Analysis of the volatile compounds' condensate exhaled air "electronic nose" based on piezoelectric sensor to assess the status of calves

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Abstract. The article discusses general principles of obtaining diagnostic information using eight chemical piezoelectric gas sensors with nanostructural coverings from exhaled breath condensate for health assessment of the upper respiratory tract of pre-month-old calves. Multidimensional information of an e-nose can be presented in various numeric and visualized matrices, characteristics of which are an integral analytical signal of the sensor array. The research devoted to the search for the techniques of extracting analytical information from multidimensional e-nose data to assess upper respiratory tract state from the appearance of the first sign of respiratory disease to tracheobronchitis and bronchopneumonia. The piezoelectric sensor array is characterized by high sorption activity with priority biomolecules (which are the markers of the abnormal metabolic processes), low cost along with reliable repeatability of sorption properties from batch to batch, the simplicity of application, and fast response and recovery time. Here we present the results of simple algorithms used for assessment of upper respiratory tract state by a 2-minute analysis of odour over 1-ml biosample without sample preparation. It was shown that traditional quantitative parameters of e-nose are not adequate for simultaneous sample grouping and volatile compounds qualitative composition establishing. The additionally calculated sorption parameters $A_{ij}$ are more informative in the analysis of biosamples volatile: using for identification of volatile biomarkers, describe the health state correlating with clinical diagnosis. The sequence of information processing: signals of each sensor, integral characteristic, “visual prints,” additional sorption parameters – allows assessing the calf health virtually in situ without transporting samples to specialized laboratories.

1. Introduction
The highly volatile metabolome of living organisms is one of the readily available and most informative units of bio-samples of both human and animals. Currently, the analytical practice offers several highly sensitive methods of quantitative definition of volatile organic and inorganic molecules. There are the methods of chromatography with various types of detectors, mass-spectrometry,
spectroscopy, gas sensors and their arrays in the artificial intellect systems – electronic noses (e-noses).

E-noses – artificial sensor systems consisting of chemical gas sensors of various number and combinations – have been widely used for detection and comparison of profiles of volatile compounds in different living samples. The progressive development of the theoretical foundations of sensory methods and implementation of analytical various sensor-based devices into different areas of analysis is directly linked to the development of electronics, big data processing algorithms, and chemometrics methods. It is getting more and more essential to align conditions of sensor array work to normal and to non-laboratory; the development of means of personal application, so-called «pocket lab», is becoming increasingly appealing. It became possible due to the wide application of e-noses, especially in the analysis of the food products and their production, as well as ecological monitoring.

In the contemporary animal husbandry, the problem of animal health control is considered to be of equal importance to the problem of early diagnostics into human diseases. On the farms the calves are especially vulnerable to respiratory diseases. Early detection of respiratory tract illness, disease stage assessment, the speed of disease development, and treatment path optimization are highly demanded in the contemporary animal husbandry [1].

2. Problem formulation
In clinical and laboratory medicine, interest to sensor systems has rapidly increased recently. To date, there have been accumulated many materials about informatively of various samples, their stability, and diagnostic reliability. The most popular objects of diagnostics are exhaled breath, exhaled breath condensate [1-4], urine [5-6], sweat, local mucus [7-9], and other biosamples [10].

It is not enough to solve the clustering tasks of volatile organic compounds (VOC) traces (prints) fixed by e-noses, assignment of a set of data to a particular group of objects is incredibly essential to reliably define the presence of significant biomolecules-markers of any particular pathological processes [11]. Exhaled breath and its condensate are the most popular subjects in e-nose research. It has been established that their content depends on the state of the upper respiratory tract and the diseases of the gastrointestinal tract [12].

According to e-nose signals in the exhaled breath or its concentrate, we can establish not only the problems with the upper respiratory tract but also health problems of the whole organism.

It is essential to select from digital sensor array data information about the general state of the body – such as «stress, exhaustion, weakness, inflammation», also to identify volatile compounds compositions, typical of both general states and exact diagnosis (diseases).

This work aims to assess informatively of simple algorithms for processing data from 8-piezo sensor array when analyzed exhaled breath condensate of calves in order to establish pathology of upper respiratory tract and severity of the disease.

3. The object of the study
The research objects were calves from one of the husbandries located in Voronezh region (Russia). The animals were observed for seven days; there were chosen two measurement control points: initial one (biosamples are marked as 1n-5n) and the second one accomplished in 6 days (marked as 1(2)-5(2)). After clinical examination, animals were divided into two groups: normal upper respiratory tract (n=2) and complicated, inflammatory processes of the trachea and lungs (n=3). By the second measurement control point, the health state of animals had changed; yet only one out of five calves was evaluated as relatively healthy. The sampling of exhaled breath condensate was done in each monitoring point from calves with fixing 19 clinical indicators (temperature, cough, functional probe with apnea; WI score, Hildebrant index and others).
4. Materials and methods

4.1 Specification of the E-nose

Exhaled breath condensate (EBC) of the calves was studied using developed odour analyzer «Diagnose-Bio-8» with e-nose methodology (Russia, Figure 1a) in the mode of “frontal analyte input” (VOC frontal spontaneous entry into the peri-sensory space in the closed detection cell). As a measuring array, we have chosen the set of piezoquarz BAW-type resonators with the basic oscillation frequency of 14,0MHz, 6 of which are covered by nano-structural sorbents and two ones with polymer films from «Living system» «LS©» set [13]. The chosen sensors possess high sensitivity to various classes of volatile organic compounds (alcohols, aldehydes, acids, ketones, amines, arenas) [13-14]:

- Sensors 1 and 8 – phases of carboxylated carbon nanotubes of different weights, marked in the tables and text asMCNT1, MCNT2.
- Sensors 2 and 7 – zirconium nitrate phases of different weights, Zr1, Zr2.
- Sensor 3 – dicyclohexane-18-Crown-6, DCH18C6.
- Sensors 4 and 5 – biohydroxyapatite phases of different weights, HA1, HA2.
- Sensor 6 – polyethylene glycol succinate, PEGsc.

The base lines of sensors in an array are stable (± 1 Hz) within all the period of active measuring (80 sec) (Figure 1b). Initial analytical information of e-nose device is chrono-frequency-grams– output curves of piezo sensors for the whole measurement time – dependence of oscillation frequency changes(-ΔF, Hz) of each sensor on time (Figure 1b from the software of device). Then selective chrono-frequency-Gramin formation is exploited in processing and is making the decision.

4.2 Sample preparation for the analysis

The samples of EBC were selected from 5 calves aged between 2 and 3 weeks old; frozen at the temperature of -20°C, after delivery to the laboratory samples were defrosted at room temperature and finally thermo-stabilized to 18°C. After defrosting EBC samples of 1 cm3 volume were selected by the sterile syringes and relocated to a sample substrate. When changes of oscillation frequency were reflected in the software of the device, the e-nose open cell was placed onto the sample. After completion of the measurement, the cell was relocated to a stand for spontaneous desorption of vapours from peri-sensor space and sensors.

![Figure 1](image-url)

**Figure 1.** Photo of the 8-sensor-nose “Diagnose-Bio-8” with the frontal input of vapours from samples (a) and chrono-frequency-grams of sensors in an array without loading, (b) for each own sensor colour.
4.3 Measurement regime
The total measuring time of VOC samples is 200 sec in “frontal analyte input” mode: the first 40 seconds is keeping sensors above the sample. In front of the frontal entry of volatile sample components into peri-sensor space, the period between 41st second and 200th second is spontaneous desorption of volatile compounds from sensors with an open detection cell without sample. Repeatability of responses is 3-5% for the least sensitive sensors up to 10% for most sensitive one (1st and eighth sensors respectively). Typical chrono-frequency-grams for EBC sample are shown in Figure 2.

4.4 Analytical information of electronic nose
Total analytical signal of the sensor array is formed as «visual print» of maximum signals using the integral algorithm of responses processing by eight sensors. To establish the total sample odor, we applied summary «visual prints» of maximum (Figure 2b), which are built on the maximum responses of sensors during loading (first 40 sec of measurement). This allows establishing significant similarities and differences of the volatile fraction content of odour over analyzed samples [13]. Areas of “visual prints” are calculated automatically in the device software.

For evaluating the differences in the odor of analyzed samples, there were chosen the following qualitative characteristics of VOC mixture content: 1) «visual print» shapes with the characteristic distributions along the response axes, which are defined by a set of compounds in a biosample. 2) $A_j$ identification parameters, calculated by the sensor signals in vapours of the analyzed samples, were applied to differentiate the separate compound classes in the mixture.

The quantitative response of the sensor array: 1) maximum sensor signals $\Delta F_{\text{max}}$, Hz – for assessment of the content of individual classes of organic compounds in the gas phase via normalization method, and 2) area of «visual print» of maximum, $S_{\text{max}}$, Hz$^2$ [13]. Additionally, out of the entire numeric matrix of sensor responses, device software extracted the most informative parts by fixating measurement time (masks – algorithms of fixating and selecting sensor responses at a particular set time – “light” (acetone, alcohols, aldehydes), “heavy” (acids, amines)).

The approach allows informatively increasing when answering the questions about the presence and content of the exact compounds in EBC samples. Such reduced “visual prints” were also calculated in the software – Slight, Sheavy, Hzs. The time point of measurement for masks searched during preliminary training of sensor array by 45 vapours of individual organic biomolecules of various classes, water, and ammonia. The sensor responses are fixed, processed and compared in the analyzer software «MAG Soft».

![Figure 2](image)

**Figure 2.** Analytical signals of sensor array: chrono-frequency-gram (a), processed and visualized traces of VOC (b, c) (from device software)
5. Discussion of the results

5.1 Samples ranking by direct analytical signals of electronic nose
Measurement of each EBC sample was done two times with a 5-minute interval (repeat). It was established that during that time, the content of volatile compounds in the sample decreases sharply, yet sensor responses are significantly different from ones for water (Table 1). EBC biosamples belong to unstable objects for which repeated measurements can significantly differ from the initial ones because of volatile compounds volatilization. The more compounds are in a sample, the greater the chances of sensor signals are for the repeated measurements. It was established that the content of the highly volatile compound (which the sensor array is configured to) is typical of all EBC samples. Since changes are not homogenous, it is complicated to select the most contrasting samples. That signifies that the preliminary ranking of calves according to clinical signs into groups was not reliable. The changes in the second monitoring point were much more informative.

While repeated sampling completed 7 days later, the samples of exhaled breath condensate show changes differently. According to the exchanges, samples could be divided into several groups:
- The 1st group includes EBC samples in which VOC content increases significantly by the second measurement point (in 6–7 days), which is proved by growth of signal values for each sensor and of integral signals from e-nose. One stand second calves belong to this group.
- In the 2nd group VOC content in EBC increase in significantly – third and fifth calves.
- The sample of the 4th half constitutes the 3rd group, the VOC content in its EBC decreased so insignificantly that it may be considered as constant.

The division into the groups becomes differential by various time masks applied for the extraction of useful information from chrono-frequency-grams, which show the contribution of the different VOC classes into sorption.

Table 1. Quantitative signals from the sensor array for EBC samples

| EBC samples (sampling date) | Sensor number in the array and $\Delta F_{\text{max}}$, Hz | Integral response of the sensor array |
|-----------------------------|-------------------------------------------------|--------------------------------------|
|                             | $1$     | $2$     | $3$     | $4$     | $5$     | $6$     | $7$     | $8$     | $S_{\text{max}},$ Hz$^2$ | $S_{\text{light}},$ Hz $\cdot$ s | $S_{\text{heavy}},$ Hz $\cdot$ s |
| Drinking water              | 59      | 35      | 32      | 113     | 128     | 16      | 28      | 141     | 12740                | 3533                | 2818                |
| 1n (16.1.2020)              | 121     | 65      | 65      | 213     | 240     | 34      | 54      | 296      | 49090               | 21325               | 4668                |
| 1n repeat                   | 101     | 57      | 51      | 169     | 200     | 26      | 47      | 250      | 33413               | 12000               | 3778                |
| 1(2) (22.1.2020)            | 136     | 73      | 72      | 239     | 275     | 38      | 62      | 333      | 62528               | 23616               | 8090                |
| 1(2) repeat                 | 119     | 65      | 67      | 197     | 247     | 33      | 54      | 288      | 47272               | 16628               | 4415                |
| 2n (16.1.2020)              | 100     | 55      | 50      | 177     | 206     | 28      | 48      | 250      | 34533               | 11925               | 6296                |
| 2n repeat                   | 72      | 38      | 35      | 123     | 157     | 19      | 34      | 179      | 17778               | 5534                | 1857                |
| 2(2) (23.1.2020)            | 133     | 72      | 69      | 230     | 271     | 37      | 60      | 321      | 59025               | 23129               | 5708                |
| 2(2) repeat                 | 106     | 57      | 54      | 166     | 216     | 27      | 49      | 264      | 36068               | 13717               | 3661                |
| 3n (16.1.2020)              | 141     | 79      | 82      | 258     | 274     | 38      | 61      | 338      | 67342               | 25876               | 8113                |
| 3n repeat                   | 99      | 55      | 53      | 164     | 207     | 27      | 46      | 245      | 33005               | 10489               | 1866                |
| 3(2) (23.1.2020)            | 141     | 78      | 83      | 261     | 182     | 39      | 63      | 341      | 69210               | 29727               | 8969                |
| 3(2) repeat                 | 101     | 56      | 54      | 169     | 212     | 26      | 47      | 248      | 34320               | 10775               | 2390                |
| 4n (18.1.2020)              | 147     | 78      | 83      | 256     | 280     | 41      | 64      | 344      | 69847               | 28670               | 8740                |
| 4n repeat                   | 114     | 64      | 66      | 198     | 237     | 32      | 52      | 275      | 44693               | 15621               | 4107                |
| 4(2) (24.1.2020)            | 122     | 69      | 64      | 221     | 263     | 37      | 56      | 303      | 53329               | 20793               | 7122                |
| 4(2) repeat                 | 91      | 48      | 44      | 150     | 196     | 26      | 42      | 223      | 27693               | 8872                | 2227                |
| 5n (18.1.2020)              | 103     | 55      | 47      | 167     | 212     | 30      | 48      | 262      | 34954               | 12628               | 3584                |
| 5n repeat                   | 83      | 44      | 39      | 143     | 176     | 24      | 38      | 208      | 23482               | 8387                | 3503                |
| 5(2) (24.1.2020)            | 101     | 58      | 56      | 185     | 210     | 25      | 43      | 243      | 35226               | 11289               | 6209                |
| 5(2) repeat                 | 84      | 48      | 44      | 142     | 184     | 22      | 40      | 215      | 24787               | 7806                | 3157                |
Thus, when increasing the total analytical signal of e-nose which interpreted as the worsening of the state, these change can be different as for biosamples of first and second calves (Table 2). Detailed analysis of the data showed that in 5-day time health state of calf 1 worsened more versatile (by several compound classes) than the state of calf 2. Where in significant changes in the state of calves 3 and 4 are not established. Within observation time, calf five state altered in the content of difficultly desorbed compounds, whereas according to the initial integral signal of sensor array state of its biosample was stable. Consequently, it proves low reliability of initial quality indicator of VOC content in biosamples – an area of “visual print.” Application of this indicator for long monitoring of animals’ state leads to an increase in false negative and false positive results in health status assessment.

5.2 Informatively of volatile organic compounds «visual prints» in the gas phase over biosamples

Visualization of numeric data obtained from sensors within different measurement time is the most informative way to present and process the results about biosample state. Resolving properties of an array of sensors is determined by their number and individual characteristics. On the one hand, the different affinity of individual sensors to VOC vapours provides detection selectivity; on the other hand, it leads to low sensitivity regarding target components and noise responses.

Resolving properties of an array of sensors is determined by their number and individual characteristics. On the one hand, the different affinity of individual sensors to VOC vapours provides detection selectivity; on the other hand, it leads to low sensitivity regarding target components and noise responses. The contribution of such sensors into the final analytical signal is ambiguous. If one sensor in the array fails, data correction in previous measurements is required. It is especially critical when analyzing living objects who second itinerant forms rapidly and irreversibly.

Let us analyze the possibilities of storing analytical information about biosample state with the help of the most informative sensors. For all sensors, we calculated average response: 70±1 Hz (Table 1) using the internal standard – water which is the main component of biosample matrices. Sensor signals which are two or more times less than this average response value were excluded from the integral signals of the array. To store information, we increased the number of points on chrono-frequency gram up to 7 ones, all of which was included in «visual print» (Figure 3).

Table 2. Relative changes of integral signals from sensor array for EBC samples in different points of sampling

| EBC samples (sampling date) | Relative changes in area, % | Data Analysis |
|-----------------------------|-----------------------------|---------------|
| Drinking water 1n (16.1.2020) | For $S_{\text{max}}$, Hz$^2$ 3 For $S_{\text{light}}$, Hz.s 3 For $S_{\text{heavy}}$, Hz.s 11 | Repeatability |
|                            | +27                         | Significant change in quality composition |
|                            | +11                         | |
| Characteristic 2n (16.1.2020) | Significant 71 Insignificant 94 Significant -9 | Quality composition change |
| Characteristic 3n (16.1.2020) | Significant 3 Insignificant 11 Significant 11 | |
| Characteristic 4n (18.1.2020) | Insignificant -24 Border line significance -28 Insignificant -19 | Without major physiological changes |
| Characteristic 5n (18.1.2020) | Significant 1 Insignificant -11 Significant 73 | Quality composition change |
| Characteristic 5(2) (24.1.2020) | Insignificant 1 Insignificant 1 Significant 73 | |
Figure 3. Comparison of changes in geometric parameters of «visual prints» of sensor signals in vapours of calves’ biosample taken in different control points and built on signals from 4 sensors. The red shape is the first control point, and the blue shape is the second one.

Such prints help to visualize the ratio between separate branches of Chrono-frequency-gram (sorption and desorption analytes), as well as to visualize the alterations in the content of the samples for various monitoring points. For example, in sample 2(2) the concentration of sorbing molecules significantly grew, which lead to an enlargement of part of sorption on VOC «visual print». While the component for desorption hardly changed, which corresponds with the calculations given in Table 2. For sample 3 VOC print based on signals from 4 sensors did not change; in the second control point of the 4th sample VOC prints are smaller; content of substances increased and their nature altered which triggered the growth of contribution from desorption branch of chrono-frequency-gram for calf’s 1 sample. Such data obtained from half of the sensor array allows adequately fixing the changes in the chemical composition of biosample in different monitoring points and also reduces the expenses for manufacturing a full array; however, the number of identified compounds will narrow.

5.3 Identification of EBC volatile compounds by sensor signals from e-nose
One of the most vital diagnostics tasks is the establishment of the presence and alteration of compound-markers concentration. In research, the pathologies of body organs and systems along with diagnosis are carried out based on a set of individual VOC. There is a wide range of compounds of some diseases and states, for instance: decan; nitrogen; nitrogen oxide; ammonia; ethyl methyl ketone; butyric, acetic and acetoacetic acids; dodecane, 2-ethyl hexanol, 6-methyl-5-hapten one acetone and many others. For identify individual compounds or even a whole class of similar compounds in biosamples, e-nose sensor array was trained on vapours of 45 individual biomarkers of normal and pathogenic states of tissues and organs. For each combination, we calculated sets of Aij identification parameters for the chosen sorption conditions. Analogous parameters were calculated for analyzed samples. In Table 3 there are given sets of identification parameters for two observation points, for the most typical samples 5, 4 and 1. By comparing parameters values for samples and compounds, we determined their most probable presence in the samples, both freshly taken from the sampler and after five minutes of contact with air. The changes in qualitative and quantitative EBC samples composition were established for samples of calves 3, 4 and 5; while the samples of calves 1 and 2 did not alter in qualitative VOC content.

| Sample | A12 | A13 | A27 | A28 | A35 | A37 | A47 | A56 | A57 | A78 |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1n     | 1.9 | 1.9 | 1.2 | 0.22| 0.27| 1.2 | 3.9 | 7.1 | 4.4 | 0.18|
| 1(2)   | 1.9 | 1.9 | 1.2 | 0.22| 0.26| 1.2 | 3.9 | 7.2 | 4.4 | 0.19|
| 4n     | 1.9 | 1.8 | 1.2 | 0.23| 0.30| 1.3 | 4.0 | 6.8 | 4.4 | 0.19|
| 4(2)   | 1.8 | 1.9 | 1.2 | 0.23| 0.24| 1.1 | 4.0 | 7.1 | 4.7 | 0.18|
| 5n     | 1.9 | 2.2 | 1.2 | 0.21| 0.22| 0.98| 3.5 | 7.1 | 4.4 | 0.18|
| 5(2)   | 1.7 | 1.8 | 1.4 | 0.24| 0.27| 1.3 | 4.3 | 8.4 | 4.9 | 0.18|
Especially significant alterations in the content are characteristic for EBC samples of calf 5 in different measuring points. For these samples, a significant change in the desorption is observed, which is connected to accumulation or emerging of amines, cyclic ketones, and acids. These compounds are the markers of various diseases of upper respiratory tract, moreover in sever stage of development or in an acute form of an illness. Let us compare the characteristics of calves’ biosamples: their health states, identified substances, clinical diagnosis in the final observation points (Table 4).

Condensate of exhaled breath concentrates volatile compounds of both native and pathogenic micro-flora in the respiratory system; biomarkers of both process changes and their intensity is a representative test to reflect the state of the nasopharynx, bronchi, and lungs. According to e-nose data on qualitative and quantitative changes of volatile organic compounds of condensate in exhaled breath in last monitoring point, calves 3 and 5 are in the worst health status which corresponds to an acute form of pneumonia, as well as inflammation of other organs (sample 5). Unobvious changes in VOC content is characteristic of the calf 1 samples. Also, according to the clinical signs, the health state of this animal is not critical. That is why VOC content alterations in EBC biosamples by the signals of the chosen sensors can be exploited as the reason for assessment of the state of the upper respiratory tract and in other animal organs; here with nature of identified compounds demonstrates the character of changes. The high concentration of cyclic amines, sulfur characterize Acute form of bronchopneumonia and nitrogen-containing aldehydes, acids; accompanying illnesses are shown in ethanediol and methylglyoxal accumulation, which is the markers of bile duct spasms, pancreas and gallbladder disorders.

Table 4. Comparison of calves’ health state according to sensor data and established diagnosis

| Animal number | Diagnosis of upper-respiratory tract | Additional disease | Assessment by E-nose data |
|---------------|-------------------------------------|-------------------|--------------------------|
| 4             | Healthy upper respiratory tract     | alkyl-, cyclic amines | Stable, mild stress |
| 1             | Bilateral chronic confluent catarrhal-purulent bronchopneumonia | Acute catarrhal cholecystis | ammonia, alkyl-, cyclic amines, pyridine-carbaldehyde, methoxyalkylamines | Stress, spasm |
| 2             | Bilateral chronic confluent catarrhal-purulent bronchopneumonia | Acute catarrhal bronchopneumonia | 1,2 - dimethyl cyclohexylamine, cyclohexylamine, methylalkylamines, aliphatic acidsC2-C5, methylglyoxal, ethanal, 1,2 - dimethylcyclohexylamine, cyclohexylamine, methylalkylamines, aliphatic acidsC2-C5, methylglyoxal, ethanal, aliphatic acids, pyridine-carbaldehyde | Weakness, severe inflammation |
| 3             | Bilateral acute confluent serous-catarrhal bronchopneumonia | Acute gallbladder in inflammation | 1,2 - dimethyl cyclohexylamine, cyclohexylamine, methylalkylamines, aliphatic acidsC2-C5, methylglyoxal, ethanal, aliphatic acids, pyridine-carbaldehyde | Weakness, exhaustion |
| 5             | Right-sided severe acute serous pneumonia | Acute gallbladder in inflammation | 1,2 - dimethyl cyclohexylamine, cyclohexylamine, methylalkylamines, aliphatic acidsC2-C5, methylglyoxal, ethanal, aliphatic acids, pyridine-carbaldehyde | Weakness, exhaustion |
6. Conclusion
VOC profile from calves’ EBC fixed by 4-8 piezoelectric sensor signals and applied in diagnostics of diverse respiratory diseases. The device itself and the absence of special sample analysis preparation make this method perspective for animals’ state monitoring on farms. The sensor array can be exploited for fixating, accumulating, collecting and storing information about the health state of calves and other animals, as well as for creating their electronic health cards.

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