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Comparison of various pattern recognition techniques based on e-nose for identifying bacterial species in diabetic wound infections

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Abstract

This paper presents the diabetic foot ulcers bacteria species identification by using two different types of electronic nose (e-nose) which are PEN3 and Cyranose320. There are three types of bacteria that are commonly found in diabetic foot ulcer in a form of wild and standard American type culture collection bacteria which are *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus* (*S. aureus*) applied in this research. E-nose with array of gas sensors offers great potentials in the medical field via odor measurements. The detections of diabetic foot ulcers can be rapidly analyzed according to volatile organic compound (VOC) released by the bacteria. The conventional method of diabetic foot infection diagnostic exploit in the laboratory microbiological tests such as swabbing and biopsy culture usually consumes three days to attain the result are hectic and inconvenient. Hence, rapid detection of diabetic foot ulcer using e-nose could increase the effectiveness, efficiency, and reliability for further treatment purposes such as antibiotic treatments. Data collected from both e-noses are processed through various pattern recognition techniques such as linear discriminant analysis, K-nearest neighbor, probabilistic neural network, support vector machine, and radial basis

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function. Gas chromatography mass spectrometry is used to detect the VOC released from ulcer bacteria to validate the results collected from the e-noses.
Keywords: diabetic wound, electronic nose, pattern recognition techniques.

1 Introduction

Over the years, a number of diabetes mellitus patients is growing rapidly. In Malaysia, an alarming number of up to 600 thousand diabetes patients have been reported in the year of 2012 by the Ministry of Health and the numbers are still increasing [1]. Table 1 shows the number of diabetes mellitus patients in Malaysia upon April 2012. This chronic and dreadful disease has resulted in various complications to the patient where they would have higher chances in getting ulceration of foot. Diabetic foot ulcers typically arise on any foot of diabetic patient due to poor nerve signaling, improper fitting shoes, repetitive movement with stress and poor circulation, which often caused by harmful pressure on parts of the foot [2, 3]. Since diabetic mellitus causes some delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength, hence the wound will not be healed normally unless amputations are done to the ulcerated foot [4, 5]. In order to avoid chances of the diabetic foot ulcer getting worse during the conventional method of diagnostic diabetic foot ulcer which last for 3 days, rapid detection of diabetic infected foot should be used as a modern medical inspection method. This can be done by using an e-nose such as Cyranose320 or PEN3 where they have various arrays of gas sensors to detect odors released by the bacteria on the infected foot [6–8].

Table 1: Diabetic Mellitus Statistic in Malaysia upon April 2012 [1].

States	Number of Patient
Johor	81,013
Kedah	77,931
Kelantan	24,774
Melaka	31,427
Negeri Sembilan	39,393
Pahang	43,871
Perak	68,372
Perlis	10,338
Pulau Pinang	31,895
Sabah	9,205
Sarawak	64,848
Selangor	104,137
Terengganu	16,944
Kuala Lumpur	23,728
Labuan	535
Total	**628,411**

Gas sensors or transducers that usually exist in the e-nose including quartz crystal micro balances, surface acoustic wave sensors (SAWs), metal oxide semiconductor sensors (MOSs), metal oxide field effect transistors, conducting organic polymers, and mass spectrometry [9, 10]. E-nose mimic olfactory system in human to detect odors and classify them as a smell. Various sensors will detect the specific odor and each of them will have different selectivity patterns and certain threshold to indicate and produce data with information on the odors that e-nose detected. Comparing modern real time e-nose detection method of diabetic infected foot with conventional method which are swabbed and biopsy culture, modern detection clearly provides easy approach without hectic procedures and shorter duration where the chances of worsen in foot ulcerations could be minimized [11].

Furthermore, the swabbing method might assess to further unwanted infection and cause damage to healing tissues. Biopsy methods on the other hand are invasive, inappropriate, and inconvenience to be used as the detection method. Bacteria that usually found on diabetic foot ulcers are the Proteus mirabilis, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter spp*, *Beta-hemo streptococcus*, and *Klebsiellapneumoniae*. E-nose is able to detect the odors release from a particular bacteria and the statistical result obtain from e-nose could be used in the supervised artificial neural network (ANN) such as radial basis function (RBF) and support vector machine (SVM) to gain information, or data regarding classifications of the odors regardless the information that provided by the e-nose sensors. Aroma technique in obtaining data information has proven to offer positive feedback and progressively develop in various industries such as medical pathology, chemicals detections, food industries, and agriculture plant productions. The increasing development over the years in the e-nose shows that aroma based technique is widely acceptable to support certain industries in detecting odors [6, 12].

2 Discussed problems

The detection of diabetic foot ulcer among diabetes mellitus patients should be in rapid method to avoid any further complications, infections, damage, and spreading of the bacteria in the foot ulcer. Conventional method of diagnosis diabetic foot ulcer through laboratory microbiological investigations such as swabbing and biopsy culture normally require three days, and the procedures are considered hectic and inconvenient. Besides, the time consuming method could worsen the wound in a glance of time. Hence, rapid detection of diabetic foot ulcer using e-nose potentially increases the effectiveness, efficiency, reliability, and time that can be used for further treatment purposes such as antibiotic treatments. Appropriate on time treatment can absolutely reduce the chances of diabetic foot ulcer patient to undergo amputation, and detection of diabetic foot ulcer could be less sophisticated with further reduced procedures.

2.1 E-nose

Since the year 1982, measuring of a smell or flavor is possible without human olfactory system after the intelligent and magnificent creation of e-nose. The human olfactory system has the function to detect and recognize fragrance, odor or aroma which is further interpreted by the brain as a certain specific smell. By using the concept of the human olfactory system, e-nose has engineered to obtain the ability to detect, distinguish, analyze, and classify complex and simple odors or vapors by detecting the aromatic VOC. Due to the high vapor pressure and low boiling point, VOC would easily be released and evaporated as a vapor and odors as communications or a chemical defense between microorganism [13, 14].The aroma or odors that released would be detected by the various types of sensors in the e-nose. Those array sensors are capable of detecting certain VOC as they are only sensitive in particular VOC. E-nose acts as a useful diagnostic tool in rapid detection of odors, detection of microbial species, detection of harmful and dangerous chemicals, as well as odors that unlikely to be detected by the human olfactory system due to certain threshold concentration. Unlike other analytical instruments, e-nose allows the identification of mixtures of organic samples as a whole where it is identifiable to a source that released the mixture without having to identify individual chemical species within the sample mixture [15, 16]. An e-nose system typically consists of three main components which are the multisensor array, an information-processing feature extraction unit, and odors classification unit that sequentially operate. Those components complete the intended function of e-nose in detecting odors.

The multisensor array contains an array of sensors with broad sensitivities that provide dynamic responses of the interaction between an odor sample and the sensing elements [17, 18]. The first developed sensor array was a MOS, which could be used to detect 20 odors [9, 19, 20]. Up until now, there is e-nose with 32 sensors that are widely and commercially available, which are made up from various types of materials with the abilities to detect and process up to thousand types of odors. There are few types of transducers available in the e-nose. Each of these sensors has different selectivity and sensitivity threshold patterns where it could yield a unique "odor signature" for the VOC in the headspace of each sample under test [21, 22].

Information-processing feature extraction unit could be used to apply for several purposes such as to process the information obtained from the array sensors, eliminate the interfering environment factor toward the array sensors, and preclassify the recorded data [3, 23, 24]. Some of the information processing unit that are frequently used are the linear discriminant analysis (LDA). As for classification unit, pattern analysis constitutes a really important building block in the development of gas sensor instruments given its ability of detecting, identifying, and measuring volatile compounds [18, 25]. ANN could be used in pattern classification of the data obtained [26–28].

3 Proposed methodology

The flow chart of methodology process is as Figure 1. The main processes of this experiment are:

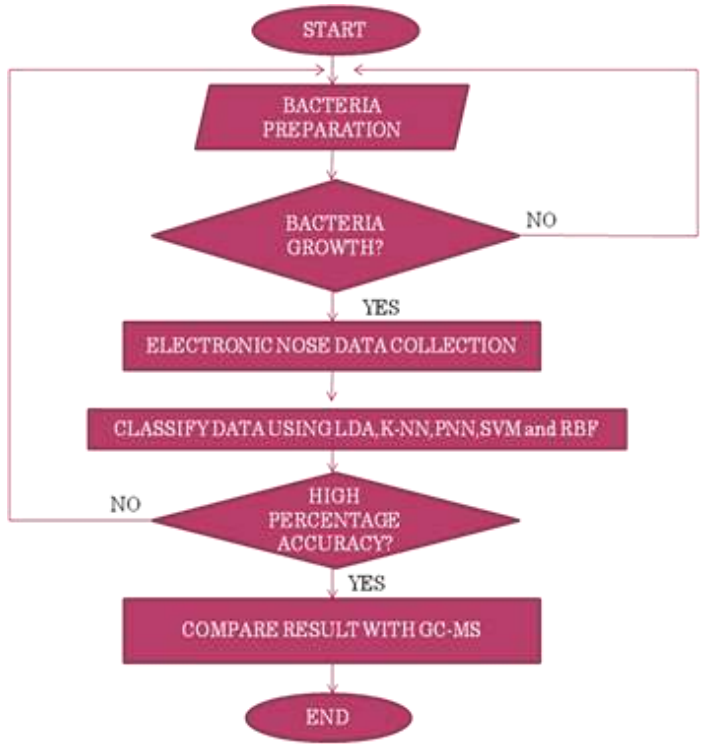

Figure 1: Methodology flow chart.

1. Bacteria sample preparations
2. Data collections using e-nose (PEN3 and Cyranose 320)
3. Data analysis using LDA, K-nearest neighbor (KNN), probabilistic neural network (PNN), SVM, and RBF
4. Result comparison between e-nose and GC-MS

3.1 Bacteria sample preparation

The bacteria sample preparations are done in both wild and standard American type culture collection (ATCC) bacteria for *E. coli*, *S. aureus*, and *P. aeruginosa*. Wild bacteria are the bacteria that are directly found on diabetic foot ulcer of diabetic mellitus patients while standard ATCC bacteria is the bacteria from purified DNA microbial strain isolated under aseptic conditions for cultured and grown in an optimum environment of the laboratory. Both standard of ATCC and wild bacteria of *E. coli*, *S. aureus*, and *P. aeruginosa* have different steps in sample preparations. Table 2 shows the number of data collected bacteria samples per batch in blood agar medium for wild and standard ATCC bacteria. In this experiment, two batches of bacteria samples are prepared and cultured in different weeks for data collections.

Table 2: Number of Bacteria Sample in a Blood Agar Medium.

Sample Per Week	Standard ATCC Bacteria			Wild Bacteria		
	E. coli	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. aureus*	*P. aeruginosa*
Batch 1	6	6	6	6	6	6
Batch 2	6	6	6	6	6	6

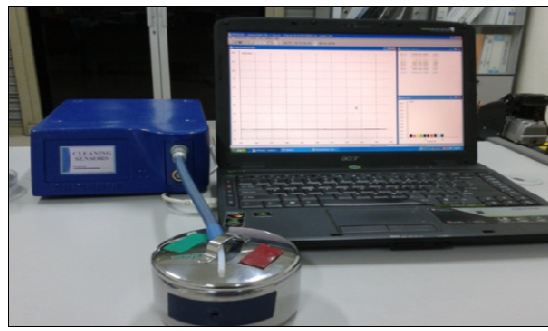

Figure 2: PEN3 equipment setting for data collections.

3.2 Data collection using PEN3 and Cyranose320

As for data collections, e-nose is used to collect data of VOC released by each bacterium at the interval time of 6 h for six times. Six Petri dishes for each bacteria is divided into two groups of samples where the first group of sample is used for time interval of 6th, 18th, and 30th hour while the second group is used for time interval of 12th, 24th, and 36th hour. This is important to ensure that the bacteria could perfectly grow in the incubator. For each data collection on a specific time interval, three Petri dishes are used for each bacterium where five data collections are done on each Petri dish.

The experiment setup during data collections using Airsense PEN3 required a laptop, RS232 cable, PEN3 silicon tube, and a container to store sample. Bacteria sample is placed inside the fully covered container so that bacteria odors are not released into the surrounding air. PEN3 silicon tube on the other hand is connected in between container and PEN3 and Cable RS232 is used to connect the laptop to PEN3. Figure 2 shows the equipments setting for data collections.

The measurement parameters along with software WinMuster V 1.6.2.14 as shown in Table 3 is used for data collection. Since data collection is done in time interval of 0.5 s for 60 s, hence each data collection from PEN3 produced 120 numerical data for each sensor in the form of an Excel file. From the Excel file obtained, only the last 20 data which is from 50 to 60 s of data collected is used for data analysis. For each data collection of one type of bacteria with three Petri dishes in specific 6-h time interval, there are 300 data collected for each PEN3 sensor.

Table 3: The PEN3 Parameter Setting for Bacteria Assessment.

Cycle	Time (s)	Pump Speed
Sensors cleaning	170	400 ml/min
Measurement time	60	400 ml/min
Flush time	85	400 ml/min
Baseline time	10	400 ml/min
Time interval	0.5	—

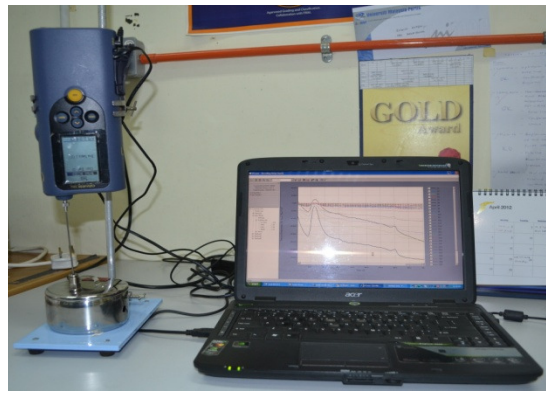

Figure 3: Cyranose320 equipment setting for data collections.

Table 4: The Cyranose320 Parameter Setting for Bacteria Assessment.

Cycle	Time (s)	Pump speed
Baseline purges	10	120 mL/min
Sample draws	60	120 mL/min
Idle time	0.5	—
Air intake purges	40	160 mL/min

The experiment setup (as shown in Table 4) during data collections using Cyranose320 required a laptop, RS232 cable, and a container to store sample. Bacteria sample is placed inside the fully covered container so that bacteria odors are not released into the surrounding air. The container is connected to Cyranose320 for data collections and Cable RS232 is used to connect laptop to Cyranose320. Cyranose320 is used along with PcNose software. The e-nose was set to be 37 °C as that is the optimum temperature for bacteria growth. Figure 3 illustrates the equipments setting for data collections using Cyranose320.

3.3 Data analysis using various pattern recognition techniques

For the data analysis, SPSS 17.0 is used for feature extractions while for determining the classification performance, Matlab R2012a is used. As for feature extractions, LDA is used and the discriminant values produced is used for clustering purposes and further classification analyses including in graphical form are implemented using Matlab R2012a. KNN, PNN, SVM, and RBF are applied for assessing the accuracy of classification results.

4 Experimental results and analysis

Results in a form of graphs are shown for visual classifications and the classification accuracy in numerical and percentages which are produced by using the above mentioned feature extractions (linear discriminant function), and classification techniques such as KNN and ANN (PNN, SVM, and RBF) are elaborated. Both wild and standard ATCC bacteria for *E. coli*, *S. aureus* and *P. aeruginosa* are analyzed under the same time variant which is every 6 h to study the odors classifications between wild and standard ATCC bacteria. Then, GC-MS results are shown to compare the results obtained by e-nose.

4.1 Analysis results for PEN3

This section discusses regarding PEN3 data analysis for 6th, 12th, 18th, 24th, 30th, and 36th hour. By using LDA, feature extractions result in the form of the graphs are shown in Figure 4(a)–(f). From the 6th and 24th hours of bacteria growth result obtained, it is clear that bacteria are fully classified into corresponding groups even in the first 6 h of evolution. The blank blood agar (without bacteria) also can be separated into its corresponding classification groups, which indicate the differences of bacteria odors or VOC released by the respective bacteria.

4.2 Analysis results for Cyranose320

Apart from PEN3 analyses, Cyranose320 is also being applied in this research to investigate the performance and accuracy of the result attained. Hence, data analysis was collected for 6th, 12th, 18th, 24th, 30th, and the 36th hour as presented in visual graphs with color indicator. Analyses were done using LDA as the feature extraction for classifications of bacteria as presented in Figure 5(a)–(f).

Based on the results obtained, the analyses shows different grouping of bacteria species can be classified into different regions in the early 6 h of bacteria growth. These findings show that accurate classification of bacterial species can be obtained even at the first 6 h of bacteria growth. This somewhat proved that the particular VOC is already produced by those causative bacteria that could serve as biomarkers for wound infections.

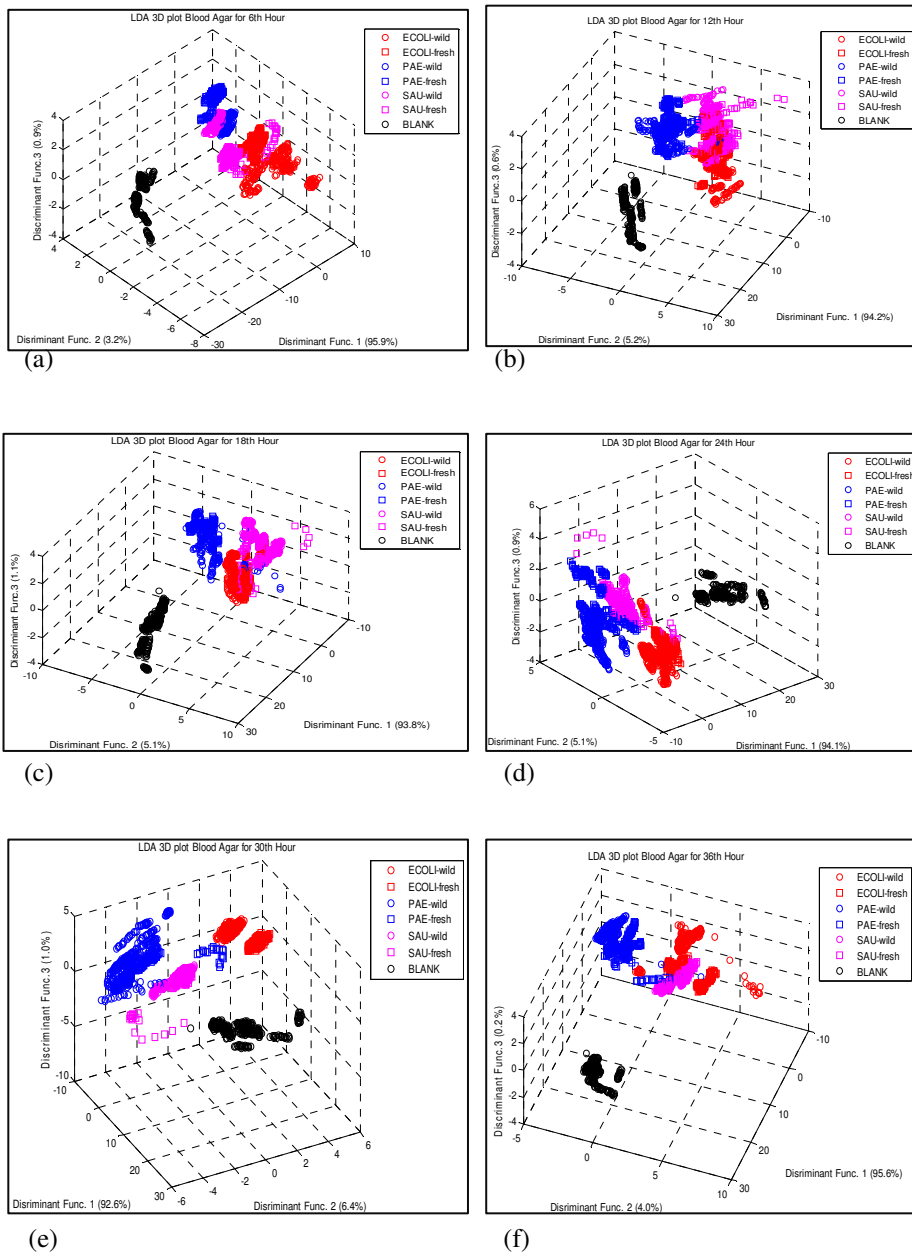

Figure 4: (a) LDA plot for 6th hour; (b) LDA plot for 12th hour; (c) LDA plot for 18th hour; (d) LDA plot for 24th hour (e) LDA plot for 30th hour; and (f) LDA plot for 36th hour.

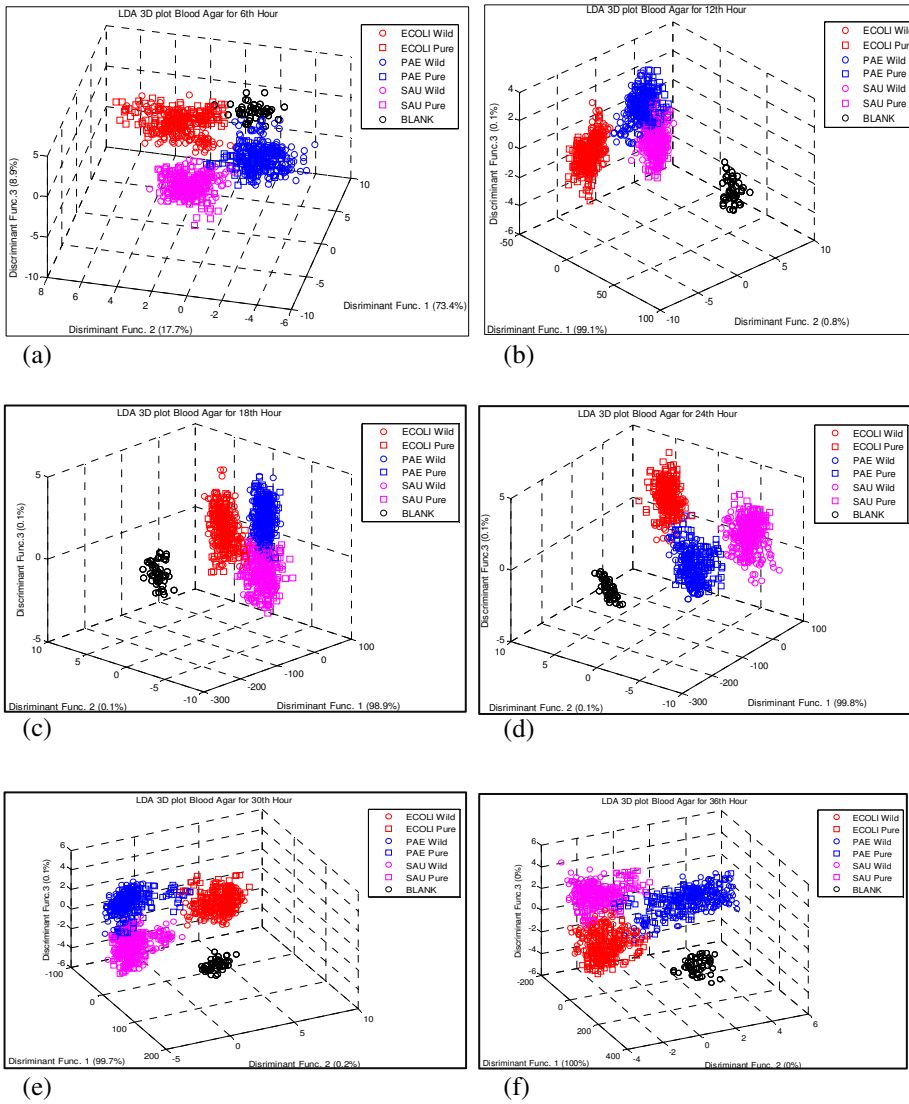

Figure 5: (a) LDA plot for 6th hour; (b); LDA plot for 12th hour (c) LDA plot for 18th hour; (d) LDA plot for 24th hour; (e) LDA plot for 30th hour; (f) LDA plot for 36th hour.

4.3 Hybrid LDA-neural network : evaluation and classification performance

This section is divided into two parts. Tables 5 and 7 demonstrate the classification findings using raw data without performing any dimension reduction for both respective e-noses. Whereas Tables 6 and 8 illustrate the

Table 5: Percentage Accuracy Uses Raw PEN3 Sensor Data as Input.

Method	6th Hour		12th Hour		18th Hour		24th Hour		30th Hour		36th Hour	
	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test
LDA	98.6	98.2	98.7	98.6	99.6	99.8	97.6	97.1	99.5	99.4	99.4	99.3
KNN	100	100	99.9	100	100	100	100	99.9	100	100	100	100
PNN	100	100	100	99.9	100	99.3	100	99.8	100	100	100	100
SVM	98.1	99.9	99.7	100	100	100	99.7	99.9	100	100	100	100
RBF	100	100	100	100	100	100	100	100	100	100	100	100

Table 6: Percentage Accuracy for Bacteria Classifications by Using the LDA Discriminant Value from PEN3 as Input.

Method	6th Hour		12th Hour		18th Hour		24th Hour		30th Hour		36th Hour	
	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test
LDA+KNN	99.9	100	99.9	100	100	100	100	99.9	100	100	100	100
LDA+PNN	100	98.7	100	98.4	100	99.2	100	99.6	100	99.5	100	99.4
LDA+SVM	98.2	99.4	99.8	100	100	100	98.2	98.4	100	100	100	100
LDA+RBF	100	100	100	100	100	100	100	100	100	100	100	100

Table 7: Percentage Accuracy for Bacteria Classifications by Using Raw Cyranose320 Sensor Data as Input.

Method	6th Hour		12th Hour		18th Hour		24th Hour		30th Hour		36th Hour	
	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test
LDA	98.3	96.7	99.5	98.9	93.4	91.9	96.9	95.7	99.5	96.5	98.3	97.8
KNN	96.5	87.3	95.7	83.5	93.2	80.5	93.7	80	96.2	86.8	92.8	83.3
PNN	100	89.2	100	83.6	99.7	81	100	78.5	100	87.5	99.5	85
SVM	99.7	99.6	100	100	99.4	99.2	95.4	97.8	98.6	100	98.8	99.8
RBF	100	100	100	100	100	100	100	100	100	100	100	100

Table 8: Percentage Accuracy for Bacteria Classifications by Using the LDA Discriminant Value from Cyranose320 as Input.

Method	6th Hour		12th Hour		18th Hour		24th Hour		30th Hour		36th Hour	
	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test	Train	Test
LDA+KNN	99.2	97.8	99.6	98.9	96.5	94.3	98.2	97.6	99.4	99.1	99.1	98.9
LDA+PNN	100	84.6	100	84.7	100	80.7	100	84.8	100	84.6	100	82.4
LDA+SVM	97	97.5	98.6	98.9	92.4	95.2	94.2	97.6	96.4	98.9	98.3	98.4
LDA+RBF	100	100	100	100	100	100	100	100	100	100	100	100

classification results using extracted features which was performed using LDA and followed by the respective classification approaches.

Obviously, during the 6 h of bacteria growth, the classification percentage accuracy of VOC released from bacteria is high. Of all the methods according to the percentage accuracy, generally RBF shows the highest percentage of accuracy approximately 100% for bacteria classifications for both training and testing. The results obtained from this experiment are promising where both e-nose (PEN3 and Cyranose320) could classify different types of bacteria even before the optimum time of growth for bacteria which is 24 h.

This would greatly helpful in the diabetic foot ulcer detection in a shorter time compared to conventional method that expended up to 3 days long due to some compulsory medical ethic processes that could not be avoided. PEN3 data collections for 6 h of bacteria growth shows classifications of bacteria where it is visible that each bacteria has its own clustering. Wild and standard ATCC conditions of those bacteria are plotted in a same clustering too. This has greatly proven that even in the first 6 h, PEN3 is also able to classify the bacteria by sniffing the VOC released.

4.4 Result validation using GC-MS

To make confirmations regarding the classifications made of various types of bacteria that commonly found on diabetic foot ulcer, GC-MS is used to identify the VOC released by each bacterium. Figures 6–8 show the GC-MS result for wild and standard ATCC condition of each bacteria, respectively. The upper graph indicates that VOC compound that is present in the wild type strain bacteria while the bottom part shows the present compound in standard ATCC bacteria.

The chromatograms from the GC-MS is allowed for detailed analysis of the potential biomarkers. Figures 6–8 show interesting result for three different bacteria species from wild type strain and standard ATCC bacteria. Here, *E. coli* emits alcohol (1-hexanol-2-ethyl), alkane (3-trifloroacetoxydodecane), and benzaldehyde as biomarkers for that particular bacteria. The dominant compound

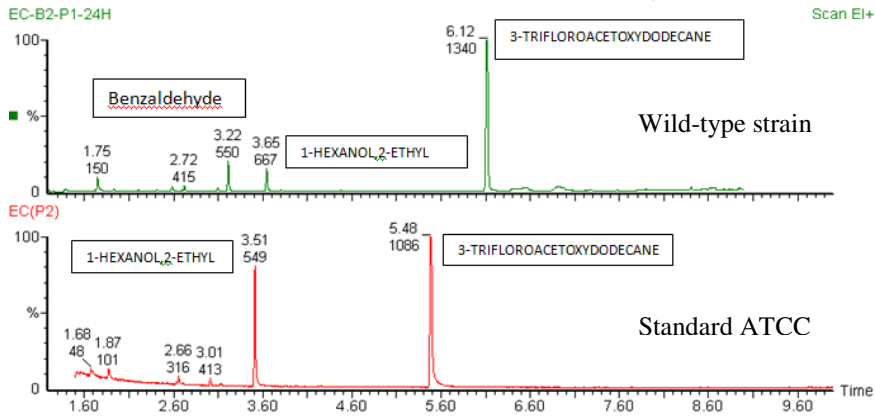

Figure 6: Chromatograms of wild and standard ATCC strain of *E. coli* bacteria.

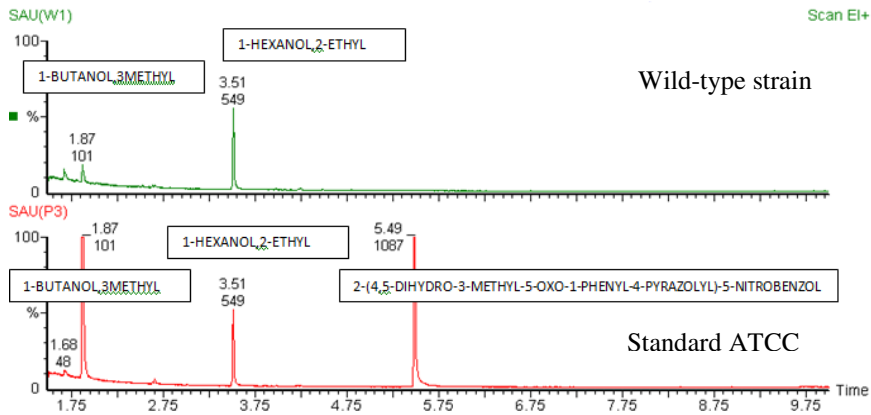

Figure 7: Chromatograms of wild and standard ATCC strain of *S. aureus* bacteria.

produced by *S. aureus* consists of alcohol (1-butanol,3-methyl) and ethyl (1-hexanol,2-ethyl) group which is different from *P. aeruginosa* that contains alcohols (1-hexanol,2-ethyl) and alkane (3-trifloroacetoxydodecane) group as biomarkers. Since there are differences of compounds found for each specific bacteria, this shown that each bacteria releases different types of VOC which acts as their odors signature that make them different from one another.

Normally, optimum time of growth for bacteria is within 24 h. However, this study only needs 6 h to culture the bacteria species and further classification can be implemented. This quick process is useful for diabetic foot ulcer detection compared to the conventional method which would last for 3 days due to some compulsory medical ethic processes that could not be avoided.

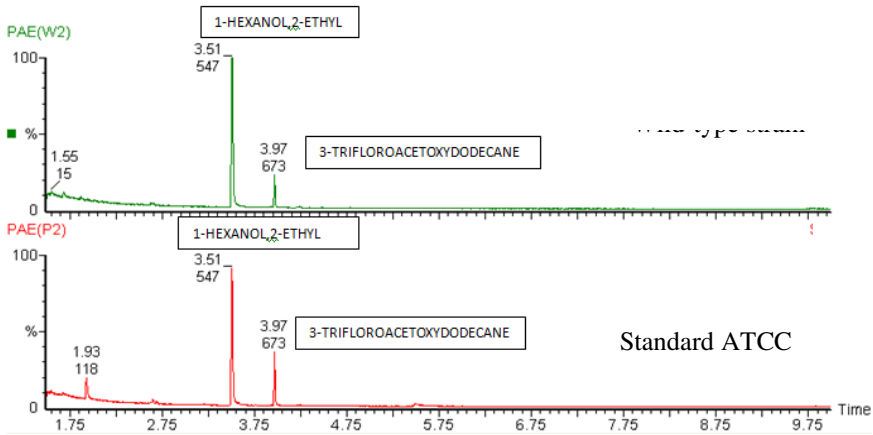

Figure 8: Chromatograms of wild and standard ATCC strain of *P. aeruginosa* bacteria.

5 Conclusions

Early detection of bacteria species in diabetic foot ulcer is very critical in an effort to reduce the number of imputation of diabetic patient. An attempt to resolve the problem is to conduct an experiment to investigate the nature of bacterial morphology from the perspective of sensor technology. From the study, it can be concluded that both e-noses that is PEN3 and Cyranose320 are able to mimic the human sense of smell and useful for diabetic foot ulcer bacteria detection. Human sense of smell is closely related to function of nose that can specify different odorants by detecting the smell, sent the information to the brain, and interpret the specific odorant detected. The same processes are adopted by both e-noses.

In this experiment, our most concerned is that both e-noses could detect the bacteria even before the optimum time of growth within 6-h time. This would greatly improve the detections compared to the conventional methods that would last for 3 days. The alternative method for diabetic foot ulcer detections could possibly reduce the number of patient's to undergo amputations due to improper time of treatment and antibiotic. In the conventional approach, before the clinical testing result is obtained, physician would provide general medication to patient that may not respond to certain antibiotic, and this would caused worse infection. Then only after the clinical testing result is available, suitable treatment and medication can be provided to the patient. For classification purposes, LDA, KNN, PNN, SVM, and RBF were applied. Basically, these methods are divided into two; parametric method (such as LDA) is assumed to follow the Gaussian conditional densities and the nonparametric methods (like KNN, PNN, SVM, RBF) with an assumption of an unknown group conditional densities. Therefore, in this experiment, we wish to employ both approaches to see which method is suitable and preferable.

From the findings, if the raw data from the e-noses is used for classification, LDA appeared to perform better than other methods. However, if classifications using extracted features are performed, RBF seems to outperform the rest of the methods. Two conclusions can be drawn from the findings. First, if the actual data, that is, without any modification is used (all information are being used) it seems that method that follows the Gaussian distribution is suitable for classification of bacteria species. Second, by using the extracted features, where only important features are applied (with certain loss of information), nonparametric approach, that is, RBF best classifies the bacteria species.

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