

Design and Fabrication of a Conductometry System for Fast Detection of Pathogenic Bacteria in Human Urine



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Abstract

Background: Common methods for identifying the infectious bacteria in human urine are mainly time-consuming and costly. Therefore, the most reliable method for detecting the urinary tract infections is the urine culture, which requires at least 48 hours to identify infectious factors.

Objectives: It is important to detect the bacteria in urine rapidly, simply, and accurately.

Materials and Methods: In this work, the variations in the electrical conductivity and dielectric coefficient of the urine sample due to changes in the concentration of infectious bacteria have been studied. Furthermore, an appropriate measurement system was prepared for impedancemetry and conductometry.

Results: We showed that the detection time was reduced to about an hour. Finally, the accuracy of the device for diagnosis and precision of measurement were evaluated and compared by the detection method for bacterial culture.

Conclusion: In this work, the detection time was reduced to about 1 hour.

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Background

Urinary tract infection is known as the second most common human infection, annually affecting 150 million people around the world. Moreover, its annual health care costs are considerable, and it occurs among all age groups.^{1,2} The urinary tract infection refers to the presence of at least 10^5 bacteria (CFU/mL) in the culture.³ Acute urinary infection is anatomically divided into two groups: lower urinary tract infection (acute bladder infection and infection of the urethra), and upper urinary tract infection (acute pyelonephritis, perirenal, and intrarenal abscesses).¹

Urinary tract infection-causing bacteria encompass both gram-negative (*Enterobacteriaceae* and *Pseudomonas* families) and gram-positive (*Enterococcus*, *Staphylococcus* families and a number of fungi and viruses) species.⁴ Other microorganisms with a lower prevalence consist of *Salmonella*, *Candida*, *Corynebacterium*, *Urolicium*. Certainly, there is a possibility of the infection caused by unusual pathogens such as *Mycoplasma hominis*, *Mycobacterium tuberculosis*, and BK and JC viruses.⁵ More than 80% of urinary tract infections are caused by gram-negative bacilli, the most prevalent of which is *Escherichia coli*.^{6,7}

Failure in the diagnosis and timely treatment of patients with urinary tract infections can result in severe consequences such as disorders of the urinary tract, hypertension, renal disorders, and uremia. In addition, they may be the factor of premature labor and even abortion among pregnant women.⁵

One of the most important and accurate ways to diagnose infection is urine culture. In this way, very small amount of urine is cultured on a sterile environment and checked out after 24 to 48 hours. Urine culture is positive when the number of bacteria colonies is $\geq 10^5$ (CFU/mL). In given cases, it is only conducted based on signs, diagnosis, and treatment in definite cases. In complex cases, it is useful to confirm the diagnosis by urinalysis and to detect nitrite in the urine, leukocytes or leukocyte esterase (LE). The other diagnostic test of urinary infections is microscopic, which detects red blood cells, white blood cells, or the bacteria.⁸

In global healthcare, the accurate and appropriate diagnosis of bacterial infection is highly important. Although a rapid diagnosis can assure that treatment is administrated at the earliest moment, the most suitable treatment will be obtained by an exact diagnosis of a bacterial infection. Regrettably, such methods

for identifying bacteria in clinical populations have restrictions in terms of time, cost, and complexity. These methods include culturing, polymerase chain reaction (PCR) as well as immunological methods, which have some limitations. To meet the key requirements of being rapid, scientific, expensive and simple to use, no single method has been discovered yet. To address this challenge, sensor technologies, including label-free detection, are widely investigated as a means for detecting bacteria rapidly.

In addition, chemiresistors are chemical sensors composed of a thin film material that changes its electrical resistance in reaction to changes in the nearby chemical environment.^{9,10}

The use of biosensors is another way to detect the bacteria incorporating a biological recognition mechanism into a physical transduction technique. It should be noted that the type of transduction used in biosensor may be micromechanical, thermometric, electro-chemical, piezoelectric, magnetic or optical. The electrochemical transduction methods among these techniques are much less time-consuming and more sensitive than the others.^{7,9,10}

Based on plate count, the standard methods for enumeration of bacteria could not satisfy the requirements of microbe-detection anymore since those procedures are time-consuming and grueling. To improve the total assay time and further amplify signals, many interesting and new methods for enumeration such as enzyme-linked immune sorbent assay (ELISA), TaqMan real-time PCR, fluorescence analysis, as well as flow cytometry detection have been developed accordingly.^{11,12}

Nevertheless, such methods require expensive consumables, apparatus and high cost per test at all times. In this regard, the exploration of a timesaving and low-cost bacterial enumeration method is still important. Owing to its flexibility, good environmental stability, remarkable electrical conductivity, low cost as well as optical properties, much attention has been paid to the polyaniline as one of the substantial polymers.^{11,12}

Staphylococcus epidermidis and *E. coli* as the most prevalent pathogenic bacteria have diameters of 1-2 μm . Given that water (or other liquid) accounts for 12% of the structure of a bacterium, its population increment in the urine will affect the electrical conductivity and dielectric constant of a suspension. Accordingly, if the other salts separated from urine and only liquid containing probable bacteria are obtained, the bacteria may be analyzed through measuring its electrical conductivity statistically.

To detect the urinary infection, the conductometric method was examined in the present study accordingly. In the following, the electrical conductivity measurement system was designed and the obtained results were introduced.

Materials and Methods

To perform the conductometry and detect the infectious bacteria, laboratory work has been divided into two parts, including (1) preparation of standard microbial suspension and (2) the suitable system to measure the electrical conductivity and capacitance caused by changing infectious bacteria concentration.

Preparation of Microbial Suspension

The objective was to create a uniform concentration to prepare the intended microbial samples in various dilutions. In this method, the standard strains of *S. epidermidis* (ATCC 12228) and *E. coli* (ATCC 25922) were purchased from Iranian Fungal and Bacterial Collection in the form of lyophilized vials. Under sterile conditions, the lyophilized powder was transferred from brain heart infusion (BHI) broth (Acc. ISO 6888 GanuCult code: 1.10493.0500) medium to the 2 mL, then it was incubated at 37°C for 48 hours. In the next step, the stock culture was prepared using the grown mother culture. To perform this method, 8 test tubes (the first tube containing 8 cc deionized water and the others containing 4 mL deionized water) were sterilized and prepared. Next, some of the intended bacterial colonies (which were prepared) were transferred to the insemination environment using sterilized loop, and its opacity was controlled by a 0.5 McFarland standard tube (1.5×10^8 CFU/mL). The content of the first tube was mixed well, and then 4 mL of it was added to the next tube under sterile conditions. In addition, the previous step was repeated for the subsequent tubes respectively. The lower the ambient opacity, the more diluted the initial suspension was. Each tube was two times more diluted in terms of microbial concentration compared to the previous tube.¹³

Measuring System

As mentioned in the previous section, changes in the concentration of bacteria cause changes in the electrical conductivity as well as dielectric constant of the samples. The changes produced in the dielectric constant of the suspension can be measured by calculating the capacitance. To this end, a double-glazed cylindrical container of internal and external cylindrical plates was made of copper, and the capacitive plates were prepared. Furthermore, the space between the capacitor plates was filled up with the suspension and evaluated by the electrical circuit displayed in Figure 1. A sine wave with 300 kHz frequency and a peak voltage (up to 5 V) were prepared through a signal generator and applied to the measurement circuit. The phase difference between the waveforms of V_c and V_m is determined and measured by the capacitance relationship 1, and finally, the changes caused by the suspension concentration variation were obtained.

$$\Phi = \text{tg}^{-1}(\text{RC}\omega) \quad (1)$$

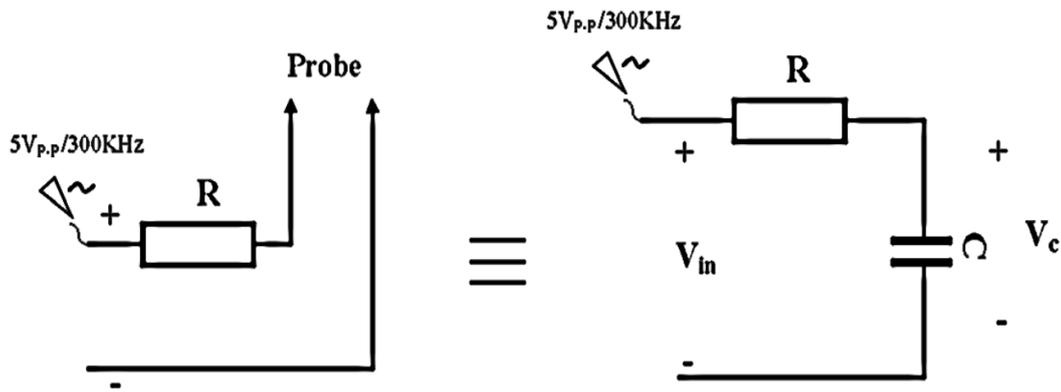


Figure 1. Electrical Circuit of the Phase Difference and Capacitance Measurement System.14

To measure the electrical conductivity, Metrohm 914 and an electrode (made of stainless steel) with measurement range of 0-300 $\mu\text{S}/\text{cm}$ have been used. This device has an appropriate degree of accuracy (0.01M KCL) and cell constant (0.1 cm). Under sterile conditions, the standard specimens were prepared from two common bacteria in the urinary tract infection called *E. coli* and *S. epidermidis* with the varying dilutions, and their electrical conductivities were measured. All the experiments were conducted at 297°K.

Results and Discussion

Using the method mentioned in the previous section, the test specimen with different concentrations was provided and evaluated for the presence of *E. coli* and *S. epidermidis*. In Figures 2a-b, the results of electrical conductivity and capacitance measurement are presented, respectively. As the bacterial concentration was changed from 1.5×10^8 CFU/mL to 0.75×10^5 CFU/mL, the electrical conductivity related to the *E. coli* and *S. epidermidis* has been shifted from 56 to 14 and 68 to 28, respectively. This method was repeated more than 20 times. The maximum deviation from the nominal value was 10%. Therefore, we managed to measure the changes of bacterial concentrations up to a minimum of 0.5×10^5 CFU/mL. In the basic state, urine is sterile and lacks any bacteria. In most cases, the number of infectious bacteria in the patient’s urine sample was

more than 10^5 CFU/mL and was equal to capacitance. Using such a method, the infectious bacterium in the patient’s urine specimen can be detected. The major salts in the patient’s urine specimen included crystals, red blood cells, white blood cells, bacteria, and chemical compounds. In the real specimen, first, the crystal (if any) was separated using the low-speed centrifuge for 5 minutes. Then, the specimen separated from the crystal was poured into another tube, and red and white blood cells were lubricated and finally bacteria were settled.

Then, the superficial liquid (including the lubricated cells) was discarded, and the bacteria remained. To assure the absence of lubricated cells, a portion of deionized distilled water was added to the sample, and the rotation was repeated for 10 minutes through a medium-speed centrifuge (1500 rpm).

Therefore, the supernatant fluid was discarded again, and the residual bacteria were completely mixed with approximately 4 mL deionized water to make a uniform fluid. Then, the electrical conductivity was measured. Figure 3a displays the measurement results from 15 human urine specimens. As can be seen, the designed system was well suited. It should be noted that the only time-consuming measurement part was the process of isolating the probable bacteria from the urine specimen. This process took a maximum of 1 hour. It is considered an effective and fast method compared to conventional

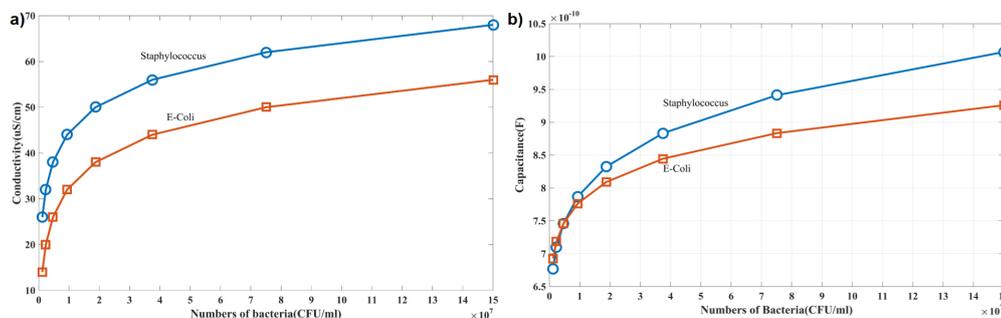


Figure 2. Electrical Conductivity Change Curve; (a) Capacitance of Samples; (b) Caused by Different Concentrations of *E. Coli* (□) and *S. epidermidis* (○).

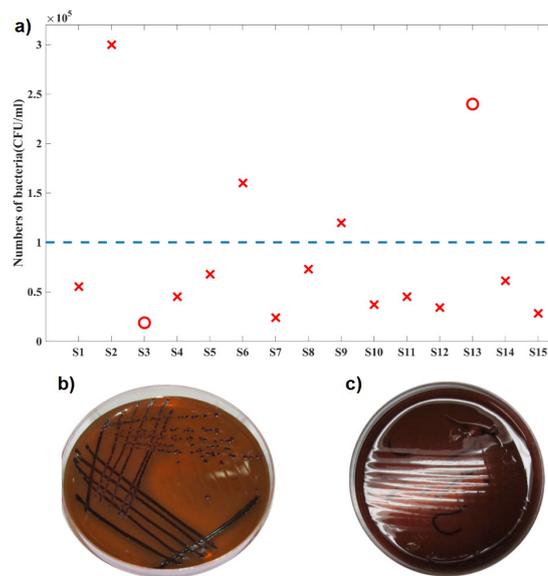


Figure 3. (a) The Result of Conductometry of 15 Real Samples; (b) the Result of S13 Sample Culture; (c) the Result of S3 Sample Culture.

commercial methods like bacterial culture (taking at least 48 hours). Followed by studying, the measurement system was prepared and used to test 15 urine specimens of healthy and patient subjects (Figure 3a).

The dashed lines (Figure 3a) represent the boundary and threshold of the critical state of infectious bacterial concentration in the urine sample, i.e., value = 10^5 CFU/mL. As can be seen, the samples S1–S15 are healthy and infectious according to the obtained results. To verify the results, the S13 sample was studied in bacteria culture method. In such a sample, the colony is formed owing to the presence of infection (Figure 3b). The results are confirmed through the conductometry method. Therefore, the urine specimens were carefully analyzed using the conductometry method and capacitance measurement (through the designed system) in a short time (about 1 hour), and the presence of infectious bacteria was examined.

Conclusion

In the present study, pathogenic bacteria in the patient's urine specimen were identified using conductometry and impedancemetry methods. To this end, first, a suitable measurement device was made for conductometry and impedancemetry. In the following, probable bacteria were separated from the real sample through the deionized water and then were centrifuged. Then, the healthy and unhealthy specimens were analyzed using the mentioned method. We placed the precipitated sample, which was mainly bacteria, in distilled water to be measured. Moreover, the obtained results were confirmed. In the presented method, the detection time was reduced to 1 hour. Therefore, it has very high efficiency compared to conventional methods such as bacterial culture (needing

at least 48 hours to detect).

Conflict of Interest Disclosures

The authors declared that they have no conflict of interests.

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Ethical Approval

This study was approved by Ahvaz Jundishapur University of Medical Sciences and Islamic Azad University, Mahshahr.

Authors' Contributions

M. Enami performed experiments and writing the manuscript, Dr. M. Amin is a supervisor, Dr. P. Shabani is an advisor and M. Gashtil performed some of the experimental details. All the authors discussed in the manuscript and approved for submission of it.

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