

## Electronic Device for Non Invasive Urea and Ammonia Estimation.

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**Abstract :** The uremia is a condition which shows symptoms and signs that result from toxic effects of elevated levels of nitrogenous and other wastes products in the blood. The levels of urea in the blood have to be measured to assess the extent of the disease. Conventional method to measure the blood urea nitrogen (BUN - Chaney and Marbach ) require a venous puncture and tested in a laboratory. The Electronic device is non invasive instrument that has been developed as a simplified model of the human olfactory system. Urease which is an enzyme applied over the skin, it helps the hydrolysis of the urea into ammonia, carbon dioxide and water. The device smells the ammonia through a sensor or the electronic nose which is connected to an Analog to Digital Converter and Microcontroller with Liquid Crystal Display (LCD). The concentration of ammonia is displayed, by using a pre calibrated equation, urea level is calculated. The urea levels were tested in the serum samples by conventional method, direct method (method II) and non invasive method (Method III). The detection of urea level by the device through e-nose vis a vis with other methods is statistically proved by Kruskal-Wallis ANOVA Origin 8 Data analysis graphing software at 0.05 level of significance. The output result indicates that there is no significant difference in the efficiency of detection of urea by the three methods. The designed electronic mode stands better and patient friendly device on comparison with invasive mode of detection.

### INTRODUCTION

Uremia or uremic syndrome is the illness associated with kidney failure in particular the nitrogenous waste products associated with the renal failure. The signs and symptoms of uremia are fatigue, peripheral neuropathy, decreased mental acuity, seizures, anorexia, nausea, decreased sense of smell and taste, cramps, unsteadiness, sleep disturbances, coma, reduced muscle membrane potential, amenorrhea, sexual dysfunction, reduced body temperature, altered amino acid levels, bone disease due to phosphate retention, secondary hyperparathyroidism, and vitamin D deficiency, insulin resistance, increased protein-muscle catabolism, effusion in pericardium, pleura and abdomen, itching, hiccoughs, oxidative stress, anemia due to erythropoietin deficiency, shortened red-cell survival, granulocyte, lymphocyte and platelet dysfunction etc.

The nitrogenous waste products build up in the blood stream, poisoning all the tissues in the body. Urea level in the blood has got a direct relationship to the extent of damage to the kidneys. Uremia could be life threatening. Kidney function is assessed by Glomerular Filtration Rate (GFR) which is usually expressed by creatinine clearance level (Normal 80-120 ml/min/kg). Other laboratory tests to consider for abnormalities prevalent with clinical uremia including hemoglobin, calcium, phosphorous, Parathyroid Hormone (PTH), albumin, potassium, phosphate and serum bicarbonate values. Urine analysis including microscopic examination should be performed on all cases to evaluate for the presence of protein, cellular casts, fat globules, ketones, acid hematin crystal, myoglobin and to assess pH. Conventional BUN method of urea estimation requires a laboratory

set up. The Electronic nose is an instrument that has been developed as a simplified model of the human olfactory system. It can discriminate odour and measure its intensity. Here we used electronic nose to sense ammonia from the skin after converting sweat urea by urease into ammonia, carbon dioxide and water. Urease<sup>10</sup> is an enzyme that helps the hydrolysis of urea into ammonia, carbon dioxide and water.

### E-NOSE DEVICE AND ITS APPLICATION

Electronic-nose systems has been designed specifically to be used for numerous applications in different industries<sup>9</sup>. Electronic nose also has been developed for a wide range of medical applications<sup>3</sup>. It has provided many benefits to commercial, agricultural, biomedical, cosmetics, environmental, food, manufacturing, military, pharmaceutical, regulatory and various scientific research fields. The development and utilization of many new electronic nose (e-nose) applications in the health care and biomedical fields have continued to rapidly accelerate over the past many years. Innovative e-nose technologies are providing unique solutions to a diversity of complex problems in biomedicine that are now coming to fruition.

There are devices to smell alcohol content in a person breath such as breathalyzer, detect lung cancer, bomb sniffers in airports, smell phones etc. The data from such devices are admissible as evidence in a court of law.

Urinary tract infections has been investigated by Aathithan *et al* Pavlou *et al* proposed the use of the electronic nose as a potential diagnostic tool by distinguishing traces of blood in urine samples, and for the rapid identification of *E. coli*, *Proteus* spp. and *Staphylococcus* spp. infections at very high levels of confidence. Aathithan *et al.* analyzed 534 clinical urine specimens of which 21% had significant bacteriuria. The sensitivity and specificity of the electronic nose compared with conventional cultural counts were

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83.5% and 87.5% respectively, but the e-nose diagnosis done at significantly lower costs.

It is well known that pathogenic microbial species produce a wide range of Volatile Organic Compounds (VOCs) However, the use of VOC chemical analyzers, is still very expensive, requires highly-skilled personnel and is time consuming. The connection between differences in the aroma of diseased vs. healthy human tissues and diagnostic detection of human pathogenesis is supported by studies using the extraordinarily keen olfactory abilities of well trained dogs<sup>12</sup>. In the case of pneumonia diagnosis, Hockstein *et al.* discriminated between diseased and non-diseased patients with an accuracy as high as 91.6%. The severity of asthma also was investigated by use of the electronic nose in young and older patients with mild and severe asthma.

## MATERIALS AND METHODS

Thirty six men and women, whose age ranged from 19 to 71 years were studied. This sample was drawn randomly from patients at KJ Hospital, Chennai, South India during March 2013. Of these 16 were men and 20 were women. Subjects with major illness were excluded. The other exclusion was those who had any skin diseases and history of allergic skin reactions especially to pulses. All the subjects were explained about the proposed study. As the study was under evaluation we have done both blood urea estimation by conventional method as well as by the electronic nose. The subjects were made to sit in relaxed position and one of the forearm was chosen for venous puncture and 2 cc blood was drawn and serum was separated by centrifuging at 1500 rpm for 15 minutes. The other forearm chosen for application of urease.

## UREASE PREPARATION AND APPLICATION

Urease is an enzyme that helps the hydrolysis of urea into ammonia, carbon dioxide and water.

Urease was extracted from horse gram. Horse gram (50g) was macerated well in mortar. Acetone was added to the horse gram and stirred for about 3 to 4 minutes. The content was then filtered through Whatman No.1 filter paper. When half the amount is filtered the set up was transferred to a refrigerator and filtration was continued. The filtrate was left overnight at 0° C. Urease crystals formed at the bottom which is centrifuged at 22° C and washed with 5 ml cold water and 31.6% acetone. The crystals were dissolved in 5 to 10 ml distilled water and centrifuged to remove any insoluble residue. About 5 ml of the solution was transferred to another test tube and with the use of a cotton bud the urease was applied about an inch diameter over the skin on the other forearm. No skin preparation is required for the application of the urease. The area was washed on completion of the study. No skin or systemic reactions were noted in our study.

## ELECTRONIC DEVICE

The Electronic nose is a non-invasive urea estimation device by smelling ammonia from the patient's skin, (Figure 1) consists of two main parts namely the sensing system and the odour recognition system. The sensing system is Figaro TGS 2444 gas sensor, (Figure 2) a thick film metal oxide semiconductor which offer low cost, long life and good sensitivity to target gases while utilizing a simple electrical circuit. The sensing material SnO<sub>2</sub> (tin dioxide). The output voltage of the sensor is given to an embedded circuit which uses an Analog to Digital Converter through a Microcontroller to an LCD that displays the quantum of ammonia. The software program with

graph and a pre calibrated equation incorporated will give direct measurement of urea. The simple block diagram of electronic nose is given below.

## BLOCK DIAGRAM OF ELECTRONIC NOSE

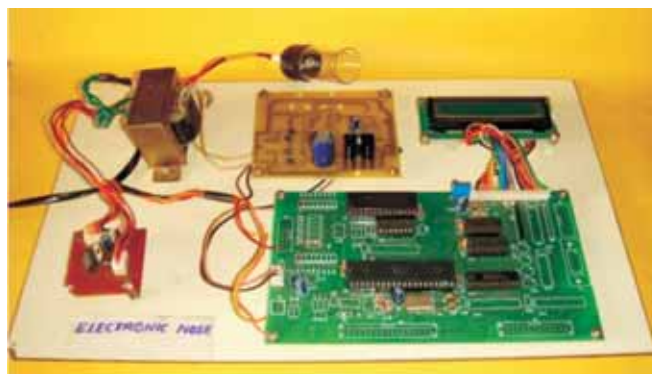
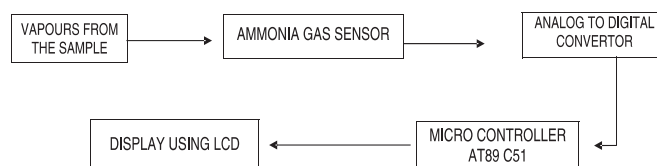


Figure 1: Electronic device – E Nose



Figure 2: Gas sensor

## CONVENTIONAL METHOD ( METHOD – I )

In this conventional GLDH-UREASE method, about 2ml of serum is mixed with urease, the urea can be measured from the mixture using a BUN Analyzer.

## DIRECT METHOD ( METHOD – II )

In this method ( Figure 3) the urea level is estimated by adding urease enzyme 1ml to 1 ml serum in a test tube, urease reacts with urea in the serum and liberates ammonia, carbon dioxide and water. The sensor is placed over the open end of the test tube and the emanating ammonia is sensed and the output voltage is converted to digital form by the analog to digital converter (ADC) and the quantum of ammonia is displayed on the LCD The pre calibrated equation will show the amount of urea. The out put voltage will vary depending on the concentration of ammonia.



Figure 3 - Directly smelling ammonia from the serum

### NON - INVASIVE METHOD ( METHOD III )

In this method, the urease applied over the forearm skin which liberates ammonia and other compounds from the urea in the sweat. The electronic nose ( Sensor) is directly placed over the skin ( Figure 4) where the urease is applied. The sensor smell ammonia and the out put voltage is fed through the ADC to the microcontroller to the LCD which indicates the quantum of ammonia and with the equation the urea is estimated.



Figure 4 – Non invasive method of smelling ammonia from the skin

## RESULTS

### Calibration study – Standard urea solution

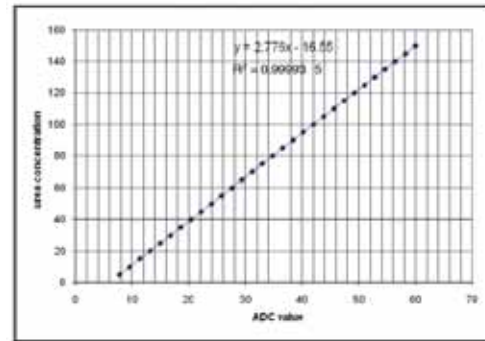
Known quantity of urea (10mg to 100mg) is taken in different test tubes, urease is added into it one by one. When sensor is placed on each tube, the liberated ammonia in each tube gives some specific readings. As the urea concentration increases, the readings were also increased. All the readings were noted.

A standard XY graph (below) is drawn by taking urea concentration in Y – axis and corresponding digital reading in X – axis.

By applying the linear equation  $Y=mx+c$ ; value of Y was determined by substituting the values of X.

The unknown quantity of urea in the blood samples was calculated by applying the equation obtained from the standard

### STANDARD XY GRAPH



The results obtained clearly shows that out of 36 patients tested with both the genders in the age group 19 to 78, 14 were found to be uremic patients. ( Table 2) Remaining 22 were found to be normal. Threshold value of 50 was set as the starting parameter in ADC. When the ADC value crosses 70, it is predicted as uremic. The urea was estimated by subtracting the initial value from the finally displayed value.

A comparison with conventional method of urea estimation to know the accuracy of direct method and graphical method showed 96% and 94% accuracy respectively . The proposed mode of detection of urea by electronic nose is on par with currently used BUN method. The status was statistically proved by Kruskal – Wallis ANOVA Origin 8<sup>(17)</sup> (Data Analysis Graphing Software) at 0.05 level of significance ( Table 1). The output results indicate that there is no significant difference in the sensitivity of detection of urea by the three methods.

The sample comes from the same population. There was no significant difference among the groups on value (Chi –Square 0.97883). At 0.05 level of significance the inference was that the data's were not significantly different. Therefore the proposed methods; Direct method and Non-Invasive method are equally good as compared to the conventional BUN method used currently for urea estimation in diagnostic labs. Based on this we can propose that the new device can be successfully used to replace the existing conventional method.

Table-1: Adc Values Vs Urea Concentration

S.NO	ADC VALUES X-AXIS	UREA CONCENTRATION(mg) Y-AXIS
1	8	5
2	10	10
3	11	15
4	13	20
5	15	25
6	17	30
7	19	35
8	20	40
9	22	45
10	24	50
11	26	55
12	28	60
13	29	65
14	31	70
15	33	75
16	35	80
17	37	85
18	38	90
19	40	95
20	42	100

Table-2: Urea Concentration In Various Blood Samples

S.No	Gender	Age	Initial ADC Value	Final ADC Value	Actual ADC Value	Method I	Method II	Method III	Interference
1	M	51	50	67	17	28	31	30	Absent
2	F	45	50	65	15	25	25	23	Absent
3	M	78	50	78	28	62	61	60	Present
4	M	21	50	63	13	18	20	21	Absent
5	F	42	50	69	19	35	36	35	Absent
6	F	19	50	62	12	17	17	25	Absent
7	M	65	50	92	42	102	100	99	Present
8	M	52	50	88	38	88	89	90	Present
9	F	21	50	60	10	11	11	13	Absent
10	F	30	50	66	16	28	28	25	Absent
11	M	54	50	78	28	61	61	60	Present
12	M	60	50	62	12	16	17	18	Absent
13	F	35	50	65	15	24	25	26	Absent
14	M	42	50	87	37	88	85	87	Present
15	F	20	50	65	15	22	25	24	Absent
16	F	52	50	62	12	18	17	15	Absent
17	F	22	50	69	19	34	36	33	Absent
18	F	22	50	62	12	16	17	28	Absent
19	F	53	50	63	11	14	14	13	Absent
20	M	23	50	68	18	33	33	31	Absent
21	F	49	50	72	22	45	44	41	Present
22	F	38	50	70	20	38	39	39	Absent
23	F	26	50	67	17	29	31	29	Absent
24	F	23	50	75	25	52	53	50	Present
25	F	53	50	83	3	75	75	72	Present
26	F	62	50	72	22	42	44	42	Present
27	F	71	50	92	42	99	100	97	Present
28	F	58	50	86	36	83	83	80	Present
29	F	35	50	77	27	56	58	55	Present
30	M	47	50	84	34	75	18	75	Present
31	M	44	50	81	31	68	69	65	Present
32	M	56	50	67	17	32	31	29	Absent
33	M	21	50	64	14	22	22	20	Absent
34	M	23	50	68	18	31	33	31	Absent
35	M	40	50	65	15	24	25	22	Absent
36	M	37	50	62	11	14	14	11	Absent

Method I – Concentration of Urea by Conventional method – BUN Method

Method II - Concentration of Urea by Direct method – Directly smelling ammonia from serum sample

Method III - Concentration of Urea by Non-Invasive method – Directly smelling ammonia from skin

## DISCUSSION

Conventional method to measure urea (BUN) devised by Chaney and Marbach in 1962 is time honored proven gold standard in estimating the level of urea in serum. It is accurate and reproducible method followed world over. All the automated urea estimation devices are based on the same principle that involves collection of blood and separation of serum. A patient with renal failure requires estimation of urea level at regular interval which involves multiple sample collection and need the help of the doctor or lab technician every time.

This innovative device which is pain free, patient friendly and can be repeated any number of time. No technological expertise is involved in handling or operating the instrument, even it could be done at home by self. This device helps non-invasive, accurate and fast detection of ammonia and urea in the blood. By this one could initiate treatment without waiting for time consuming laboratory test. This will prove a screening tool for assessing urea level in suspected uremic patients and ammonia level in suspected hepatic failure patients. Wider applications of this methodology will go a long way as a patient friendly accurate modality in the above mentioned

conditions.

Since it is in the evaluation process we have collected the blood sample by vein puncture to measure the urea level by conventional method. This device is going to be a major breakthrough for the renal failure patients who are on dialysis and on other supportive therapies.

## CONCLUSION

Conventional method of urea estimation requires a standard laboratory set up. Our objective is to overcome such difficulties by using a simple gas sensor and microcontroller based device to estimate urea automatically by simply capturing the odour from the skin. This method is absolutely non-invasive, user friendly, simple, time and cost effective. The patients with diabetes mellitus, hypertension, kidney infection, renal failure etc. may use this apparatus in their home itself. The e-nose proved to be a patient friendly, non invasive, cost effective and simple device for the estimation of blood urea.

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