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Bacteria Classification Using Electronic Nose for Diabetic Wound Monitoring

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Keywords: Electronic Nose, Bacteria Infection, Diabetic Foot, Principle Component Analysis (PCA), Linear Discriminant Analysis (LDA).

Abstract. Array based gas sensor technology namely Electronic Nose (E-nose) now offers the potential of a rapid and robust analytical approach to odor measurement for medical use. Wounds become infected when a microorganism which is bacteria from the environment or patient's body enters the open wound and multiply. The conventional method consumes more time to detect the bacteria growth. However, by using this E-Nose, the bacteria can be detected and classified according to their volatile organic compound (VOC) in shorter time. Readings were taken from headspace of samples by manually introducing the portable e-nose system into a special container that containing a volume of bacteria in suspension. The data will be processed by using statistical analysis which is Principle Component Analysis (PCA) and Linear Discriminant Analysis (LDA) methods. The most common bacteria in diabetic foot are Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa, and many more.

Introduction

In modern medicine, early detection and qualitative discriminant of bacteria present in infections is mostly important. Modern medicine faces the problem and challenge of achieving effective disease diagnoses through early detections of pathogenesis or disease conditions in order to facilitate the application of rapid treatments, but at the same time dramatically reducing the invasiveness of diagnostic treatments [1]. Smell used to be a common diagnostic tool in medicine, and physicians were trained to use their sense of smell during medical training. However, odor diagnostics have been relegated to secondary status as a diagnostic method. Array-based gas sensor technology now offers the potential of a robust analytical approach to odor measurement for medical use. The technology has been used to examine odors emitted from the body, such as from breath, wounds, and body fluids, and to identify possible problems, such as bacterial vaginosis [2,3].

Wounds are injuries to body tissues caused by diseases, processes, or events such as burns, punctures, chronic leg or decubitus ulcers, or as the result of surgery. Wounds become infected when micro-organisms from the environment or from the patient's body enter the open wound and multiply [3]. The symptoms related to an infection include abnormal flushing of the skin, heat, pain, tenderness, and abnormal odors, such as fruity odors that often indicate the presence of staphylococcus or foul odors due to the presence of gram-negative bacteria [2]. Standard techniques for microbiological detection are surface swabbing and wound biopsy culture. Surface swabbing is the most frequently used technique, mainly because it is quite inexpensive, but also because it is not invasive. However, swabbing can only assess surface infection, and it is very time consuming.

Biopsies are invasive and inconvenient [1,2]. The current methods for detecting infection take minimum two to three days before microbiological results are available. It consumes more time to determine which antibiotic or drug is appropriate. Moreover, patients suffer extended hospitalization and this will cause greater costs.

The term electronic nose (E-nose) describes an electronic system that is able to mimic the human sense of smell. E-nose system use a number of different gas sensors depending on application, e.g. metal oxide chemoresistors, conducting polymer chemoresistors, etc [5]. The E-nose has been developed to improve on and thus provide a better emulation of a human system for sensory analysis. Researchers are currently developing a new generation of artificial E-nose in order to build smaller and cheaper systems that will find application in the consumer marketplace [5].

Volatile organic compounds (VOCs), produced by bacteria as waste products or primary metabolites (e.g.,acetone, ethanol, or acetic acid), or as secondary metabolites(e.g., signaling molecules), may be produced in different quan-tities and combinations by each bacterial species or serovar, generating characteristic odors.

Proposed Methodology

PEN-3 Electric Nose. The PEN3 electronic nose from Airsense Analytics GmbH is a very compact instrument (255 × 190 × 92 mm), light-weight (2.1 kg) and portable olfactory system. It consists of an array of 10 different doped semi-conductive metal-oxide gas sensors (MOS) positioned into a very small chamber with a volume of only 1.8 mL [7]. The instrument operates with filtered, ambient air as a carrier-gas at a flow rate of 10-400 mL min-1,sample-chamber temperature of 0-45 °C, and sensor-array operating temperature of 200-500 °C [7]. The sensing reaction is based on an oxygen exchange between the volatile gas molecules and the metal coating material. Electrons are attracted to the loaded oxygen and result in decreases in sensor conductivity. Instrument sensitivity to various gas analytes ranges from 0.1-5.0 ppm.

Sample Preparation. The strain use in this study were Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa, which are the most common bacteria found on diabetic foot. Monocultures of all strains were cultured in standard petri dishes for 24 h at 37°C. In this experiment, the medium for cultured the bacteria is Muller Hinton Agar. 15 samples of each bacteria were used. The blank sample which is consisting of only medium without bacteria is used as a control for this experiment. For each batch it will be repeated for three days. Table 1 shows the number of samples used.

TABLE 1. The number of bacteria samples.

Sample per week	Staphylococcus aureus	Escherchia coli	Pseudomonas aeruginosa
Batch 1	5	5	5
Batch 2	5	5	5
Batch 3	5	5	5

Pre-Run Procedures, Data Collection and Statistical Analysis. The instrument was pre-warmed for 10 minutes at the beginning of each run session. A uniform run schedule was used for all samples based on the following run cycle: sensors cleaning, 170 s; sampling run time, 120 s. Data from the sensor array were collected at 0.5 s intervals and all data were averaged from 10 replications per sample. A conventional 10-s sampling interval between 30 and 40 sec into the run was utilized. The sample is located in the special designed container that has a small tube for connecting to the PEN3 device for 10 min for headspace generation. The headspace gas was pumped over the sensors at a carrier gas flow rate of 400 mL min-1and the run cycle was controlled by Winmuster 1.6.2.14 software (WMA Airsense Analytics GmbH).

Principal component analysis (PCA) and linear discriminant analysis (LDA) were performed by MATLAB software to discriminate between the different classes of samples. PCA allowed the extraction of useful information from the data and to explore their structure (including correlation

between variables and the relationship between the subjects), whereas LDA maximized the variance between the sample categories (aroma classes) and minimized the variance within the same category or class [8]. The diagram presented in Figure 1 is a representation of the experimental setup used for the experiment.

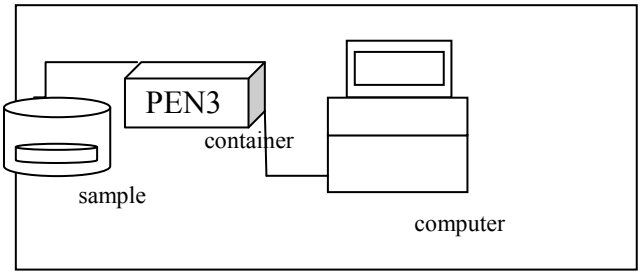

Figure 1: Experimental setup

Experimental Results

The PEN3 electronic nose instrument consists of 10 sensors array that are coated with semi conducting metal oxide is a MOS-type electronic nose. Metal oxide semiconductor sensors consist of three layers: a silicon semiconductor, a silicon oxide insulator and a catalytic metal through which the applied voltage creates an electric field [7]. The sorption of gas molecules provoke changes in conductivity brought about by combustion reactions with oxygen species on the surface of the metal oxide sensors. The transistor is used to record the modulation of the electric field when the polar compounds interact with the metal. Typical PEN3 sensor output graphs for representative types of bacteria are presented in Figure 2 (a) - (d). The graphs show comparisons between the typical response of ten sensors during measuring each type of bacteria and blank. In Figure 2, the data obtained are the changing ratio of conductivity between G and G_0 (the conductivity of the sensors when the sample gas or zero gas blows over). Each curve represents a different sensor transient. The curves represent sensor conductivity of one sensor of array against time due to electro-valve action when the volatiles from the bacteria reach the measurement chamber. In that transition, the clean airflow that reaches the measurement chamber is substituted by airflow that comes from the concentration chamber, closing a loop circuit between both chambers. It can be seen that after an initial period of low and stable conductivity (when only clean air is crossing the measurement chamber), conductivity increases sharply and then stabilizes after 30 s. The each sensor signal generally stabilizes and was considered to use in analysis of electronic nose. In this research, the signals of each sensor at response 30s to 40s were used in analysis of electronic nose. Different types of bacteria show the different sensor output. The shape of graph is different because each of the bacteria produces different odor and its volatile also different.

The capability of the PEN3 e-nose in distinguishing volatiles released from different types of bacteria were further analyzed using Principle Component Analysis (PCA) as indicated in Figure 3. Figure 3 shows the PCA volatile mapping of pseudomonas aeruginosa (red labels), escherchia coli (green labels), staphylococcus aureus (blue labels), and blank (purple labels) for (a) day 1, (b) day 2, (c) day, and (d) combination all day. PCA is a linear combinatorial method, which reduces the complexity of the data-set. The inherent structure of the data-set is preserved while its resulting variance is maximized [8]. The PCA analysis for day 1 indicates that the principle component 1 explained 62.55% and principle component 2 explained 33.27% of total variance with 95.82%. PCA result shows the early clustering of the different types of bacteria. For day 2 the total variance is 97.0% which is 63.67% for principle 1 and 33.13% for principle 2. Total variance for PCA analysis for day 3 is 96.46% respectively. The principle component 1 and principle 2 is 63.63% and 32.83%. The result of PCA analysis for combination of 3 days shows 63.87% for principle 1 and for principle 2 is 33.13%. The total variance is 97.0%. After analyzing these results using PCA analysis, it is found that the E-Nose can detect the different bacteria successfully. This can be observed from the certain bacteria grouping and clustering.

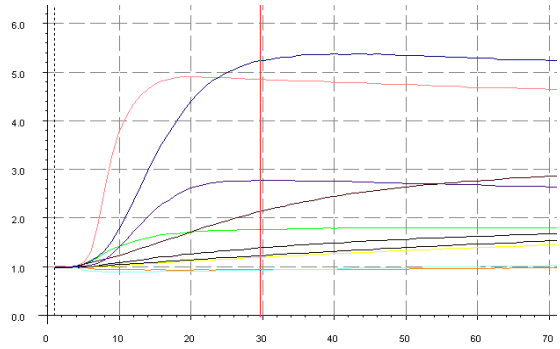

Figure 2 (a): Sensor output graph for pseudomonas aeruginosa

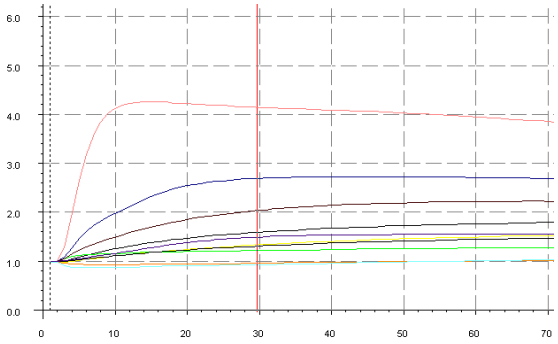

Figure 2 (b): Sensor output graph for aeruginosa staphylococcus aureus

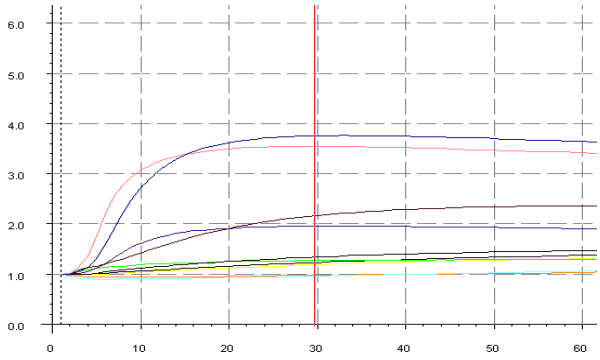

Figure 2 (c): Sensor output graph for escherchia coli

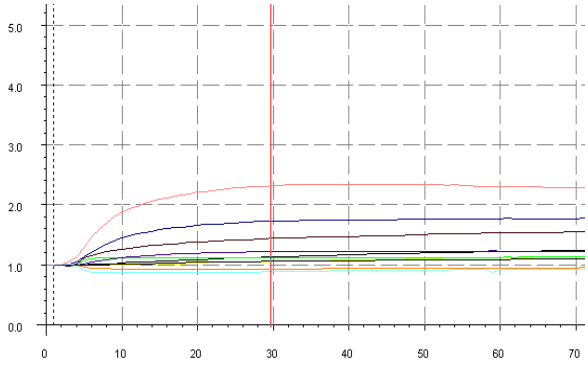

Figure 2 (d): Sensor output graph for blank

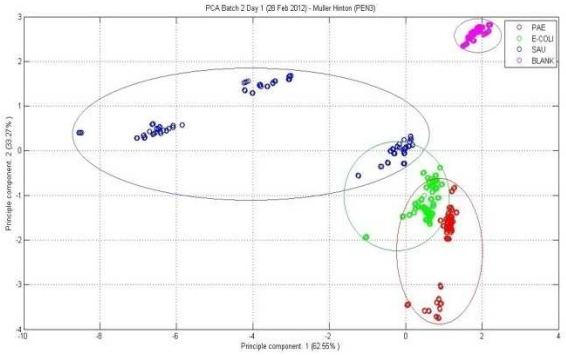

Figure 3 (a): PCA plot for day 1

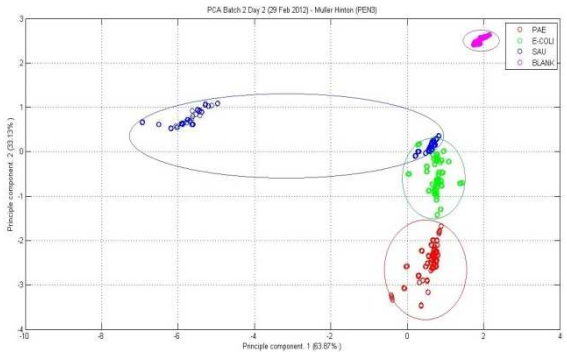

Figure 3 (b): PCA plot for day 2

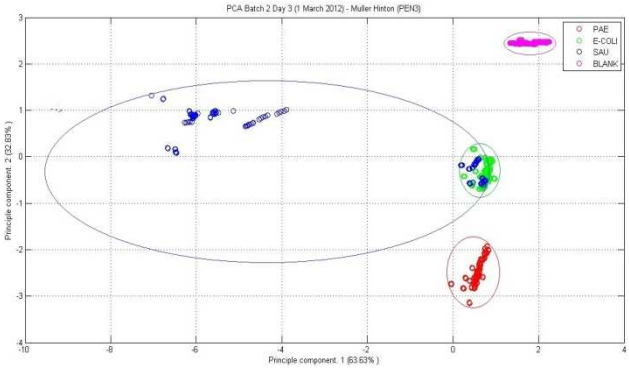

Figure 3 (c): PCA plot for day 3

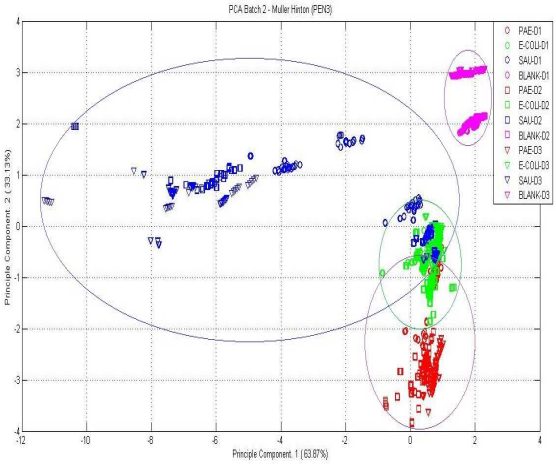

Figure 3 (d): PCA plot base on volatiles of different bacteria for different days

Then, the same set of data is analyzed by using Linear discrimanant analysis (LDA). Figure 4 determines the LDA plot for (a) day 1, (b) day 2, (c) day 3, and (d) combination of 3 different days.

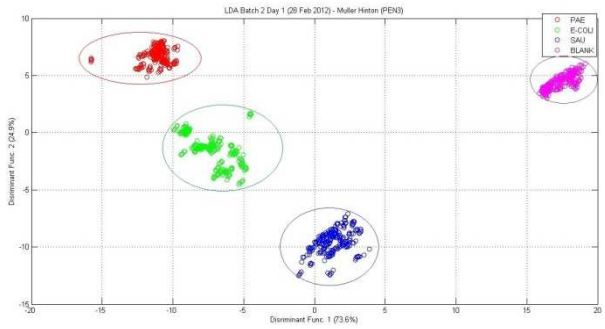

Figure 4 (a): LDA plot for day 1

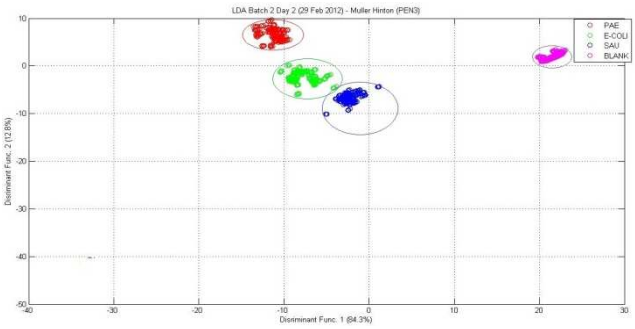

Figure 4 (b): LDA plot for day 2

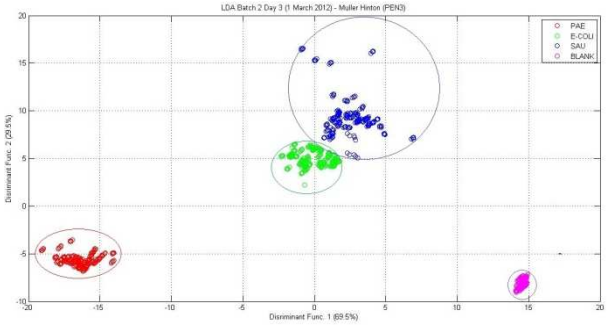

Figure 4 (c): LDA plot for day 3

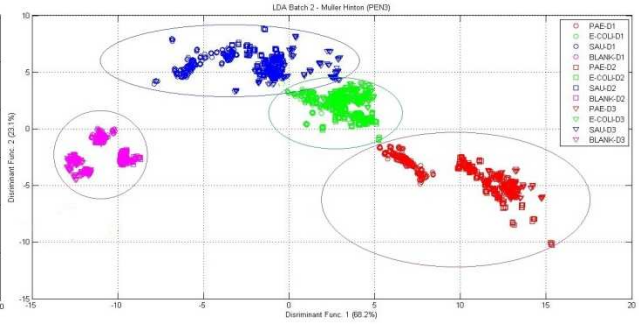

Figure 4 (d): LDA plot for combination of different day

By using Linear Discriminant Analysis (LDA), the result shows better clustering compared to the Principle Component Analysis (PCA). There is less number of overlapping between different groups for each different day. The total discriminant function for day 1 is 98.5%. LDA function 1 and function 2 accounted for 73.6% and 24.9% respectively. For the second day, the LDA function 1 show 84.3% meanwhile 12.8% for LDA function 2 of total 97.1%. The analysis LDA result for day 3 displayed that total variance function is 99% respectively which consists of 84.3% for LDA function 1 and 12.8% for LDA function 2. The LDA analysis for combination of 3 different days show that LDA function 1 displayed 68.2% and 23.1% for LDA function 2 of total of 91.3%.

Conclusion

The purpose of this experiment is to distinguish between different types of bacteria according to their volatile organic compound by using PEN3 electronic nose. The obtained result proved that the PEN3 electronic nose can detect and differ successfully the different types of bacteria for different days. The electronic nose was able to detect the difference in volatile produced by the bacteria using PCA analysis but it is not clear and has some overlapping of the group. It achieves a clear and better separation in all the cases using LDA analysis. Sensors 2, 6, 8 and 10 in bacteria have the highest influence in the current pattern profile. Hence, nearly a subset of few sensors can be chosen to explain all the variance. This result could be used in further studies to optimize the number of sensors and find the better performance. In the future, we will proceed with the experiment where the E-nose will be placed to the diabetic infected foot of patients and the measurement will be taken. The successful of this method can lead to in-house fabrication of the application specific e-nose that would be low in cost and highly tuned for bacteria species detection for diabetic patients. This experiment can be further replicated for extensive clinical trials throughout the selected hospital in Malaysia.

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