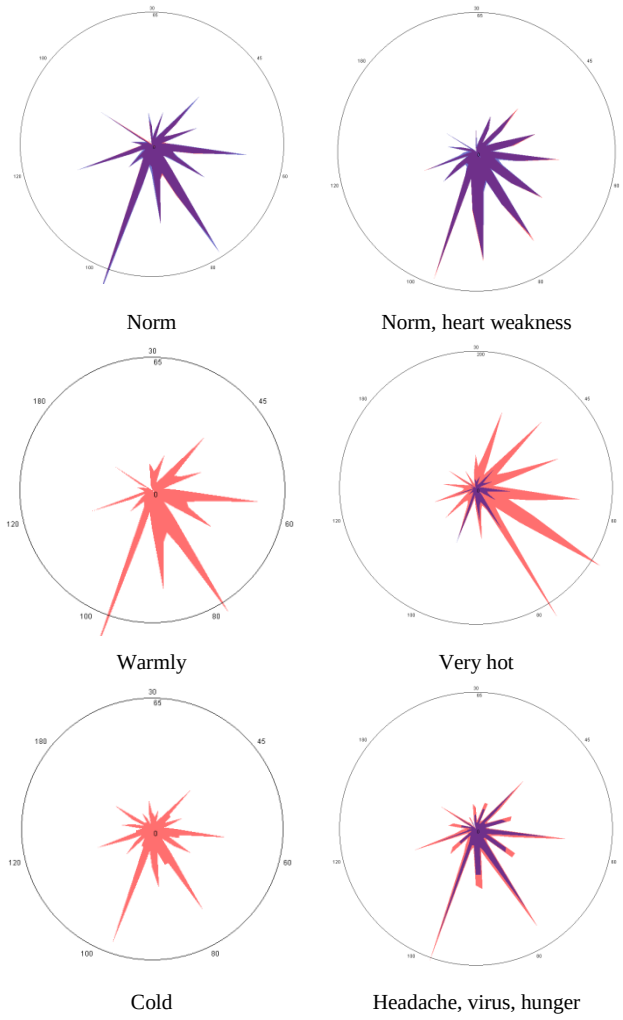


Figure 4. Statistically significant typical changes in the shape of the integral signals of the sensor array of the portable e-nose ("General state" algorithm) for different conditions of one person (for left forearm – the blue color and for right –red color).

Masks and a set of sensors for constructing multidimensional visual signals were selected based on the results of preliminary training of the array of sensors for individual substances – biomarkers of metabolic disorders and the disfunction of individual organs, the presence of inflammatory processes in the gaseous phase of biosamples [33][34][35][36][37][38][39][40][41][42][43][44]. The proposed algorithms are different from the traditional approach to visualize the sensor signals used in the state-of-the-art methods [4]. Moreover, such visualization is more straightforward and more evident for the user.

Based on the data obtained for 400 measurements of volatile compounds secreted by the forearm zone of skin, the geometric characteristics of "visual prints" and their typical shape were determined, corresponding to the reference boundaries of the norm for different categories of people (men, women, adolescents, dependence on the type of central nervous system). The geometric shape of the "visual print" is characterized by its rays, area, and the ratio of parts relative to the line of symmetry (passes through 80s). Changes in the geometric shape of typical "visual prints" at different time intervals (for example, the first three points) are signs of a change in the composition of the volatile metabolome. The features of these changes were compared with a verbal description of the condition (headache, stress, fatigue, hunger, and others), clinical examination and the presence of disease symptoms (runny nose, cough), parameters *A(i/j)* for substances. Fig. 4 shows the comparison of "visual prints" forms for left and right hands, used for visual assessment of descriptive states. As a result, statistically reliable responses and "visual prints" forms were determined, which correspond to the physically normal functioning of the body (norm), stress, tiredness, inflammation, weakness, in total no less than 17 states. The deviation of the geometric shape of the "visual print" from the typical one is the first sign of a change in health state.

In a normal state, the qualitative and quantitative composition of the volatile metabolome on the left and right hand practically does not change - the shape and area of the "visual print" practically do not differ (Table IV, Fig. 4). During an extended stay in a warm or cold room, when a person can describe his condition as "warm" or "cold", a regular increase or decrease in the amount of excreted substances occurs, while the shape of the "visual print" does not change (Fig. 4). When the body overheats, changes in the volatile metabolome are more significant, the shape of the "visual print" changes, associated with a more intense excretion of VOCs to maintain homeostasis.

TABLE IV. THE MOST TYPICAL EXAMPLES OF CHANGES IN THE AREA OF THE "VISUAL PRINTS" WITH SOME CHANGES IN HEALTH STATUS

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 eat	11-30% more for the right hand than for the left one
Norm, easy hunger	For the right hand less than for the left one by 11-25%
Norm, severe hunger	If the gallbladder is malfunctioning, a response increase of 15-20% for the right hand is observed due to the excreted of propandial
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
Increase the air temperature up to 26-30°C	10-30%, at perspiration till 500%
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 %

In the presence of pathology and severe symptoms of the disease (viral infections, headache, heart weakness, Fig. 4), the shape of the "visual print" changes in different ways compared to the normal state, which is associated with the VOCs of intoxication of the organism. If we normalize for one person its average quantitative parameter of the smell trace (area of "visual print") by 500 measurements in different periods during two years, then the general nature of the bias of this indicator will obey the laws presented in Table IV. The geometric shape of the "visual print" is strictly individual for each person and the calculated parameters (*A(i/j)*) – for health status (Fig. 4, Table V).

The number of changed parameters and indicators depends on the degree of deviation of the health state from the norm and correlates with the severity of destructive processes during the disease. The shape of "visual print" is influenced to a greater extent by the body health, a psycho-emotional state during measurement, and gender, to a lesser extent by age. The reference limits of parameters *A(i/j)* for the degree of deviation from the norm have been established for each descriptive state, for instance: the low level of tiredness, the middle level of tiredness, the high level of tiredness, and the critical level of tiredness. Besides, the appearance of individual substances in the descriptive states can be evaluated, comparing values of parameters *A(i/j)* for 45 substances of normal and pathogenic metabolism with those for descriptive states. The ranges of parameters *A(i/j)* for identifying VOC classes and individual compounds are described in the works [25][26]. For an array of eight sensors, the maximum number of parameters *A(i/j)* is 28. Of these, the values of 21 parameters are statistically significant and reliably (according to Pearson's and Student's criteria) correlate with changes in clinical and laboratory test results or the verbal description of the state by volunteers. Namely, four parameters *A(i/j)* are associated with inflammatory processes, 4 – with malfunctions of the gastrointestinal tract (spasms of bile streams, slowed metabolism in the liver, pancreatitis, ketoacidosis), 3 – with changes in hormones or their metabolites (growth hormone, adrenaline, cortisol, sex hormones, hypothyroidism). Several parameters reflect descriptive conditions, for example, fatigue and its severity up to critical exhaustion (Table V).

For the convenience of deciding the health status of the organism, the state sphere is constructed using parameters *A(i/j)* in the software. This program is written in a high-level Java language as an Android application, designed to interact with the electronic nose device (Fig. 5) and based on previous our development [45][46]. Two algorithms have been developed to analyze received signals from an array of 8 sensors. The first allows getting the measurement results understandable to any user. Initial processing of the measurement results is carried out according to the maximum sensor responses. The body's health state decoding is displayed on the screen together with a complete set of calculated parameters in the form of a sphere (health status sphere), where each parameter corresponds to its sector (Fig. 6).

TABLE V. THE VALUE OF SOME PARAMETERS OF SORPTION *A(I/J)* IN VARIOUS STATES

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, cortisol, severe stress	-
A(1/2)	> 1.15	0.90 – 1.14 Inflammation, very hot	< 0.9 Alcohol, ketones	-
A(1/4)	-	-	<0.30 Sharp pain, inflammation in the organ (depends on the projection of which organ by Zakharyin-Ged zone was measured), weakness, exhaustion	0.38 – 0.42 Risk of diabetes, weakness, exhaustion of the body
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, high level of glucose, endocrine system disorders	-
A(2/6)	0.62 – 0.73	0.75 – 1.2 Weakness	1.3 – 1.5 Spasm in the gastrointestinal tract, pain	-
A(3/5)	5.3 – 3.2	3.0 – 1.6	1.5 – 1.7 Norm after meal, inflammation, hormones	-
A(4/5)	1.6 – 3.2	-	3.2 – 3.8 Ketones, problems with the pancreas	-
A(4/6)	< 4.5	-	4.8 – 5.2 Severe inflammation, stress, exhaustion, ketones	> 6.0 Stress, weakness, severe inflammation
A(4/8)	-	-	0.62 – 0.80 Problems with pancreas or stress and inflammation	-
A(5/6)	-	1.3-1.5 Hunger, fatigue, lack of sleep, stress	1.8-2.2 Hot, stress	-

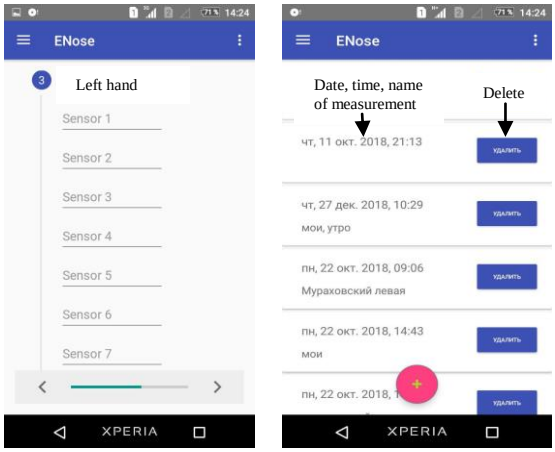
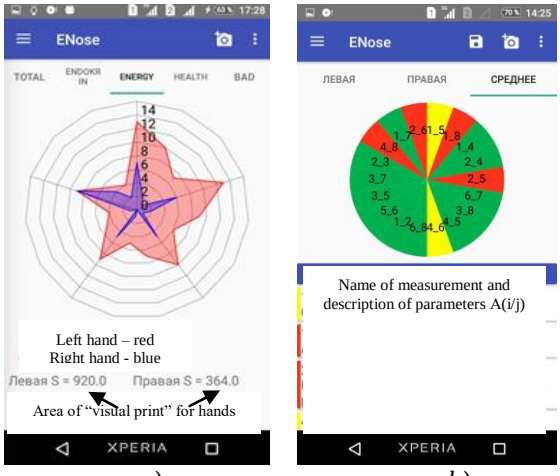


Figure 5. The dialoge windows of program to input of sensor responses and save the mesurements.

The sector's colors are determined by the numerical value of parameters in Table V. The comments about the body's health state or the volatile substances in the analyzed sample are displayed depending on the interval in which the calculated parameters fall. The complete data about measurements (entered and calculated) are saved to the database (on a personal device) (Fig. 5). The more green sectors in the health status sphere, the closer it is to norm. For instance, in Fig. 7 on the sphere of the state, 4 red and 2 yellow sectors are noted, which corresponds to many deviations from the norm: inflammation, disturbances in the functioning of the endocrine glands, a violation in the work of the excretory system. The value of parameters *A(2/6), A(4/8)* is in the red zone, which corresponds to the spasm and problems with the gastrointestinal tract or inflammation and the results of clinical and laboratory tests confirm inflammatory process in the digestive and respiratory tracts.



a) *b)*

Figure 6. The dialoge windows of program with text and graphical information and results of comparison for two measurent for left and right forearm (*a*) and for average measurement with norm (*b*) –green sector means that values of parameter included in diapazon for norm state, yellow, red color – deviation from norm.

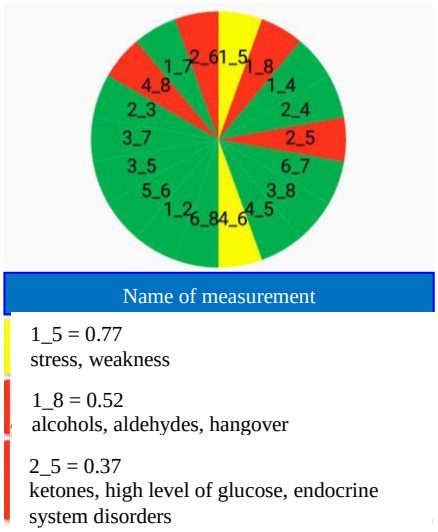


Figure 7. Example of the sphere for human health status on parameter *A(i/j)* in the dialogue window of the software.

In the example in Fig. 8, the presence of inflammation is noted, presumably of the gastrointestinal tract by the parameters *A(2/6), A(4/5), A(4/6)*, severe exhaustion and stress according to parameters *A(5/6), A(1/7)*. Parameter *A(3/5)* reflects the residual processes of food digestion. The doctor's conclusion by the examination of the volunteer with the special test was gastritis. According to the survey of this volunteer, the study was carried out after a snack due to a strong feeling of hunger. Although in the cases of monitored diseases, correlations were established between deviations in laboratory indicators and parameters of the sensor array, the study of the mechanisms of changes in the volatile metabolome in the process of biological adaptation in the case of specific diseases requires clarification.

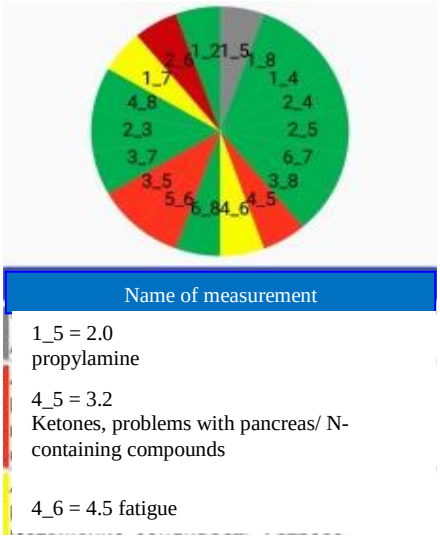


Figure 8. Example of the sphere for human health status on parameter *A(i/j)* in the dialogue window of the software.

Thus, according to the parameters of sensor signals, one can easily and quickly obtain comprehensive information about the deviation of the health status from the average norm.

The general algorithm for assessing the condition using a portable "electronic nose" consists of:

1) Measurement of the volatile metabolome of the forearm skin with an array of 8 sensors.

2) Processing of sensor chronograms – constructing of the "visual prints" and calculation of its area, parameters *A(i/j)*.

3) Comparison of the shape and areas of "visual prints" for the left and right hands according to the "General condition" algorithm

4) If there are deviations, we analyze the "visual prints" using other algorithms to determine the presumptive reason for the deviation.

5) Deviations identified by other prints are confirmed and refined by the parameters *A(i/j)* in the health status sphere.

6) If serious deviations are found (the presence of a disease, hormonal imbalance, inflammation), it is necessary to consult a specialist and take specific laboratory tests.

At the moment, according to the collected base of measurements, the sensitivity and specificity of determining descriptive states (fatigue, anxiety, stress, excitement, and others) is 98 %, clinical cases of the disease, including exacerbation of chronic illness, – up to 80%. The sensitivity of determining deviations in some blood test indicators (changes in hormonal balance, including during a monthly cycle in women, an increase in glucose levels) reaches 100%.

Thereby, using portable e-nose for scanning volatilome secreted by the skin allows determining the diverse variation in health status, including the descriptive states, the presence of individual volatile substances at ppm-level.

VI. Conclusion and Future Work

According to the responses of the electronic nose, when monitoring biosamples of nasal mucus from 17 calves during 5-9 hours, with an interval of 2-3 hours, the changes in the qualitative and quantitative composition of biosamples gas phase possible to assess atraumatic, on a place. The proposed parameter ΣF_{ref}. can be used to differentiate the diagnostic group of calves, identifying the animals that needed medical treatment.

For the first time, a fundamentally new approach is proposed for a quick assessment of the health status of the human body as a whole (normal, stress, inflammation) and the work of individual systems (reproductive, endocrine, digestive), based on the results of an assessment of the qualitative and quantitative composition of the gas mixture of biomolecules secreted by the skin in the forearm and the Zakharyin-Ged zone.

The correct interpretation and prediction of the status of biosamples and a person's state have been proved by different analyses (leukogram, biochemical, microbiological, molecular genetic analysis). The algorithms are proposed for reading and visualizing signals from an array of sensors understandable to any user.

The time of one analysis of volatile substances excreted by the biosamples or skin using portable e-nose, including visualization and processing, is up to 5 min that is faster than described in works [3][4][5][6], and without any sample preparation, unlike other modern research [3][8][9].

We believe that proposed in this work approach in the analysis by sensor array is appropriate for other biosamples, such as blood, cervical mucus, exhaled breath condensate, and urine.

In future works, we plan to expand the set of parameters when processing sensor chronograms to better specify the state of a person and differentiate states corresponding to one parameter value, such as stress, inflammation, and gastrointestinal problems. Investigation of idiosyncratic changes in humans and animals is one of the future work tasks. Also, we plan to improve the algorithm for deciding on the dominant reason for the deviation from the norm state to include it in the sensor data processing program.

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