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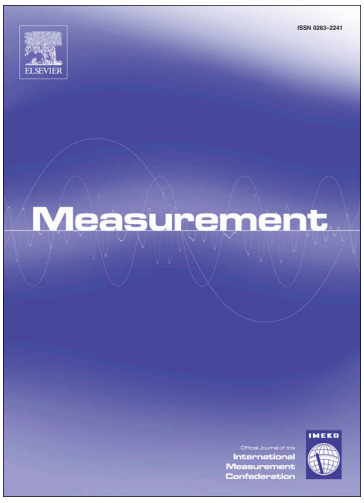

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PII: S0263-2241(22)00037-9
DOI: https://doi.org/10.1016/j.measurement.2022.110733
Reference: MEASUR 110733

To appear in: *Measurement*

Received Date: 3 October 2021
Revised Date: 25 December 2021
Accepted Date: 12 January 2022

Please cite this article as: J. Smulko, T. Chludziński, T. Majchrzak, A. Kwiatkowski, S. Borys, A. Lisset Jaimes-Mogollón, C. Manuel Durán-Acevedo, O. Geovanny Perez-Ortiz, R. Ionescu, Analysis of exhaled breath for dengue disease detection by low-cost electronic nose system, *Measurement* (2022), doi: https://doi.org/10.1016/j.measurement.2022.110733

Analysis of exhaled breath for dengue disease detection by low-cost electronic nose system

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Keywords: Electronic nose, Low cost gas sensors, Dengue disease, Exhaled breath analysis, Classification algorithms

Abstract

This paper presents a procedure and a set-up of an electronic nose system analyzing exhaled breath to detect the patients suffering from dengue – a mosquito-borne tropical disease. Low-power resistive gas sensors (MiCS-6814, TGS8100) were used to detect volatile organic compounds (VOCs) in the exhaled breath. The end-tidal phase of patients exhaled breath was collected with a BioVOC™ breath sampler. Two strategies were assessed for breath samples measurement: either direct transfer from the BioVOC™ into the sensors test chamber, or storage in Tenax TA sorbent tubes followed by VOCs release through thermal desorption and then transfer into sensors test chamber. DC sensor resistances were recorded and processed by multivariate classifier algorithms to detect infected patients. The experimental studies were run on a group of 26 individuals (16 dengue diagnosed patients and 10 control volunteers). The detection accuracy of dengue patients was over 90%.

1. Introduction

Exhaled breath intrigued medics for centuries. Its smell contains numerous volatile organic compounds (VOCs), whose analysis can provide a non-invasive tool to determine the health status. Breath composition was found to be characteristic to various diseases [1, 2] because it contains traces of the metabolic reactions taking place in the human body [3–5].

About two thousand VOCs were identified in the exhaled breath by analytical chemical tools and spectrometric methods [6, 7]. These methods identify VOCs at trace concentrations in the parts-per-million (ppm) or even parts-per-billion (ppb) range. Nevertheless, the analytical or spectroscopic techniques (e.g., Gas Chromatography-Mass Spectrometry, Ion Mobility Spectrometry) are time-consuming and require bulky and expensive laboratory equipment, limiting their use in clinical practice [4].

Inexpensive and portable systems utilizing commonly known resistance gas sensors are good candidates to detect the presence of selected VOCs in a much easier and affordable way. The e-nose technology (a technique that employs an array of cross-reactive gas sensors in order to enlarge the number of detected VOCs) applied to breath analysis was not implemented yet in clinical practice, but it can be used to systematically monitor changes in the exhaled breath for patients screening and preselection of those that should be directed to perform more accurate diagnosis tests. This is of high importance especially for the developing countries with limited health service resources affected by various spreading diseases.

There are numerous papers related to the implementation of e-noses in medical diagnosis. In the recent years many review articles summarizing these findings were published [8–11]. A general conclusion is that there is still a lack of cheap, easily accessible and, importantly, dedicated diagnostic devices, because most studies employed laboratory-conceived e-noses and prototype gas sensors, which face important drawbacks related with replication and life cycle, and limits the application of such devices in on-site settings. There are some recent works on new e-nose systems, such as portable Wolf e-nose [12], however there is still room for more adequate e-nose systems for disease diagnosis.

The present study is focused on Dengue disease, the most widely distributed mosquito-borne viral infection in humans. It is a common illness in the tropical and subtropical countries and it is affecting an estimated 100-400 million people worldwide each year [13]. Dengue causes a wide spectrum of disease manifestation. This can range from subclinical disease (people may not even know that they are infected) to severe flu-like symptoms in those infected. Although less common, some people develop severe or hemorrhagic dengue, which can present complications associated with severe bleeding and organ impairment and can even lead to death [14].

The symptoms of dengue are challenging to recognize from the common flu. Moreover, dengue is in most cases asymptomatic or mild and can pass unnoticed. For the severe form of dengue, the mortality rate reaches 20%, but it could be substantially decreased up to 1% when early diagnosed by a reliable diagnostic tool. The dengue symptoms could last for up to 10 days after a few days of incubation period, suggesting that there is time for dengue detection and effective medical treatment saving numerous lives.

Recently published research results confirmed that exhaled air analysis is a potential and fast method for early diagnosis of dengue. It was detected by an array of eight custom-made chemical gas sensors based of organically functionalized metal nanoparticles [15]. The results are optimistic but far from everyday use because of limited sensors availability. Additionally, sensors long-time stability and durability is not known. Thus, an alternative dengue detection method with low-cost and commonly available sensors is highly recommended.

Currently there is a series of newly issued, commercial metal oxide semiconductor (MOS) resistance gas sensors of reduced dimensions and power consumption [16–18]. These sensors are specific to various groups of gases and have been mainly developed for air quality monitoring, although they have

been successfully applied in breath analysis, too [12, 19]. Detection results gathered by such sensors are available within minutes after breath sample measurement, while the maintenance costs are relatively low.

The present study aimed to develop a low-cost e-nose device for dengue diagnosis that employed these recently developed MOS sensors, based on the silicon integrated circuits technology, which require very low power consumption and are of smaller dimensions, and a portable measurement set-up that used low-cost elements (e.g., microcontroller with common electronic digital I^2C interface, operational amplifiers). This strategy has higher potential for practical applications because of reduced costs and potentially increased detection effectiveness. A suitable breath collection procedure, a dedicated custom-made sensing system, and a data analysis method were developed to achieve this goal. A comparison between the analysis of the as-acquired breath samples immediately after collection (on-line measurements) and after an intermediate storage step in a sorbent material (off-line measurements) was furthermore performed.

The paper is organized as follows: Section 2 provides information about the volunteers included in this study, presents the developed e-nose measurement set-up, the procedure applied for breath samples collection and measurement, and gives an insight into sensors data analysis and the pattern recognition algorithms employed; Section 3 presents the results obtained; Section 4 discusses the main findings of this study; and finally, Section 5 summarizes the conclusions of this study.

2. Material and methods

2.1 Study population

This study was performed on volunteers recruited between 8th November and 14th December 2018 in the Erasmo Meoz hospital from Cucuta, Colombia. The experimental procedure applied in this study was approved by the ethical commission of Erasmo Meoz hospital, under protocol number 2015-136-014441-2.

In this study were included 26 volunteers aged between 18 and 54 years old. 16 of the volunteers were patients diagnosed with dengue disease that were hospitalized for at least 24 hours in the emergency unit of the Erasmo Meoz hospital. The control group was formed from 10 subjects selected among the medical staff or patients of the same hospital that were neither diagnosed with dengue nor presented any symptoms characteristic for tropical diseases, such as fever, muscle pain or vomit.

The volunteers were informed about the aim of this study and signed an informed consent before being included in the study. The subjects were transported on beds or came to the experiments room on their own. Each volunteer completed a questionnaire concerning health, addictions, occupational risks and pharmacological treatment. Their personal data were anonymized applying the K-anonymity anonymization technique. The detailed and anonymized data of the dengue diagnosed patients and control volunteers are separately presented in the Supplementary Information (Tables S1 and S2, respectively). To ensure the same breathing atmosphere for control patients as for the dengue-diagnosed patients, we collected the exhaled breath samples only from those who were hospitalized or stayed in hospital (medical staff) for at least one day, although this could affect the age balance between groups.

2.2 Measurement set-up

A modular e-nose set-up comprising two gas-sensitive modules was built for the purpose of this study (see Supplementary Information, Fig. S1). The detailed description of the applied modular set-up is available elsewhere [20]. The first module is MICS-6814 (SGX Sensortech, Neuchatel Switzerland) – a compact MOS sensors module with three independent sensors in a single housing, with tuned sensitivities for CO, NO_2 and NH_3 compounds, respectively, which present also good sensitivities to

several VOCs (e.g., C_2H_5OH, C_3H_8, C_4H_{10}) [21]. The second module is the TGS8100 (Figaro USA Inc., Arlington Heights USA) sensor dedicated to detect various VOCs (CH_4, C_4H_{10}, C_2H_5OH) as well as inorganic (CO, H_2) compounds. Both modules are characterized by small dimensions, and their total power consumption is as low as ~ 60 mW.

Each gas sensor was placed in the feedback loop of an operational amplifier operating in the current-to-voltage converter configuration. This set-up can adjust sensor polarization currents and the output voltage ranges within suitable limits for the accurate DC resistance measurement. A data acquisition board (National Instruments USB-6216) with 16-bit analogue-digital converter was employed to record voltage data. The voltage across the sensors, proportional to their DC resistance and polarization current, was sampled at 1 Hz sampling frequency.

The sensors were placed in a relatively small gas test chamber (~ 20 ml volume) made of aluminium for attenuating external electromagnetic interferences and for easy cleaning. The sensors were mounted to electronic boards attached to the metal test chamber by using silicon seals and metal screws. The system was equipped with an integrated environmental sensor BME280 (Bosch Sensortec, Reutlingen Germany) to concomitantly measure temperature, humidity, and pressure inside the test chamber during the measurements [22]. This information was necessary to identify if there were any environmental changes in the exposing environment that could affect the measurement results, to exclude the affected measurements from further consideration.

The measurement chamber was provided with electrical valves at both inlet and outlet openings. A micropump, mounted at the outlet opening, was used to suckle the breath sample coupled to the inlet opening inside the test chamber after opening the valves, by replacing with the breath sample the environmental room air introduced shortly before breath sample measurement in the test chamber. This solution was selected in order to reduce the impact of the changes in the room air atmosphere, as room air measurement was used as background measurement.

Further details of all sensors employed in the measurement setup are presented in Fig. S3 from Supplementary Information.

2.3 Breath samples collection and measurement

The hospital room where the experiments were performed was ventilated with a ventilator without opening the windows for limiting external interferences induced by sunshine variations or random air draughts.

Breath samples were taken from all volunteers between 6:00-10:00 AM after fasting overnight. A 129 mL Teflon made BioVOC™ breath sampler (Makers International Ltd, Llantrisant, UK) collected the final stage of patients breath. This procedure is very suitable for human breath analysis in research, because it gathers the last portion of breath, dominated by the alveolar air, which is the most reliable bearer of the VOCs carried by the blood to the lungs. These VOCs are related to metabolic reactions inside the body.

The breath samples were collected after 5 minutes of volunteer rest in a sitting or half-sitting position. The volunteers were asked to exhale their breath through the breath sampler until completely empting their lungs. A disposable mouthpiece was used for each volunteer to avoid their direct contact with the breath sampler. The breath samplers were cleaned after each use with 5% aqueous solution of sodium hypochlorite and naturally dried for 24 hours, therefore they could be used multiple times.

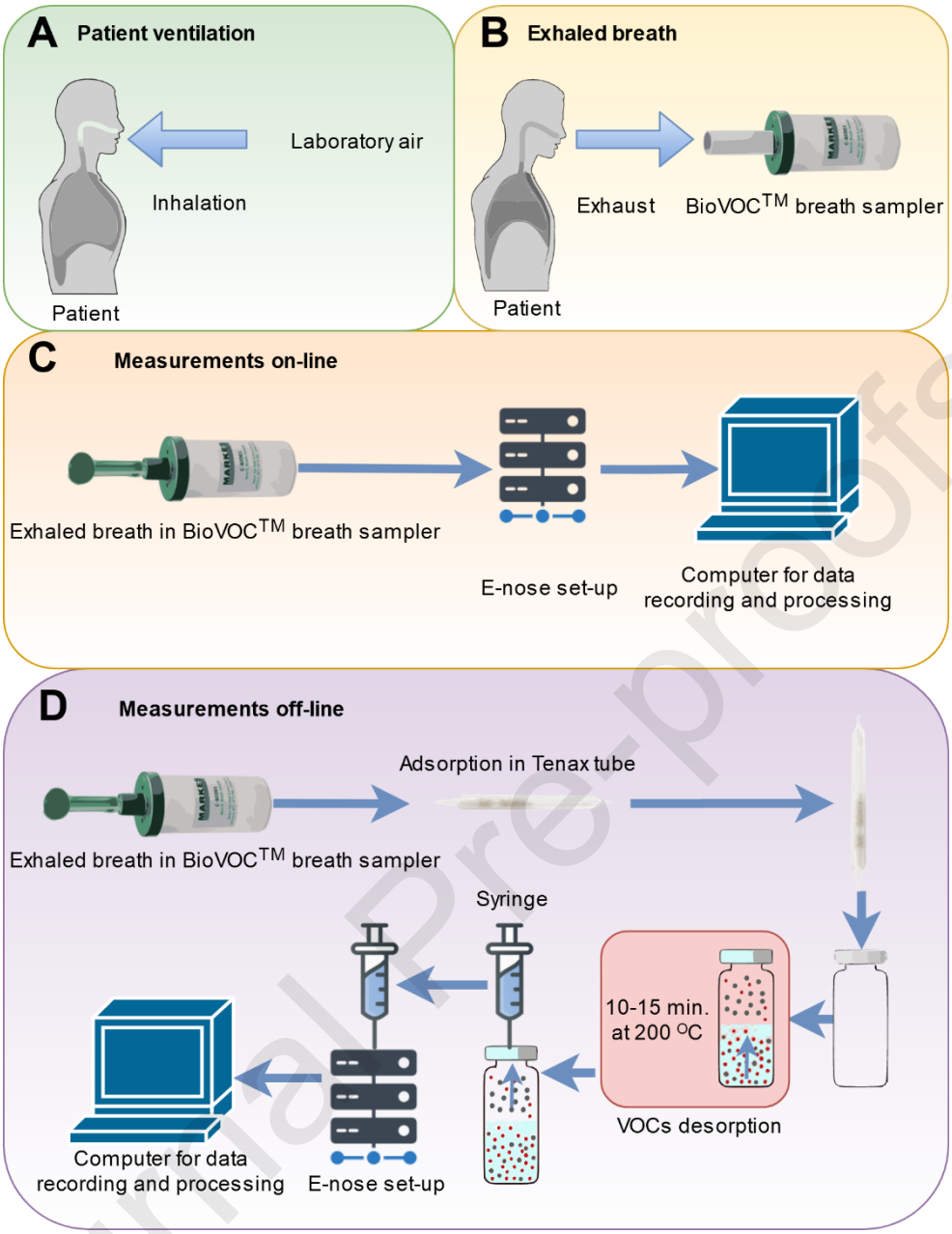


Figure 1. Schematic diagram of exhaled breath samples collection and analysis: Patient inhalation (A); Exhaled breath sampling in the BioVOC™ breath sampler (B); Immediate transfer of the breath sample from the BioVOC™ to the e-nose set-up (C); Breath sample transfer to a Tenax® TA sorbent tube and ulterior analysis with the e-nose system after VOCs desorption from the sorbent at 200 °C for 10-15 min. (D).

Two methods of breath sampling and analysis were assessed (Fig. 1). In the first one, the breath sample was directly transferred from the breath sampler into the gas chamber by means of a micropump, which was preferred instead of manually pushing the standard piston of the BioVOC™ breath sampler in order to achieve more repeatable samples injection conditions (Fig. 1C). This approach guarantees immediate point-of-care diagnostics. In the second method, the breath sample was transferred into a glass thermal sorption tube that contained a suitable sorbent material for breath VOCs preconcentration (Tenax® TA – hydrophobic porous sorbent polymer). The sample, stored in a glass vial (diameter: 8 mm, length: 110 mm; Fig. 1D), was preserved at low temperature (4 °C) for less than four months, and then analysed after desorption of the preserved VOCs. Before the analysis, the sorbent material from the Tenax® TA tube was placed inside a silicon sealed glass vial (20 mL volume) and heated at 200 °C for 10-15 min

for desorbing the preserved VOCs. The VOCs were captured from the headspace formed inside the sealed vial by means of a tight gas syringe (20 mL volume) and injected into sensors test chamber (Fig. 1D).

Following breath sample transfer into the gas chamber, this was closed using the mechanical valves, and breath measurement took place in a static mode. After each breath sample measurement, the gas chamber was purged by introducing room air inside it, whose measurement was then used to substract the baseline from the next breath measurement. This cleaning procedure is much more comfortable in clinical settings then using an external gas bottle of synthetic air or N_2 for cleaning, and increases the potential of popularizing this portable set-up procedure for breath analysis in real environments outside the laboratory conditions.

2.4 Sensor data analysis

The relative changes ($\Delta R_S/R_0$) of sensor DC resistances were considered as input data for data analysis. Denoting resistance before sample injection as R_0, the following parameters were extracted, represented in Fig. 2:

- **A:** Maximum value of the relative DC resistance change – $(\Delta R_S/R_0)_{max}$,
- **B:** Slope of the relative DC resistance change $\Delta R_1/R_0$ within time $\Delta T/2$ after breath sample injection, where ΔT represents the time necessary to reach 90% of the maximum value of the relative DC resistance change – $(\Delta R_1/R_0)/(\Delta T/2)$,
- **C:** Slope of the relative DC resistance change $\Delta R_2/R_0$ within the last $\Delta T/2$ time period after sample injection – $(\Delta R_2/R_0)/(\Delta T/2)$.

The above parameters were separately extracted for each one of the four employed gas sensors. When a sensor response (change of DC resistance) was too slow and did not stabilize in 700 s, the measurement was stopped, and the parameters were calculated considering the 700 s time point as a stable condition. The measurement time was limited to 700 s for practical reasons to avoid a long experimental time. Moreover, excessive measurement time may increase unavoidable drifts requiring more complicated data processing for their reduction [23, 24].

Data vectors created with these parameters were saved in an Excel file and used as input data for detection algorithms that were computed in Orange and Matlab software. The extracted parameters for all four sensors were normalized to get zero mean value and unity standard deviation before further processing.

Initially, the data of all subjects were presented as a heatmap. The analyzed sensors features were clustered based on hierarchical clustering (HCA) with Euclidean distance and average linkage [25], which provides a holistic representation of the breath prints by uncovering the sensor parameters with the highest impact in data classification.

Next, the random forest classification algorithm (RF) was used to make a binary classification between the dengue patients and the control volunteers. The RF algorithm creates a series of decision trees for randomly selected features [26, 27], and represents a proper solution for data classification that was successfully applied in medical diagnostics due to its low computational complexity [28–30]. The detailed principle of operation of this classifier can be found elsewhere [3]. For the aim of this study, the minimum number of trees was set to 20, as suggested elsewhere [29], by applying a script running under Orange software toolbox for Python [31].

Sensors data were additionally analyzed by applying the Classification Learner (CL) algorithms in Matlab software, in order to assess the efficiency of the arbitrarily selected RF algorithm by comparing its results with the results achieved by other available detection algorithms. The 23 classifiers available in the Matlab application were considered for this purpose. The accessible classifiers were characterized

by different computation speed, necessary memory usage and results interpretability. The detailed description of the applied classifiers is available elsewhere [32].

Given the limited number of patients available for this study, cross-validation statistics was employed for better assessing the classification accuracy between the dengue patients and the control volunteers given by the classification models. Five rounds of analysis were performed, where the data (separately for dengue diagnosed patients and healthy controls) were divided into two equal groups: one for learning (8 dengue patients and 5 controls) and one for model testing (8 dengue patients and 5 controls). Mean, median and maximum accuracy values were averaged over the five rounds of cross-validation.

3. Results

3.1 Sensor responses

Fig. 2 shows the plot of a representative sensor response to a breath sample. An abrupt drop of sensor's DC resistance was observed after breath sample injection, which after that got stabilized. In the specific case presented in this figure, the time necessary for the stabilization of sensor's DC resistance was below 500 s.

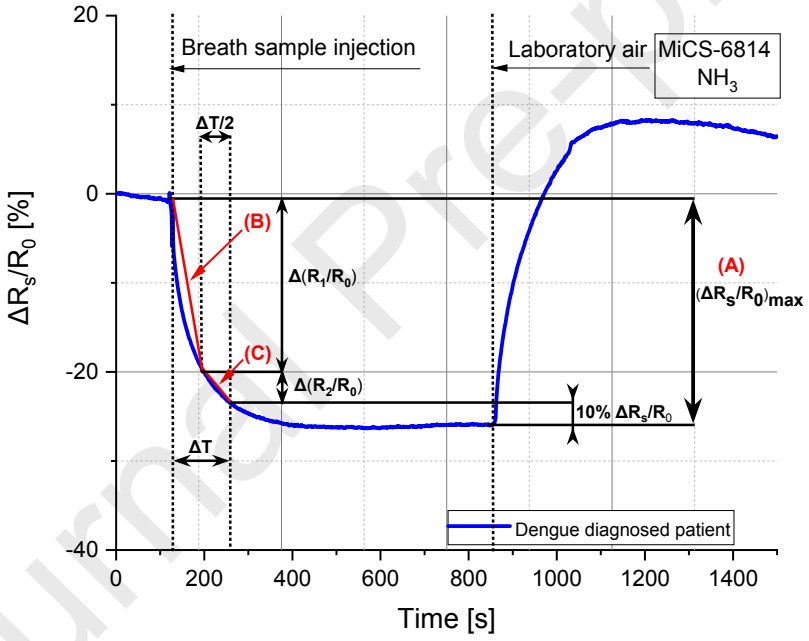
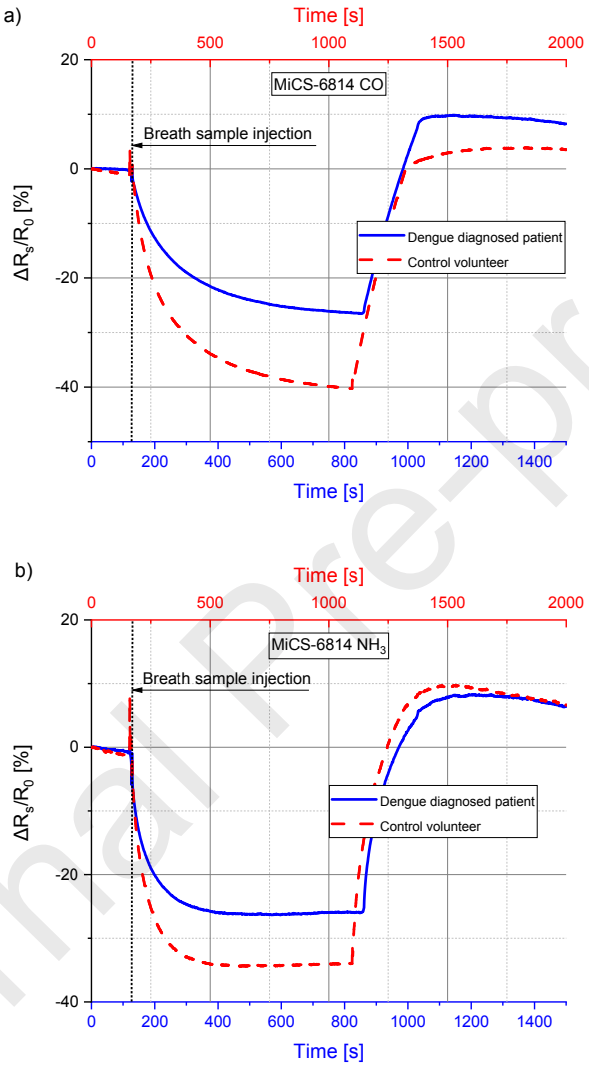


Figure 2. Illustration of the relative DC resistance change ($\Delta R_S/R_0$) for the MiCS-6814 NH_3 sensor after the injection of the breath sample (online measurement) of a dengue patient into the gas chamber (on-line measurement). The parameters used to calculate the three features extracted from sensor's response to this breath sample are represented in this graph.

Examples of relative DC resistance changes of all sensors after exposure to the breath sample of a dengue patient and a control volunteer are presented in Fig. 3, separately for each gas sensor employed. Some sensors displayed a relatively short response time and quick stabilization after breath exposures (Fig. 3b, MiCS-6814 NH_3 sensor; Fig. 3c, MiCS-6814 NO_2 sensor), while the responses of the other sensors did not stabilize during approximately 700 s (Fig. 3a, MiCS-6814 CO sensor; Fig. 3d, TGS8100 sensor). Clear differences could be observed between sensors responses to the breath samples of the two subjects. These differences were visible in both amplitude and response time, requiring the use of two different time scales on OX axis. Moreover, different times resulted necessarily for cleaning the sensors before

performing the subsequent measurement cycle, which is visible as the discrepancy observed between the sensor response $\Delta R_S/R_0$ at the starting time of 0 s and at the final measurement time of 1450 s or 2000 s (Fig. 3). The discrepancy decreased after continued cleaning to some unavoidable difference due to normal sensor time-drift or some permanently adsorbed molecules. This effect was reduced when analysing sensors responses by reporting to the resistance R_0 recorded at the beginning of the measurement cycle, just before injecting the breath sample.



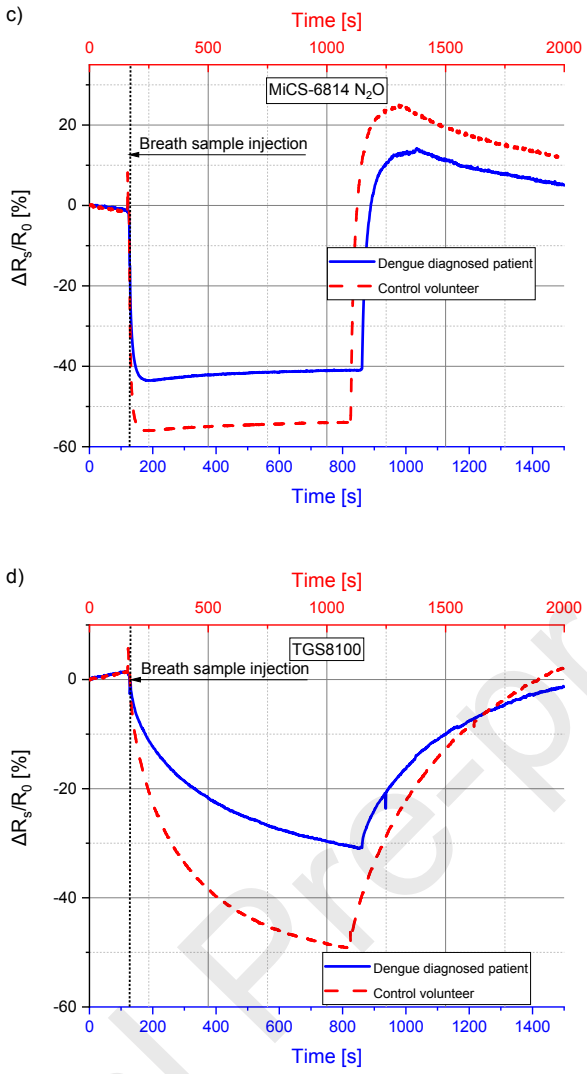


Figure 3. Illustration of relative DC resistances changes ($\Delta R_S/R_0$) observed after the introduction of exhaled breath samples (on-line measurement) of a dengue patient and a control volunteer into the gas chamber for the sensors: MICS-6814 CO (a), MICS-6814 NH_3 (b), MICS-6814 NO_2 (c), and TGS8100 (d).

3.2 Exposure conditions monitoring

Monitoring of the environmental conditions during breath samples exposure with the BME280 sensor allowed to observed how temperature, relative humidity and pressure changed in the gas chamber after each breath sample injection. The data for two representative cases of a control volunteer and a dengue patient are presented in Fig. 4. For both of them, similar and relatively tiny changes of the monitored conditions were observed after samples injection. The differences in temperature and relative humidity variations between the two subjects at the time point when sensor features were calculated were below 5% during the whole experimental study (see Figs. 4a and 4b), therefore it could be concluded that the impact of these influential environmental factors on gas sensor responses was neglected. Although the pressure in the test chamber slightly changed (Fig. 4c), this parameter does not have a critical impact on the gas sensing results.

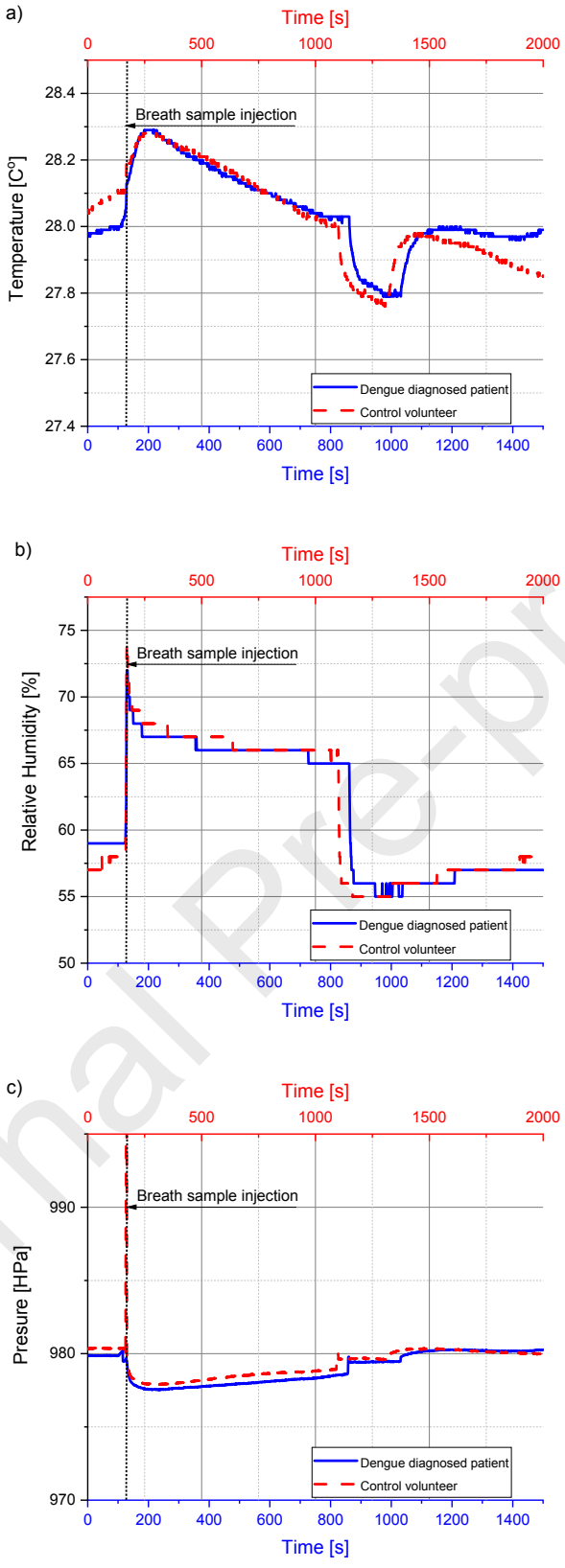


Figure 4. Environmental parameters recorded by the integrated BME280 sensor after breath sample injection (on-line measurement) of a dengue patient and a control volunteer into the gas chamber: temperature (a), relative humidity (b), and pressure (c).

3.3 Patients classification results

The heatmaps, presented in Fig. 5, display which sensor parameters were the most informative in terms of data variation. The most intense changes of the considered parameters were marked in red colour. It could be observed that in the case of the direct measurements (on-line analysis), the breath samples of dengue patients generated in general more intense sensor responses (higher normalized values) than the group of control volunteers. The highest responses were observed for the TGS8100, MICS-6814 CO and MICS-6814 NO_2 sensors, which formed a single, coherent cluster for parameter (A) extracted from sensor responses. In the case of parameters (B) and (C), their values did not differ enough to distinguish between the dengue patients and control volunteers respirations. For the off-line measurements, a separate cluster was formed for the MICS-6814 NH_3 and MICS-6814 CO sensors. These results suggest that the most suitable parameter for patients classification was related to the changes of sensor resistance induced by the breath samples (parameter (A)), which is robust to time drifts because it compares the resistance recorded shortly after the beginning of the measurement with its value few minutes before sample exposure. The other extracted parameters (B) and (C), based on the response time and the slope of relative DC resistance changes, did not provide similar good results.

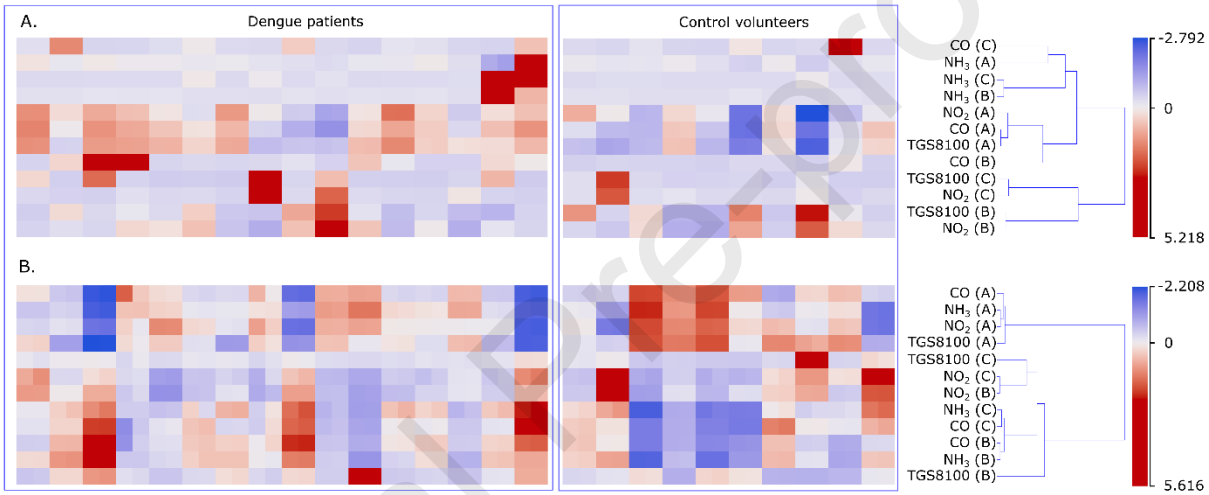


Figure 5. Heatmaps of the breath prints of the dengue patients (16 individuals) and control volunteers (10 individuals). Heatmap A represents the breath prints of the exhaled breath sample measurements performed on-line, and heatmap B shows the breath prints of the exhaled breath sample measurements performed off-line (see Fig. 1). The three different sensors available in the MICS-6814 module were marked as CO, NH_3 and NO_2. The clustering (right side) between the rows corresponds to the highest correlation between the presented parameters.

The RF algorithm achieved breath samples classification accuracies over 90% in both breath samples analysis approaches, specifically 96.2% for direct (on-line) samples analysis and 92.3% for sorbent tube (off-line) samples analysis, respectively. The classification results are indicated in the confusion matrices presented in Fig. 6. In both cases the sensitivity was 93.8%, while the specificity was 100% for direct samples analysis and 90% when the sorbent tubes were employed for samples storage, indicating 10% false positive results when the sorbent tubes were used. Nevertheless, when aiming to diagnose a viral diseases such as dengue, it is more convenient to obtain false positive results in the screening test and then to verify the result using a more advanced procedure than to overlook a potentially infected person.

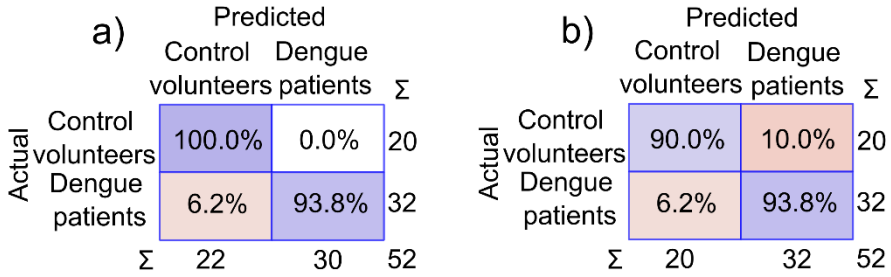


Figure 6. Confusion matrices of RF classification of the breath samples obtained from the dengue patients and the control volunteers for the measurements performed: on-line (a), off-line (b).

The *Bagged Trees* CL algorithm computed in Matlab (which is the equivalent of the RF algorithm computed in Orange) gave similar results with the RF algorithm, confirming the high accuracy of subjects classification achieved for both exhaled breath sample measurement procedures employed in this study. The detailed CL algorithms results are presented in Supplementary Information for on-line (Table S3) and off-line (Table S4) analyses, respectively. Although other of the CL classification algorithms provided better results than the RF algorithm, their computational costs and memory usage are much higher. Thus, the classifier RF (or Bagged Trees in Matlab) was selected as one of the most efficient for both on-line and off-line measurement sets of the considered data for dengue disease diagnosis.

5. Discussion

Breath samples classification into two groups of control volunteers and dengue patients was evaluated by examining their respiratory profiles with a custom made e-nose set-up based on four low cost commercial gas sensors.

The results obtained depended on the breath sample measurement procedure. Better results were observed in the on-line measurements, when the breath samples were measured immediately after collection by transferring them directly from the BioVOC™ breath sampler into the sensors test chamber. In this case, the breath prints demonstrated more intense sensor response signals in the dengue patients than in the control volunteers. The classification results provided by the RF algorithm were also better for the on-line breath samples measurement approach. This procedure is very convenient because it avoids the use of additional elements and intermediate steps for sample storage and measurement, and has the advantage of being easy to perform.

The same results were not observed when the exhaled breath samples were first preserved in Tenax® TA sorbent material and then thermally desorbed and transferred to the sensors test chamber. This was because the breath VOCs adsorption process in the Tenax® TA sorbent material has probably modified breath samples composition. While removing the humidity, the adsorbtion of some compounds, especially the polar ones, is very poor [33].

In order to counteract sensors drift over the measurements period, which is one of the most severe detrimental drawbacks of MOS sensors, sensor signals were pre-processed by computing the relative changes of sensor DC resistances after breath sample introduction into the sensors test chamber with regard to their resistances few moments before breath sample measurement. This approach is very robust to slow variations over time [34, 35]. We observed a slow trend of increasing DC resistance measured before each breath sample injection during consecutive measurement days because of sensors ageing. The change did not exceed 15% when approximated during the one-month long experiment. Therefore, we applied the above mentioned relative changes of sensor DC resistances to reduce this detrimental effect during detection process. Although other methods based on extreme learning machines can also

effectively reduce the effects of sensors drifts [23, 24], the pre-processing method applied in the present study is very simple and does not require complicated computing. Three independent parameters correlated with the exposure environment were then extracted from the pre-processed sensor responses, which provided independent information about the sensed samples that proved to be very useful for patients' classification.

Overall, the results obtained in this study indicate a remarkably high diagnostic potential of the proposed method for dengue diagnosis and underpin it for further consideration. Although the previous reported results on dengue diagnosis from exhaled breath analysis showed 100% accuracy on a population of 46 volunteers, those results were obtained with an array of hardly-reproducible custom-made chemical gas sensors based of organically functionalized metal nanoparticles, whereas the analysis was performed off-line [15]. The use of robust commercial sensors with reproducible features as in the present study, recently developed to minimize power consumption and to enable applications in portable devices for VOCs monitoring, is the main improvement introduced in this study as it opens the way to a more mature technology ready for on-site breath analysis for disease diagnosis. The development of similar commercial sensors with tuned sensitivity to the reported dengue breath biomarkers [15] (e.g., styrene, n-propyl acrylate, toluene, 1-undecyne, 1-acetyl-1H-benzotriazole and 2-ethylsulfonylethanol) could presumably further enhance the dengue diagnostic potential of the sensor system reported in the present study.

Moreover, by analysing dynamic sensors' responses to the injected breath samples and concomitantly recording the actual environmental conditions during the measurement (humidity, temperature, and pressure), corrections to sensors' responses introduced by the changes in the environmental conditions can be easily and conveniently applied. These features make the set-up reported in this study more robust for in situ applications with unavoidable variations in operating conditions, and the possibility to correlate and adjust the results recorded at different sites with different environmental conditions and/or different e-nose devices based on the same kind of sensors.

Nevertheless, it should be noted the limitation of our study in terms of relatively small number of samples and lack of long-term studies that are necessary to validate these initial results. In terms of age and race distribution, such an unbalanced group of subjects makes it impossible to verify whether these factors influenced the classification (e.g., there are age groups with only a few representatives). Even though this study was performed on a small population, it paves the way for popularizing the proposed approach and measurement set-up for dengue diagnosis. Gathering data produced by identical e-nose set-ups built with identical gas sensors would be moreover essential to assess the transfer of calibration between different e-nose systems without the need of specific calibration of each individual e-nose system with a separate dataset of samples. This could also be applied for the sensors drift removal, since one of the devices could act as the reference set-up, as was reported elsewhere [39].

We underline that the proposed e-nose system can be reproduced without a costly copy of mechanically complicated construction as proposed in the system utilizing similar commercial sensors but operating at higher temperatures in the gas chamber (40 °C) of lower volume (less than 3 mL) [17]. The alternative solution was dedicated to in-situ measurements of low volume breath samples. Our system examines the samples preserved in the sorbent of very low humidity and therefore can work at conditions closer to sensors operating temperatures, determined by their producers. Additionally, the greater volume of the breath samples preserved in the sorbent secured higher concentrations of the detected VOCs in the gas chamber during the measurements.

We believe that the proposed system can be enhanced by applying a more sensitive array of gas sensors, committed to detecting selected VOCs (e.g., for lung cancer detection [36] and monitoring its treatment [37]). The applied sensors were made of organically stabilized golden nanoparticles or single-walled carbon nanotubes capped with organic ligands [38] and operate at room temperature. These promising

sensors are not commercially available but are prosperous candidates for use in the presented e-nose system.

6. Conclusions

We present a proof of concept study that demonstrates the good potential of a relatively fast and inexpensive procedure for on-site dengue diagnosis based on exhaled breath analysis with a portable set-up that incorporated four commercial MOS gas sensors. The experimental studies were run on a group of patients diagnosed with dengue disease and a group of control volunteers. The end-tidal part of the exhaled breath was collected employing the BioVOC™ breath sampler and measured with a custom-designed e-nose set-up. Sensors responses were parametrized and analyzed with the RF classification algorithm of reasonable computational complexity, which yielded over 90% correct detection of the dengue patients within the examined group of 26 individuals. Better results were obtained when the breath samples were measured on-line immediately after collection by direct transfer into the sensors test chamber. The modified method of breath sample preservation in Tenax® TA sorbents and ulterior breath VOCs release by thermal desorption provided slightly lower results within the same group of subjects.

The developed e-nose set-up could work well under real-life conditions. Future campaigns on a larger group of patients are necessary to be performed for corroborating the promising initial results obtained in this study, employing double-blind tests to verify the diagnostic performance of our system. Thanks to its fast response, robustness and ease of implementation, the technique that we propose here could pave the way for the development of a reliable non-invasive method to diagnose dengue and other virus infections, which could be very useful for an efficient screening of epidemic outbreaks. The gas chamber was designed so that it can be easily enlarged to house additional sensors if necessary for tuning or enhancing the classification potential. Evidently, a greater number of selective gas sensors could help to effectively detect other virus infections manifested by hundreds of different VOCs in the exhaled breath. The present set-up, comprising four sensors from which multiple parameters not limited to DC resistance were extracted, was decisive for dengue detection. Some sensors were found sensitive to the VOCs, characteristics for dengue patients (TGS8100 sensitive to styrene and toluene) [15].

Author contributions

J.S. contributed sensors data analysis, manuscript writing and work coordination; T.Ch. contributed exhaled breath collection and measurements, set-up maintenance and use, sensors data analysis, figures preparation and manuscript writing; T.M. breath sampling, software scripts preparation and data results interpretation, figures preparation and manuscript writing; A.K. contributed measurement set-up design and practice; S.B. contributed to the results interpretation and manuscript writing; A.L.J-M and O.G.P-O. contributed coordination of patients recruitment and breath samples collection; R.I. contributed funds supporting and manuscript writing; C.M.D-A. contributed coordination of breath samples collection and sensing measurements.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financially supported in part by Statutory Funds, Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, by TROPSENSE project under the H2020-MSCA-RISE-2014 program, grant agreement number 645758, and by COMBIVET ERA Chair project under H2020-WIDESPREAD-2018-04 program, grant agreement number 857418. We dedicate this paper to the memory of prof. Jacek Namieśnik, who inspired us with research ideas during numerous discussions and supported financially the stay of T.M. in Cucuta, Colombia. We wish to address special thanks to the whole personnel of Erasmo Meoz hospital for their enthusiastic collaboration at volunteers recruitment.

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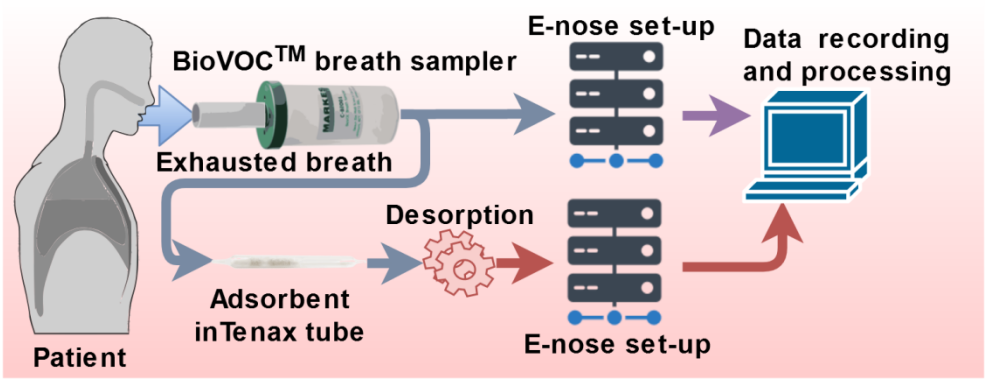

Highlights

- Commercial resistive gas sensors were applied to detect dengue disease.
- We developed a portable set-up to analyse exhaled breath samples.
- The data processing applied differential parameters to reduce drifts impact.
- The random forest classification algorithm in Orange software was utilized.

Author contributions

J.S. contributed sensors data analysis, manuscript writing and work coordination; T.Ch. contributed exhaled breath collection and measurements, set-up maintenance and use, sensors data analysis, figures preparation and manuscript writing; T.M. breath sampling, software scripts preparation and data results interpretation, figures preparation and manuscript writing; A.K. contributed measurement set-up design and practice; S.B. contributed to the results interpretation and manuscript writing; A.L.J-M and O.G.P-O. contributed coordination of patients recruitment and breath samples collection; R.I. contributed funds supporting and manuscript writing; C.M.D-A. contributed coordination of breath samples collection and sensing measurements.