INVESTIGATION OF DIELECTRIC PROPERTIES OF URINE FOR PREGNANCY AND BLADDER CANCER DETECTION

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2020

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF ENGINEERING
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2020

UNIVERSITY OF MALAYA
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ABSTRACT

Detection and monitoring of pregnancy and bladder cancer are important for medical treatment and nursing. Urine has diagnostic and prognostic values for pregnancy and bladder cancer due to the presence of biomarkers. This study investigated the dielectric properties of human urine and compared them between two groups: 1) non-pregnant healthy female volunteers and pregnant healthy female volunteers, and; 2) healthy subjects and patients with bladder cancer. An open-ended probe network analyzer was used to measure the urine dielectric constant (Ɛ') and dielectric loss (Ɛ'') at microwave frequency ranging from 0.2GHz to 50GHz at 25˚C (room temperature), 30˚C and 37˚C (body temperature). Urine samples from 110 non-pregnant healthy female volunteers and 110 pregnant counterparts were collected to determine the correlation between human chorionic gonadotropin (hCG) hormone levels and dielectric properties. For the bladder cancer study, 35 healthy subjects and 35 patients were recruited from the University of Malaya Medical Center (UMMC). In the analysis of results, subjects and patients with the presence of glucose, protein, bacteria, ketone or hemoglobin in urine were excluded. As a result, 30 healthy subjects and 30 pregnant women were involved for the pregnancy study while 10 healthy subjects and 10 subjects with bladder cancer were involved for the bladder study. Generally, the dielectric properties of urine decreased with temperature before the crossing point, and they increased again after the crossing point. In the study of pregnancy, the Ɛ' and Ɛ''were observed to be significantly different between the urine samples of the two groups across all three temperatures. The urine of pregnant women had significantly lower Ɛ' than non-pregnant women ($p<0.05$), while Ɛ'' was significantly higher in pregnant women ($p<0.05$). They correlated positively with hCG levels at low frequencies ($r_{max}=0.868$, f=2.4GHz) but negatively at high frequencies ($r_{max}=-0.877$, f=46.2GHz). In cases of bladder cancer, the dielectric properties were generally lower in healthy subjects compared to patients, especially at 25°C. Similar to pregnant women,

they decreased with temperature before the crossing point and increased thereafter. Statistically significant differences in Ɛ' and Ɛ'' were observed between healthy subjects and patients ($P<0.05$). A high level of correlation was observed between urinary exfoliated urothelial cells and the dielectric properties of the urine at 25°C. The Ɛ' correlated negatively with urinary exfoliated urothelial cells (r_{max}=-0.662, f=5GHz) while Ɛ'' correlated positively with urinary exfoliated urothelial cells (r_{max}=0.664, f=40.6GHz).

ABSTRAK

Pengesanan dan pemantauan merupakan ciri-ciri penting dalam penjagaan kehamilan dan rawatan kanser pundi kencing. Air kencing mempunyai nilai diagnostik dan prognostik disebabkan kehadiran biopenanda yang boleh digunakan untuk megkaji kesan penyakit atau komplikasi. Kajian ini bertujuan untuk menyiasat dan membandingkan sifat dielektrik air kencing diantara dua kumpulan: 1) sukarelawan sihat dan tidak mengandung dengan sukarelawan mengandung yang sihat. 2) subjek sihat dengan pesakit kanser pundi kencing. Sistem rangkaian vektor terbuka digunakan untuk mengukur pemalar dielektrik (Ɛ') dan factor kehilangan (Ɛ'') air kencing pada gelombang mikro antara 0.2GHz dan 50GHz pada 25˚C (suhu bilik), 30˚C dan 37˚C (suhu badan). Sampel air kencing diperolehi daripada 110 sukarelawan yang sihat dan tidak mengandung, dan 110 sukarelawan mengandung yang sihat untuk mengkaji korelasi antara paras hormon gonadotropin korionik manusia (hCG) dengan sifat dielektrik air kencing. Dalam kes kanser pundi kencing, 35 subjek sihat dan 35 pesakit telah direkrut daripada Pusat Perubatan Universiti Malaya (PPUM). Subjek dan pesakit dengan kehadiran glukosa, protein, bakteria, ketone dan hemaglobin dalam air kencing tidak dimasukkan dalam analisa keputusan. Justeru itu, 30 subjek healthy dan 30 perempuan mengandung terlibata dalam kajian kehamilan manakala 10 subjek healthy dan 10 subjek dengan kanser pundi kencing terlibat dalam kajian pundi kencing. Secara umumnya, Sifat dielektrik akan menurun dengan suhu sebelum menyeberangi titik silang yang kemudiannya akan meningkat semula selepas titik tersebut. Dalam kajian kehamilan, terdapat perbezaan signifikan pada Ɛ' dan Ɛ'' di antara sampel air kencing dua kumpulan pada ketiga-tiga suhu tersebut. Air kencing wanita mengandung mempunyai Ɛ' yang lebih rendah secara signifikan daripada wanita yang tidak mengandung ($P<0.05$), manakala Ɛ'' adalah lebih tinggi secara signifikan dalam wanita mengandung daripada yang tidak mengandung ($P<0.05$). Sifat dielektrik mempunyai korelasi positif dengan hCG pada frekuensi yang

rendah (r_{max}=0.868, f=2.4GHz), tetapi negatif pada frekuensi yang tinggi (r_{max}=-0.877, f=46.2GHz). Bagi pesakit kanser pundi kencing, sifat dielektrik adalah lebih rendah dalam subjek yang sihat, terutamanya pada 25℃ . Seperti kes wanita mengandung, sifat dielektrik tersebut juga akan menurun dengan suhu sebelum titik silang, sebelum meningkat semula selepas titik tersebut. Terdapat perbezaan statistik yang ketara dalam Ɛ' dan Ɛ'' antara subjek sihat dan pesakit kanser pundi kencing (P<0.05). Tahap korelasi yang tinggi telah diperhatikan antara sel-sel urotel terkelupas dan sifat dielektrik kencing pada 25°C. Ɛ' berkolerasi secara negatif (r_{max}=-0.662, f=5GHz) dengan sel urotel terkelupas, manakala Ɛ'' berkolerasi secara positif dengan sel urotel terkelupas (r_{max}=0.664, f=40.6GHz).

ACKNOWLEDGEMENTS

First of all, I would like to express my sincerest gratitude to my two supervisors, Associate Professor Ir. Dr. Ting Hua Nong and Professor Dr. Ng Kwan Hoong, for their constant guidance and encouragement during my PhD studies. They had provided valuable suggestions and innovative ideas to carry out my research. In the preparation of this thesis, they had spent much time checking each draft and provided me with valuable advice to improve my work. Without their support and instruction, this thesis could not be possibly completed.

Secondly, I would like to thank my two consultants, Associate Professor Dr. Ong Teng Aik and Professor Dr. Mun Kein Seong, who provided their professional experience and knowledge to assist my work. They had also kindly helped in the collection of urine samples from their respective institutions.

Thirdly, I would like to express my utmost gratitude to the University of Malaya for supporting me with a postgraduate research grant (PPP grant number: PG056-2015A), and to my department (Biomedical Engineering, Faculty of Engineering) for providing me with the facilities and equipment to carry out my work.

Last but not least, my greatest thanks to my beloved family back in my homeland for their consistent support and great confidence in me all the way.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA: Analysis of Variance

BCa: Bladder Carcinoma

BCG: Bacillus Calmette-Guerin

BTA: Bladder Tumour Antigen

CCD: Charge-Coupled Gadget

CT: Computed tomography

CIS: Carcinoma *in situ*

DCE: Dynamic Contrast Enhanced

DGE: Dynamic Glucose-Enhanced

DW: Diffusion-Weighted

E-Cal: Electronic-Calibration

hCG: Human Chorionic Gonadotropin

FDA: Food and Drug Administration

FDP: Fibrinogen Degradation Products

FISH: Fluorescence *in situ* Hybridization

FGFR3: Fibroblast Development Element Receptor 3

MATLAB: Matrix Laboratory

MIBC: Muscle Invasive Bladder Cancer

MRI: Magnetic Resonance Imaging

NAIP: Neuronal Apoptosis Inhibitory Protein

NLRP: Nod-Like Receptor Protein

NMIBC: Non-muscle Invasive Bladder Cancer

NMP22: Nuclear Matrix Protein 22

PCR: Polymerase Chain Response

PDD: Photodynamic Diagnosis

PMT: Photomultiplier Tube

POC: Point-of-Care

RC: Radical Cystectomy

RS: Raman Spectroscopy

SDS-PAGE: Sodium Dodecy Sulfate Polyacrylamide Gel Electrophoresis

SQ: Squamous Cells

T2W: T(2)-Weighted

TVU: Transvaginal ultrasonography

UBC: Urothelial bladder cancer

UCC: Urothelial Cell Carcinoma

UCS: Urethro-Cystoscopy

UMMC: University of Malaya Medical Centre

USPIO: Ultrasmall Superparamagnetic Iron-Oxide

VNA: Vector Network Analyzer

WLI: White Light Imaging

FACS: Microfluidic Fluorescence-Initiated Cell Sorter

ε': Dielectric Constant

ε'': Dielectric loss

ε^*: Complex Permittivity

ε_∞: Infinite Frequency Permittivity

$\Delta\varepsilon$: Magnitude of Dispersion

ε_v: Vacuum Permittivity

ω: Angular Frequency

τ: Dielectric Relaxation Time

tan σ: Loss Tangent

σ_s: Static Conductivity

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CHAPTER 1 INTRODUCTION

1.1 Introduction

hCG, namely the human chorionic gonadotropin, is a glycoprotein hormone secreted by placental trophoblast cells, supporting the corpus luteum of the ovary, which in turn will support the endometrium, maintaining the pregnancy (Cole, 2009). Once a fertilized egg is implanted in the womb, hCG levels will increase and they can be detected in large amounts in the blood and urine of pregnant women (Balakrishnan et al., 2015). Thus, hCG is used as one of the most significant medical biomarkers for determining the fact of a woman's pregnancy. Besides, through quantitatively detecting hCG, it can not only help us to understand how well the fetus grows, but also greatly assist with diagnosing the some certain complications as well the pregnancy cares.

Meanwhile, bladder cancer is relatively common and is the most malignant tumour of the urinary system. In recent years, the incidence of bladder cancer has risen steadily, with over 90% of the patients being diagnosed histologically with transitional cell carcinoma, 5% as squamous cell carcinoma, and less than 2% as bladder adenocarcinoma (Kim et al., 2008). Its incidence has been ranked No.1 among the malignant cancers of the urinary system, second only to prostate cancer in Western countries. Approximately, the initial symptoms of 70% of bladder urothelial cell carcinoma (UCC) patients are presented superficially. Even though there is 60 to 85% chance that UCC will recur, which is relatively high, over 80% UCC is still constricted in the submucosa, thus having no effect on patient's life (Boman et al., 2002).

Nevertheless, it still requires to be followed long-term and extensively, thus preventing it from aggravating and becoming invasive UCC that will threaten patients' lives.

Currently, the urethra-cystoscopy (UCS) and urine cytology is the standard approach of caring UCC, in which the USC is regarded as the gold standard. For the first two years of UCC, urine cytology is required once three to four months, and then the time span will be longer subsequently. However, this method is expensive and invasiveness. Even though the urethra cystoscopy is flexible, it still has a 10% chance of getting urinary tract infection (Ramakumar et al., 1999). Therefore, due to such circumstances, there are more new tests based on urine for UCC being developed currently, and some of these new approaches have already gained the approval from the Food and Drug Administration (FDA), which are the bladder tumour antigen stat (BTAstat), bladder tumour antigen trak (BTAtrak), nuclear matrix protein 22 (NMP22), fibrinogen degradation products (FDP), ImmunoCyt and fluorescence *in situ* hybridization (FISH) (Serretta et al., 2000; Poulakis et al., 2001). It is mostly hopeful by applying new makers to the initial research, but it seems that the reports conducted successively always cannot show the comparable outcomes. Therefore, usually patients will select other approaches, which could be the best indicator implying the differences between various urine marker studies. With the goal of reducing the quantity of UCS that needs to be followed, it is of a great importance to identify the specificity of the test based on the urine.

Moreover, the incidence of bladder cancer among males is three to four times higher than that of females (Jemal et al., 2011; Siegel et al., 2012). The most common ways to diagnose bladder cancer include urinary cytology, cystoscopy and biopsy. Urinary cytology and cystoscopy with biopsy has long been the gold standard (Dey, 2004). But most of these methods (biopsy, cystoscopy, etc.) are invasive and often cause pain, bleeding, urinary tract infections and other complications in patients. It is, therefore, of great advantage for the patient if an effective non-invasive method can be developed.

Recently, whether in the field of medicine or diet, the application and biological effects of dielectric properties have attracted increasing attention from many researchers. As a result, the wide application of dielectric property measurement has turned to one of the study hot-spots of clinical detection. Being affected by the action of external electric field, the internal electrical movement of a material will be reflected by the dielectric property of a material. There are interactions happening between the non-ionizing microwave radiation and the relevant biological fluid and tissue inside, thus obtaining the dielectric contract in accordance with the content of water.

Thus, many studies have proposed the use of dielectric properties as a non-invasive measurement of biological (both human and animal) fluids and tissues (Foster & Shchwan, 1989; Popovic et al., 2005; Rajasekharan et al., 2010; Valero et al., 2010; Kumar & Raju, 2014). The electrical property of the biological tissue, despite *ex vivo* or *in vivo*, has been summarized by Popovic et al. (2005). At the same time, through the observation of the dependent relationship of frequency and the mechanism that is responsible for the indications, the most likely field of electromagnetic interaction has been also found by him. By applying different frequencies, cancerous tissues from human body and animal body have been studied, through which their dielectric properties are able to be found and studied (Kumar & Raju, 2014).

Under various factors from environment and physiology, Abdul Rauf (2013) has studied and released the different results of dielectric property existing in human plasma and blood. In his report, the great differences among the three dielectric factors which are permittivity, dielectric loss as well as conductivity existing in human plasma and blood, the cell concentration and the erythrocyte membrane have been studied. Park et al. (2003) and Caduff et al. (2003) have tried to use electronic experimental devices to

show the relationship between blood glucose concentration and dielectric constant, of which the results are not of a great significance. Mun et al. (2015) found that the dielectric property in various blood glucose concentrations varies the most at 25^{o}C. Moreover, there are some studies preliminarily reporting the changing statuses of dielectric property existing in some specific fluids like urine (Lonappan et al., 2007a; Lonappan, 2012;), semen (Lonappan et al., 2006), mucus (Lonappan et al., 2007a; Rajasekharan et al., 2010) or saliva (Lahane et al., 2013; Ranade et al., 2016) with diseases or physiological variation. Barry (1986), Ghodgaonkar et al. (1990) and Liao et al. (2003) reported that to measure the complex permittivity of a material, different measurement techniques can be endorsed; the chosen technique may vary, depending on various factors such as the nature of the sample, different temperatures and the frequency range used.

Disease development is associated with the excretion of carcinogenic metabolites in the urine, thus, it can be used as a medical basis to support the detection of dielectric properties in urine. As for the tumour cells, the electron-negativity of the cell surface will be increasing, which however cannot be cured by the deficiency of the cell calcium channels (Mostov & Tao, 2003). Exfoliated epithelial cells in the urine are an important diagnostic marker of bladder disease. It has been pointed out that a lot of core changes in cell metabolism are closely related to the electron-chemistry exchanges existing in the cell membrane. With that being said, through directly analyzing these electrical indicators, the core information about changes in cellular biology can be explained (Sun & Morgan, 2010; Valero et al., 2010; Chen et al., 2011). During the processes of forming a cancerous cell, the electrical property in the cell membrane will be changing, which is one of the most crucial behaviors, indicating that the cells at initial cancerous stage are disrupting and deteriorating (Han et al., 2006; Szachowicz et al., 2010).

Moreover, during the metastatic process of cancerous cells, it has been shown that the biological electricity of cell membrane is also disrupting (Stern et al., 1999; Hong et al., 2011). Studies have shown that when using the TRIMprob^{TM} system in soft tissues, significant differences may be observed at 465MHz, which can characterize patients at risk for bladder and prostate cancer (Bellorofonte et al., 2005; Gervino et al., 2007). In another study that compared the impedance data of bladder epithelial cells and their corresponding biopsy tissue pathology results, it was found that the bladder benign group had lower resistance than that the malignant group, and electrical impedance spectroscopy can be used as a useful technique to identify bladder disease (Keshtkar et al., 2012).

So far, not many studies have reported on the changes in dielectric properties of hCG with temperature. Moreover, there were no studies to correlate between hCG and dielectric properties. To our knowledge, no study has been reported on the dielectric properties of urine in bladder cancer. In addition, there have also been no studies measuring the changes in dielectric properties of urinary exfoliated epithelial cells at different temperatures, or any correlation studies between urinary exfoliated epithelial cells and their dielectric constant. In our study, we measured the dielectric properties (dielectric constant and dielectric loss) of urine from pregnant women, bladder cancer patients and healthy subjects using the microwave technique at frequencies between 0.2 and 50 GHz, and at three different temperatures (25 ^{o}C, 30 ^{o}C, 37 ^{o}C).

1.2 Problem Statement

The study of dielectric properties of urine in related to pregnancy is limited while the study of dielectric properties of urine for bladder cancer is new. To the best of our knowledge, no study has reported about the changes in dielectric properties of urine and exfoliated urothelial cells with temperature, and no data has also been reported on the diagnostic and prognostic values of exfoliated urothelial cells in bladder cancer. Moreover, the correlation between hCG and dielectric properties has not been studied. No studies have reported on the dielectric properties of urine in bladder cancer. In addition, there are also no studies measuring the changes in dielectric properties of urinary exfoliated epithelial cells at different temperatures, or any correlation studies between urinary exfoliated epithelial cells and their dielectric constant.

1.3 Objectives

The purpose of this study is to investigate dielectric properties of urine from pregnant, bladder cancer and healthy subjects.

The three main objectives of this study are:

1) To identify the significant differences in the dielectric properties of urine among healthy subjects, pregnant subjects, and subjects with bladder cancer at different Microwave frequencies (0.2-50GHz) and temperatures (25 ^{o}C, 30 ^{o}C, 37 ^{o}C).
2) To study the composition of the urine samples and the effects of temperature on the dielectric constant, dielectric loss and loss tangent.
3) To investigate the correlation between urinary dielectric properties with hCG and urinary urothelial cell, respectively.

1.4 Scope of the Study

This study investigates a novel non-invasive and safe method for the diagnosis and detection of pregnancy and bladder cancer. This study observes the significant differences in urinary dielectric properties and temperatures between healthy subjects and the target subjects. Moreover, the correlation between the specific composition (hCG and urinary exfoliated epithelial cells) and dielectric properties is determined as well.

Medical personnel are better equipped to handle the collection and measurement of urine samples. The use of qualified medical personnel will help patients feel safe and comfortable during the detection process. Qualified personnel would also help to reduce the risk of contaminating samples or infecting patients. Thus the development of this new diagnostic method can help train medical personnel for detection and diagnosis of pregnant subjects and those with bladder cancer.

1.5 Outline of Thesis

There are five chapters in this thesis:

Chapter 1 briefly introduces the key concepts of this research, namely pregnancy, bladder cancer and dielectric properties. The first chapter will also expound the objectives, problem statements, and scope of this research.

Chapter 2 reviews the relevant methods of diagnosing or detecting pregnancy and bladder disease. This chapter also throws light on the existing research on dielectric properties. It will focus mainly on the dielectric properties of solutions and cells related to this experiment. The measurement techniques of dielectric properties will also be presented in this chapter.

Chapter 3 presents the experimental set-up, theory and design of the whole study method. Sample collection, data processing and procedures are also described in detail.

Chapter 4 explains the results and discussion the experiments. It then compares the results with other papers in this field of study.

Lastly, Chapter 5 summarizes the whole article and suggests improvements for any future or follow-up studies.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter describes the detection of pregnancy and diagnosis of bladder disease through the evaluation of urine dielectric properties, besides discussing their existing research. Recently, the bio-effects of microwave on biological materials are gaining attention in medical diagnostics, with research being focused on the evaluation of dielectric properties to detect diseases and other physical anomalies.

Dielectric property is inherent in all materials that can impede the flow of electrical currents, and hence, their molecules become polarized when exposed to an external electromagnetic field. It determines the extent of their energy coupling and dissipation capability. In medical and biological terms, knowledge of dielectric properties is important in the field of theoretical dosimetry, which aims to relate the effects of electromagnetic fields on the body, and to the resulting power absorption. For example, if urine, which is 95 per cent water, becomes greatly polarized, then it is possible that the water molecules are the ones storing the potential energy in an electric field (Gupta et al., 2004).

More studies are supporting the evaluation of dielectric properties as a non-invasive approach to diagnose diseases in humans and animals because diseases would alter the dielectric properties of the body (Foster & Shchwan, 1989; Popovic et al., 2005; Rajasekharan et al., 2010; Valero et al., 2010; Kumar & Raju, 2014). Ghodgaonkar et al. (1990) and Liao et al. (2003) have reported that to measure the complex permittivity of a material, different measurement techniques can be utilized; the technique selection may vary, depending on various factors such as the nature of the sample, different

temperatures and the frequency ranges used.

Permittivity is a coefficient that represents the characteristics of insulation capability, the symbol of it is Ɛ, the unit is (F/m). When an electric field is applied to a medium, induced charge will be generated to weaken the electric field. The ratio between the original applied electric field (in vacuum) and the electric field in the final medium is relative permittivity, it also commonly known as the dielectric constant (Ɛ'). Dielectric dielectric loss is to measure the parameters of the dielectric loss degree, and the symbol of it is Ɛ''. In summary, the ability of a biological sample to store energy is measured as Ɛ'. On the other hand, dielectric loss quantifies a sample's inherent dissipation of electromagnetic energy.

2.2 Overview of Dielectric Properties

2.2.1 Dielectric Properties

More than 70 years ago, Nobel laureate Peter Debye (1884-1966) began studying the phenomena of polarized substances and proposed various models to describe them. In physics, dielectric relaxation refers to the relaxation response of a dielectric medium to an external electric field. This relaxation is often described in terms of permittivity as a function of frequency, which can, for ideal systems, be described by the Debye (1929) equation. The Debye relaxation is the dielectric relaxation response of an ideal, non-interacting population of dipoles to an alternating external electric field. It is usually expressed in the complex permittivity Ɛ of a medium as a function of the field's frequency ω. By applying the Debye model, it can show how dielectric relaxation respond to each other (Buchner et al., 1999; Komarov et al., 2005; Koeberg et al., 2007).

This paper proposes using the open-ended vector network analyzer to identify the urine of pregnant women and bladder cancer patients at a frequency range of between 200MHz and 50GHz, which is based on the Debye model. The Single-Debye equation is used to resolve the problem of curve-fitting function is used to draw the figures as shown in Equation 1.

$$^{*} = \ _{\infty} + \frac{\Delta}{1+j\omega\tau} - j\frac{\sigma_s}{\omega \ _v} \quad (1)$$

where:

Ɛ* = complex permittivity

$Ɛ_{\infty}$ = infinite frequency permittivity

ΔƐ = magnitude of dispersion

$Ɛ_v$ = vacuum permittivity ($Ɛ_v$=8.854 PF/m)

ω = angular frequency (ω=2πf)

τ = dielectric relaxation time (ms)

σ_s = static conductivity

To ensure that the equation is feasible, $Ɛ_{\infty}$, ΔƐ, τ and σs need to remain in a reasonable value, which are $Ɛ_{\infty} \geq 1$, $\Delta Ɛ \geq 0$, $\tau \geq 0$ and $\sigma_s \geq 0$. When comparing the dielectric properties of biological fluids using the Debye model, the frequency range should be set between 200MHz and 10GHz at 37°C (Peyman et al., 2011). In porcine urine, the dielectric properties have been shown to conform with the Debye model at the frequency range of 50MHz to 20GHz (Peyman & Gabriel, 2012). Equation 1 is applied to determine the dielectric properties of human urine at the frequency range of between 200MHz and 50GHz at 25°C, 30°C and 37°C. The study by Mun et al. (2015) has obtained data that conforms well with the Debye model.

2.2.2 Dielectric Properties Theory

A dielectric is a material having electrical conductivity low in comparison to that of a metal. It is characterized by its dielectric constant and dielectric loss, both of which are functions of frequency and temperature. The dielectric constant is the ratio of the strength of an electric field in a vacuum to that in the dielectric for the same distribution of charge. It may also be defined and measured as the ratio of the capacitance C of an electrical condenser filled with the dielectric to the capacitance C_0 of the evacuated condenser:

$$= C/Co \qquad (2)$$

The increase in the capacitance of the condenser is due to the polarization of the dielectric material by the applied electric field. Dielectric properties of materials can be defined in terms of relative permittivity or dielectric constant. The relative permittivity or dielectric constant is the ration $\mathcal{E}/\mathcal{E}_0$. In the meter-kilogram-second (MKS) system of units, the dielectric constant of free space is 8.854×10^{-12} farad/m, while in the electrostatic system of units (ESU) system the relative and the absolute dielectric constants are the same. When variation of the dielectric constant with frequency may occur, the symbol is commonly primed. When a condenser is charged with an alternating current, loss may occur due to dissipation of part of the energy as heat. In vector notation, the angle d between the vector for the amplitude of the charging current and that for the amplitude of the total current is the loss angle, and the loss tangent, or dissipation factor, as follows:

$$\tan \quad = \frac{\text{loss current}}{\text{charging current}} = - = \frac{\sigma}{\omega \quad _0} \qquad (3)$$

Dielectric properties are frequency dependent. For moist dielectric materials, ionic conductivity is dominant at frequencies lower than 200MHz. Ionic conductivity and free water dipole rotation are combined to take part at microwave frequencies. Maximum

dielectric loss at a relaxation frequency (f_c) relates to relaxation time ($\tau = \frac{1}{2\pi f_c}$). Relaxation time is the time required for dipoles to fully orientate in an electrical field. Dielectric loss materials convert electrical energy into heat in the presence of microwave frequencies that increase temperature. The increase of temperature is directly proportional to dielectric loss (Nelson, 1996). However, ionic conductance and dipole rotation are the dominant loss mechanisms at microwave frequencies (Ryynänen, 1995).

2.3 Review of Dielectric Properties of Fluids and Tissue

2.3.1 Pure Water

The increase in permittivity of pure water is positively correlated with blood serum concentration by Oncley (1938). According to Matyushov (2012), the author has studied the dependent relationship of frequency in the simulation of dielectric properties existing in the interactions between the protein and water.

2.3.2 Biological Fluids

A mixture of substance and biological material can influence a solution's dielectric property. Cohn (1941) reported how well insulin could dissolve in different solutions as well as their dielectric properties. Bayley (1950) reported that radio frequency enables polar relaxation, which resulted in different dielectric properties in insulin and chymotrypsinogen.

Abdul Rauf (2013) has observed huge differences among three dielectric factors - permittivity, dielectric loss and conductivity-in human blood, cell concentration and erythrocyte membrane in various environments and physiologies. At 25^{o}C, Mun et al. (2015) also found variations in the dielectric properties of different blood glucose

concentrations. Moreover, there are studies reporting the changing status of dielectric properties in semen (Lonappan et al., 2006), urine(Lonappan et al., 2007a), mucus (Rajasekharan et al., 2010), blood (Lonappan, 2012) or saliva (Lahane et al., 2013; Ranade et al., 2016) due to diseases or other physiological conditions.

2.3.2.1 Urine

Lonappan et al. (2007b) used cavity perturbation to investigate the dielectric properties of urine from pregnant and non-pregnant women. Using a microwave frequency of between 2 and 3GHz, the authors observed that urine samples of non-pregnant women had lower reaction compared with those who were pregnant. Moreover, the permittivity of urine from non-pregnant women was greater than their pregnant peers. Through applying different frequencies given from 0.2 to 50 GHz, Mun et al. (2015) has investigated the dielectric property of proteinuria in urine, and there were significant differences between healthy groups and patients group with kidney disease.

2.3.2.2 Blood

In order to study how blood glucose level associates with the permittivity, Park et al. (2003) and Caduff et al. (2003) have tried to utilize the electrical equipment; however it did not provide significant findings. The hCG in blood is variable, which would also make the dielectric property change, found by Lonappan (2012). After studying semen and urine samples, he expanded his study to the dielectric properties of blood samples from pregnant and non-pregnant women, which is influenced by hCG. Using the same methods and settings as the urine samples, he observed that the blood samples of women who are not pregnant showed less reaction and higher permittivity than samples of women who are pregnant. However, the author did not study the effects of the microwave frequency above 3GHz and the study temperature was not controlled.

2.3.3 Tissue Samples

Under previous studies by Gabriel et al. (1996a), (1996b) and (1996c), the dielectric properties of human and animal tissues had been observed to be significantly different. Those studies provided a historical review of tissue dielectric properties measured using various mechanisms and electrode polarization.

The dielectric properties of the brain, heart muscle, kidney, liver, lung, spleen, uterus, bladder, skin, bone and muscle have been summarized, and the summarization of the electrical property of the biological tissue, despite *ex vivo* or *in vivo*, has been given by Popovic (2005). At the same time, through the observation of the dependent relationship of frequency and the mechanisms that help for the indications, the most likely field of electromagnetic interaction has been found by him. Kumar and Raju (2014) observed the dielectric properties of cancerous tissue in human and animal bodies at varying frequencies.

2.4 Cellular Samples

In tumour cells, there are high levels of calcium ions at the cell surface due to deficiency of ion channel pumps. This causes the cell membrane to remain in a polarized state, allowing tumour cells to be presented with different dielectric properties than healthy cells (Mostov & Tao, 2003).

Exfoliated bladder epithelial cells in the urine are vital samples to diagnose bladder disease. Diseased cells have undergone core changes in their metabolism, especially those relating to electron-chemical exchange on the cell membrane. Therefore, analyzing the electron-chemical properties of the cells may reveal key information exchanges in their metabolism (Sun & Morgan, 2010; Valero et al., 2010; Chen et al.,

2011). Electron-chemical change in the cell membrane is considered an indicator of early stage cancer (Cure, 1995; Han et al., 2006; Szachowicz et al., 2010). In the course of metastasis, the increasing number of cancerous cells is also related to the disruption of cell membrane bio-electricity (Stern et al., 1999; Hong et al., 2011). Electrical impedance spectroscopy is a useful technique to identify bladder disease. For example, when the impedance data of cancerous bladder cells were compared, it was found that electrical resistance in benign tumours was lower than malignant ones (Keshtkar et al., 2012).

2.5 Detection and Monitoring of Pregnancy

Pregnancy is mainly confirmed through urine or blood test. At present, the most common method is pregnancy urine test strip. This method is mainly used to detect morning urine, because the content of hCG value in morning urine will increase rapidly after pregnancy, and this method becomes cheap and accurate (>99%) for detecting pregnancy in the current. Meanwhile, it can also be used to detect pregnancy through blood sample. Blood sample is mainly used to check the content of progesterone and hCG value. The most important indicator is the presence of hCG produced by the placenta. Quantifying a woman's hCG level is of great help not only in observing the growth and development of the baby, but also providing good reference in diagnosing and treating complications (Lonappan, 2012; Qasim et al., 1996). Pregnancy could be detected by radio-receptor assay for the determination of hCG and luteinizing hormone in plasma (Saxena et al., 1974). The method of evaluating early pregnancy by serial hCG determinations were evaluated by receiver operating characteristic curve analysis, it indicated that a more precise definition of the normal values of hCG doubling time results in improved early detection of pregnancy (Donald et al., 1985).The gestational age and the serum human chorionic gonadotropin at which an intrauterine pregnancy can be detected using transvaginal ultrasonography (TVU) is used, and it can be concluded

that TVU can detect an intrauterine gestation earlier than what has been previously reported with TVU (Gregory et al., 1988). The hCG in blood is variable, which would also make the dielectric property change (Lonappan, 2012). After studying semen and urine samples, he expanded his study to the dielectric properties of blood samples from pregnant and non-pregnant women, which is influenced by hCG. Using the same method and setting as the urine samples, he observed that the blood samples of women who are not pregnant showed less reaction and higher permittivity than samples of women who are pregnant. Pregnancy is heavily regulated by hormones and they may contribute to its symptoms like nausea, vomiting, headache, swollen breasts, food craving and lethargy.

2.6 Diagnosis and Monitoring of Bladder Cancer

Bladder cancer is the sixth most common malignant tumour cancer in men and the 17th most common malignant tumour cancer in women (Bray et al., 2018). It is the most frequent cancer of the urinary system in Western countries, second only to prostate cancer. Its incidence ranks at first place among malignant cancers of the urinary system, second only to prostate cancer in Western countries. In recent years, the incidence of bladder cancer has risen steadily. More than 90% of bladder cancer patients are diagnosed with transitional cell carcinoma, 5% as squamous cell carcinoma and less than 2% as adenocarcinoma (Kim et al., 2008). Among first-time patients, 70% to 85% are presented as non-muscle invasive bladder cancer (NMIBC), while 15% to 30% are muscle invasive bladder cancer (MIBC) (Witjes et al., 2014). NMIBC is a superficial cancer and its pathological stages include Ta (papillary), T1 (infiltration of lamina propria) and carcinoma *in situ* (CIS). Ta patients comprise 70% of cases, T1 roughly 20% and CIS about 10%. MIBC stages include T2, T3 and T4 (Burger et al., 2013; Witjes et al., 2014). Up to 80% of NMIBC patients have been observed to suffer relapse within five years, where 30% of Ta patients develop MIBC, while all T1 and CIS patients are

likely to suffer MIBC (Babjuk et al., 2013; Knowles & Hurst, 2015). The standard treatment for NMIBC is trans-urethral resection, whereas radical cystectomy (RC) and/or chemotherapy is performed for MIBC (Weihong et al., 2014; Witjes et al., 2014).

The occurrence of bladder cancer is a complex and multifactorial process due to intrinsic genetic factors or external environment factors. Smoking and prolonged exposure to aromatic amines have been proven to be major causes. Smoking is believed to be responsible for 30% to 50% of the cases, where it increases the risk by two to four-fold. The incidence is proportionate to the intensity and frequency of cigarette consumption.

Hematuria is the most common symptom of primary bladder cancer. Its nature includes full course, intermittent and painless gross hematuria, sometimes accompanied by blood clots (Jacobs et al., 2010; Babjuk et al., 2013). Other clinical manifestations include microscopic hematuria, lower urinary tract symptoms and infection (Kamat et al., 2014). Statistics show that bladder cancer has a high rate of occurring, progressing and recurring. Therefore, it is extremely important to accurately diagnose patients in the early stage and monitor those with high-risk postoperative disease. Bladder cancer can be diagnosed through biopsy, cystoscopy, urine cytology, fluorescence in-situ hybridisation (FISH), urine protein analysis (BTAstat, BTRtrak and NMP22) and radiation imaging like CT and MRI scans (Babjuk et al., 2013; Fei et al., 2014).

Cystoscopy is the gold standard for diagnosis, but it is invasive and causes pain, bleeding, infections and other complications. Besides, it is sometimes difficult to detect tumours on the top and forearm of the bladder, which limits its clinical application. Urine cytology is a non-invasive test to detect tumour cells shed in urine. Although it is simple and low cost, it has low sensitivity and diagnostic efficiency. It will, therefore, be of great advantage if

an effective, accurate non-invasive diagnostic method can be developed.

2.6.1 Epidemiology and Efficiency of Diagnostic Methods

In Western countries, the four major malignancies afflicting men are prostate, lung, colon and bladder cancers. Figure 2.1 shows the frequency of bladder cancer in 25 countries compiled in 2018. Firstly, it is obvious that the disease has very strong preponderance towards men, where the incidences are more than double, even triple, that of women. In Europe and North America, bladder cancer comprises about 5% to 10% of all male malignancies. Secondly, countries with homogeneous white populations seem to top the frequency rate, confirming the vulnerability of white men towards the disease. Even in the USA, the disease frequency among white men is also almost double than blacks. It is not very prevalent in Asian countries like China, Japan and India.

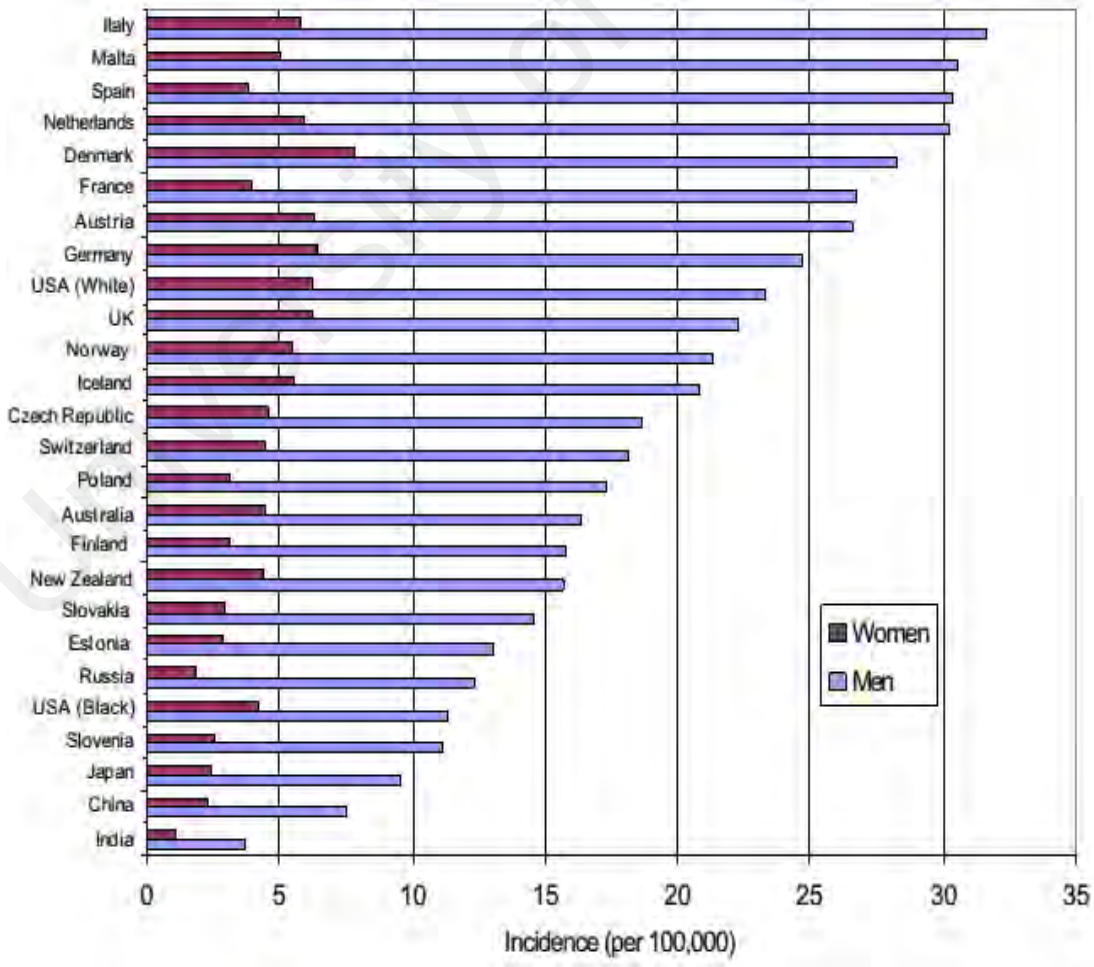


Figure 2.1: Age-institutionalized frequency rates of bladder cancer (Adjusted from Tumour Frequency in Five Continent) (Bray et al., 2018).

In Figure 2.2, the cumulative risk of bladder cancer in people aged below 75 is 2% to 4% in men and 0.5% to 1% in women. This figure is quite low compared with the danger of lung cancer, for instance, which is 8% for men and 2% for women. The middle age at conclusion is 65 to 70 years (Bray et al., 2018).

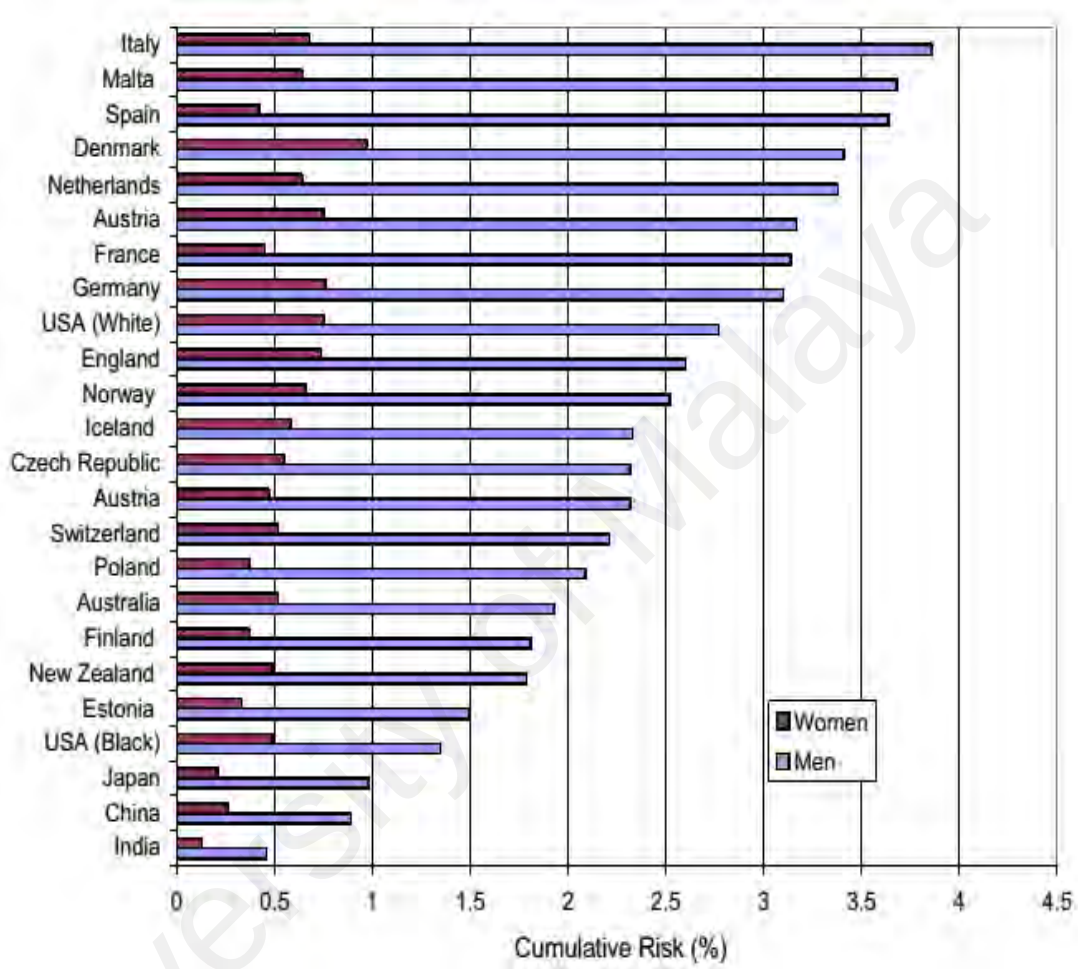


Figure 2.2: Combined risk of bladder cancer in people aged below 75 (Adjusted from Disease Frequency in Five Continents) (Bray et al., 2018).

Bladder tumours may be caused by carcinogenic substances and have variable history among patients. Although most cases present as shallow tumours of early stage, they have high chance of progression that may eventually lead to fatality. This study compiles the conclusions of various studies on disease from the Arranging and Evaluating, and Finding Advisory group of the Bladder Tumour Accord Gathering. The suggestions are based on the quality of the current accessible confirmation (Bray et al., 2018). It summarizes the detection methods and their sensitivity/specificity, and discusses the advantages and disadvantages of cancer cell detection methods.

2.6.1.1 Urine Microscopy

Urine microscopy is vital in determining conditions affecting the kidneys and urinary tract. This procedure is performed by nephrologists and pathologists, where the urine of patients is examined at the microscopic level for abnormalities, such as the presence of cancerous cells, erythrocytes, leukocytes and puss (Perazella, 2015). The equipment needed for the procedure include:

- Sterile Dressing pack
- 10ml syringe
- Alcohol swab
- Sharps box
- Urine specimen pot
- Specimen bag and correct microbiology form; and,
- Re-usable transport container

Urine can be collected by spontaneous micturition, bladder compression, catheterisation and cytocentesis, where a needle is placed into the bladder through the abdomen. Urine samples should be acquired aseptically and patients do not face any risk of injury or infection unless an invasive method was used (Vasudevan, 2014).

The disadvantage in urine microscopy includes contamination of samples by bacteria and exudates from the lower urinary and genital tracts. If there is a high content of bacteria in the urine, it may complicate diagnosis on whether the patient is having a urinary tract infection. Table 2.1 shows the sensitivity and specificity of urine microscopy determined in several studies on bladder cancer. The values are around 90%, which makes the procedure a good diagnostic tool for urinary tract diseases.

Table 2.1: Sensitivity and specificity of urine microscopy for bladder cancer

Authors	Year	Sensitivity/specificity (%)	References
Nataraju et al.	2018	87 / 92	(Nataraju et al., 2018)
Becker et al.	2016	87.5 / NA	(Becker et al., 2016)
Williams et al.	2010	91 / 96	(Williams et al., 2010)

2.6.1.2 Urine Cytology

Urinary cytology is performed under a microscope to screen the urine of a patient with bladder cancer for cancer cells. If the sample includes abnormal cells, the doctor will ask the patient for another sample. A case in point is urine tests, for instance, the UroVysion test, ImmunoCyt test and NMP22 test. The equipment for the urine cytology utilizes 3x 250ml Sterile Containers and the procedure are as follows:

- Take three sample containers from the Collection Centre that is closest to patient's place.
- The first urine you have in the morning is not recommended.
- In the next several hours, drink more water.
- Put the next urine in the container you have collected from the centre.
- About 70ml of urine is required.
- Keep the container lid tightly closed.
- Write down the number and date on every container.
- Patient's last name, first given game and date of birth are also required to be written down on the containers.
- Send all the collections back to the center on the same day; otherwise the samples should be refrigerated.
- It does not require collecting the urine consecutively.

Urine cytology evaluates sediments for the presence of cancerous cells shed along the urinary tract. The test involves the utilisation of fluorescence in-situ hybridization (FISH), where the cells are stained with florescence-labeled urine biomarkers (DNAs, antigens or antibodies) to detect chromosomal or protein anomalies. However, urine cytology is not routine in clinical practice because it is a complicated method (Councli, 2014). Table 2.2 shows the sensitivity and specificity of urine cytology from several studies.

Table 2.2: Sensitivity and specificity of urine cytology for bladder cancer

Authors	Year	Sensitivity/specificity (%)	References
Kumar et al.	2017	13.3 / 100	(Kumar et al., 2017)
Lee et al.	2015	86 / 73	(Lee et al., 2015)
Anai et al.	2014	83 / NA	(Anai et al., 2014)
Hajdinjak	2008	42 / 96	(Hajdinjak, 2008)

Normally, the specificity is lower for the low-and-high-reexamination ulcer in the samples that are invalid or the subsequently-coming settings, which is generally thought to be the margin of a positive cytology. Then again, the affect-ability for poor quality injuries was higher in voided specimens interestingly with high review sores, which were identified all the more frequently on instrumented examples (Brimo et al., 2009).

Urine collection methods have been observed to affect the quality of samples for analysis. Table 2.3 shows the advantages and disadvantages of different sampling methods. Non-invasive methods are safe and easy, but they tend to produce low quality samples prone to contamination. Catheterization and bladder washing may produce samples with high cellularity and good preservation, but leave a lot of instrumentation artifacts.

Table 2.3: Comparison of urine cytology specimens (Brimo et al., 2009)

Specimen Type	Advantages	Disadvantages
Voided urine	-Non-invasive -No instrumentation artifact	-Low cellularity -Vaginal contamination -Poor preservation
Catheterized	-High cellularity	-Invasive and high risk -Instrumentation artifacts -Poor preservation
Bladder washing	-High cellularity -Good cell preservation	-Invasive and high risk -Instrumentation artifact
Upper tract washing	-High cellularity -Good preservation -Selective sampling	-Invasive and high risk -Instrumentation artifacts
Brush cytology	-Selective sampling	-Invasive and risky -Air drying possible with direct smear

2.6.1.3 Urine Biomarkers

Urine biomarkers comprise DNA, antigens and antibodies that bind to specific sites of cancerous or abnormal cells found in patient samples, such chromosomes, nuclear protein and cellular wall. These biomarkers are usually tagged with fluorescent probes so the cells can be visualized in FISH assays. Although there are more than 30 biomarkers available to detect bladder cancer, only a few can be used in urine cytology (Schiffer et al., 2009).

Most popular biomarkers come in commercial kits and have been approved by the US Food and Drug Administration only for use in conjunction with cytoscopy. They include the UroVysion Bladder Cancer Kit, which contains DNA probes to detect aneuploidy for chromosomes 3, 7, 17 and loss of the 9p21locus; BTAstat kit, which contains bladder tumour antigen; NMP22 antibody kits; and, the ImmunoCyt/uCyt+ kit that comes with M344, LDQ10 and 19A211 monoclonal antibodies. It is universally acknowledged that bladder cancer should be diagnosed with urine tests such as the UroVysion test, ImmunoCyt test or NMP22 test. Each of these examinations is conducted by recognizing chemicals, proteins and changes in chromosomes in the urine (Council, 2018). Commercially available tests include:

- Urine cytology
- FISH
- NMP22
- BTAstat
- BTAtrak
- ImmunoCyt/uCyt+
- CertNDx

When surveying a biomarker's effectiveness, researchers should take the local population into consideration because that may influence results at a later stage of illness. Although the mark of the machine may be low, several biomarkers have been subjected to focused research. In Table 2.4, it can be seen that the use of urine biomarkers have high sensitivity in detecting bladder cancer, including low grade tumours. However, it is tedious and expensive to use, and subjected to high inter-observer variability (Vrooman & Witjes, 2008). Table 2.5 shows the advantage and disadvantage of different urine biomarkers (Smith et al., 2013).

Table 2.4: Sensitivity and specificity of urine biomarkers for bladder cancer

Urine markers	Sensitivity/specificity (%)	References
BTAtrak	72-99 / 12.1-78	(Tsui et al., 2007; Babjuk et al., 2008; Smith & Guzzo, 2013)
BTAstat	56-83 / 72-85.7	(Raitanen & Group, 2008; Smith & Guzzo, 2013)
NMP22	51-100 / 73-90	(Tsui et al., 2007; Smith & Guzzo, 2013)
UroVysion	80-86 / 61-86	(Hajdinjak, 2008; Pajor et al., 2011)
ImmunoCyt	68.1-72.5 / 65.7-72.3	(Comploj et al., 2013; He et al., 2016)
UBC	53.8 / 97.2	(Babjuk et al., 2008)
TMPRSS2-ERG Gene Fusion	45.4 / 34.8	(Nguyen et al., 2011)
Abbreviation: BTA-bladder tumour antigen; NMP22-nuclear matrix protein 22; UBC-urothelial bladder cancer.		

Table 2.5: Advantages and disadvantages of different urine markers (Smith et al., 2013)

Marker	Advantage	Disadvantage
ImmunoCyt	Sensitivity	High inter observer variability
Telomeras	Sensitivity	Influenced by inflammation and age
BTAtrak	Sensitivity	Influenced by inflammation and age
BTAstat	On bench test	Influenced by benign genitourinary con
NMP22	Sensitivity	Need further study

Abbrevation:
BTA-bladder tumour antigen;
NMP22- nuclear matrix protein 22.

2.6.1.4 Cystoscopy

Cytoscopy is an endoscopy of the bladder through the urethra, which allows clinicians to view the inner surfaces of the urinary tract. Performed using a cystoscope with the patient under local anaesthesia, it is the gold standard in diagnosing bladder cancer and other urinary tract diseases. Many cystoscopes have extra tubes to guide other instruments for surgical procedures, which allow transurethral resections to remove a tumour once it is discovered.

In general, the specificity was lower for low- and high-grade lesions in voided specimens or in a follow-up setting, and this was true regardless of the considered threshold of a positive cytologic diagnosis. Moreover, in the invalid samples, the sensitivity of low-level lesion is greater than that of the high-level one, and such detection is more common in the instrumental samples (Brimo et al., 2009). Table 2.6 shows the sensitivity and specificity of various cytoscopy techniques compiled from various studies. Although

it is the gold standard and easy to perform, it can be used in conjunction with other methods to strengthen diagnoses (Lokeshwar et al., 2010). However, this method has its disadvantages; it may miss tumours that are small and flat, and as instrumentation is involved, it carries a risk of causing urethral injury and urinary tract infection (Ciudin et al., 2013).

Table 2.6: Sensitivity and specificity of various types of cystoscopy

Authors	Method	Sensitivity/specificity (%)	References
Ciudin et al.	Air cystoscopy	88 / 97	(Ciudin et al., 2013)
Horstmann et al.	PDD cystoscopy	92 / 57	(Horstmann et al., 2014)
Shen et al.	NBI or WLI cystoscopy	87.8 / 77.1 (NBI) 68.3 / 82.9 (WLI)	(Shen et al., 2012)
van Rhijn et al.	Urethra-cystoscopy	75 / 83	(van Rhijn et al., 2009)
Allam et al.	Cystoscopy	100 / 94.4	(Allam et al., 2009)
Abbreviation: PDD-photodynamic diagnosis; NBI-narrow band imaging; WLI-white light imaging.			

2.6.1.5 Computed Tomography Imaging

Computed tomography (CT) is usually not used for screening bladder cancer but for identifying the stage once it is diagnosed via cystoscopy. A CT urogram is generated by reconstructing multiple images of the patient's urinary tract (blood vessels, soft tissue); these images are then sent to a computer for reconstruction of detailed three-dimensional images (Bélanger et al., 2016). The advantages of this method include: (1) fully eliminating overlapping images outside the target area; and (2) the statistics gained from one CT image, which comprises multiple contiguous or one

helical scan, are considered images in the axial, coronal or sagittal planes, relying on the diagnostic task, which is seen as muti-planar reformatted imaging.

The disadvantages include: (1) ionizing radiation exposure that may bring higher risk of cancer, (2) higher risk as the radiation exposure is higher than X-ray scans; and, (3) injection of contrast agent may cause patients to suffer kidney damage or allergy(Sankowski et al., 2013). Table 2.7 shows a review of the CT staging and its sensitivity and specificity.

Table 2.7: Sensitivity and specificity of CT scan for bladder cancer

Authors	Method	Sensitivity/specificity (%)	References
Lu et al.	CT for staging or re-staging	82 /89	(Lu et al., 2012)
Harkirat et al.	CT for re-staging	86.7 / 100	(Harkirat et al., 2010)
Swinnen et al.	CT for staging	46 / 97	(Swinnen et al., 2010)
Sadow et al.	CT for detection	79 /94	(Sadow et al., 2008)

2.6.1.6 Magnetic Resonance Imaging

Like CT scans, the staging of bladder cancer can also be determined by magnetic resonance imaging (MRI) (Huettel et al., 2004). Briefly, the MRI scanner operates with the following components:

- The main magnet will generate a powerful magnetic field around the patient. The magnetic field will polarize and excite the hydrogen atoms of water molecules in the patient's body. The excited hydrogen atoms will emit a radio frequency signal, which is detected by the receiving coil;

- The gradient coils spatially encode the position information by varying the main magnetic field. The contrast between different tissues is determined by the rate at which excited hydrogen atoms return to the equilibrium state. Contrast agents may be used to make the image clearer;
- The shim coils ensure the homogeneity of the main magnetic field;
- The shielding coils create another magnetic field to cancel the field from the core coils that is not focused on the target area of the body;
- The computer reconstructs the radio signals detected by the receiving coils and converts them into images;
- A Faraday shield protects the MRI scanning room; and,
- There are various cooling mechanisms in the main magnet, the scanning room and workstation.

MRI scanners generate a powerful magnetic field and metal items, even in trace quantities, will interfere with the imaging process. The items include metal watches, jewellery (bracelets and necklaces), piercings (earrings, nipple rings or nose rings), dentures, audiphones and wigs (some which may contain metal). Before starting the MRI scan, it is essential to remove all metal from the patient's body to avoid interference in the imaging process, which may affect accurate diagnosis (Huettel et al., 2004). As MRI does not involve ionizing radiation, it presents no risk to pregnant women or children. However, special care must be taken for patients with body implants, such as pacemakers and joint replacements, which are made of metal. The disadvantages of MRI scanners are their enclosed spaces, which may not be suitable for claustrophobic patients; the requirement for large amounts of power to generate the magnetic field; and, the high cost of performing a scan, which relegates it to a secondary tool to confirm diagnosis (Katti et al., 2011).

Table 2.8 shows a review of the MRI method and its sensitivity and specificity in determining the stage of bladder cancer. Compared to CT scan, MRI has higher specificity and sensitivity probably due to it high quality imaging that makes it easier to diagnose diseases accurately.

Table 2.8: Sensitivity and specificity of MRI for bladder cancer

Authors	Method	Sensitivity/specificity (%)	References
Lee M et al.	MRI for staging	80.8 / 77.8	(Lee et al., 2017)
Daneshmand et al.	DGE-MRI for staging	87.5 / 91.5	(Daneshmand et al., 2012)
Rajesh et al.	MRI for staging	78.2 / 93.3	(Rajesh et al., 2011)
Watanabe et al.	MRI for staging	70 / 79	(Watanabe et al., 2009)
Abbreviation: DGE-dynamic glucose-enhanced			

2.6.1.7 Ultrasound Scanning

Ultrasound, also known as sonography, captures live images of the body using high-frequency soundwaves. The ultrasound machine is small and mobile, allowing clinicians to view real-time the organs and tissues of patients in a simple clinical setting (Rigby & Housami, 2009). Due to its ease of use, it has become a popular tool in maternal care to confirm pregnancy and monitor the growth of the foetus. High-frequency soundwaves are directed towards organs or tissues in the body, and they are reflected and captured by the transceiver. The reflected soundwaves are reconstructed into live images by the ultrasound computer (Ahmad et al., 2008). The advantages of this method are that there is no ionize radiation and no injection of contrast medium (dye) is required. The ultrasound system does not produce ionizing radiation and does not

require injection of contrast medium (Eber & Villasenor, 1991). Table 2.9 shows a review of ultrasound method and their sensitivity and specificity.

Table 2.9:. Sensitivity and specificity of ultrasound

Authors	Method	Sensitivity/specificity (%)	References
Gupta et al.	Ultrasound for staging	90 / 93	(Gupta et al., 2016)
Nicolau et al.	Ultrasound for detection	67.6 / 80	(Nicolau et al., 2011)
Nicolau et al.	Ultrasound for detection	88.5 / 88.9	(Nicolau et al., 2010)
Kocakoc et al.	3D ultrasound for detection	96.2 / 70.6	(Kocakoc et al., 2008)

2.6.1.8 Combined Methods

(a). Urine Biomarkers and Cytology

A combined diagnosis system increases the accuracy of detecting cancer or other diseases in the human body. The combined use of urine markers and cytology can improve bladder cancer detection. To select a tumour marker that is be economically affordable, the medical equipment must check the bladder pathological area completely (Ward et al., 2016). Screening and examination methods that are powerful and specific should be made immediately and completely available to doctors. However, urine cytology is sometimes affected by lower efficiency due to voided quality (van Rhijn et al., 2009). Table 2.10 compares the sensitivity and specificity of the use of urine biomarkers and combined with cytology.

Table 2.10: Sensitivity and Specificity of urinary markers combined with cytology

Method	Sensitivity/ Specificity (%)	Reference
NMP22 + Cytology	73-94 / 84-90	(Sullivan et al., 2010; Yafi et al., 2015)
BTAstat + Cytology	91-93 / 78-90	(Sullivan et al., 2010; Yafi et al., 2015)
BTAtrak + Cytology	68-72 / 53-75	(Sullivan et al., 2010; Miyake et al., 2012)
FDP + Cytology	68-89 / 50-78	(Razzaghi et al., 2008; Sullivan et al., 2010)
ImmunoCyt + Cytology	72.8-90 / 64.4-78	(Comploj et al., 2013; Yafi et al., 2015; He et al., 2016)
UroVysion FISH + Cytology	61.9-72 / 83-89.7	(Hajdinjak, 2008; Dimashkieh et al., 2013)
Abbreviation: NMP22-nuclear matrix protein 22; FDP-fibrinogen degradation products.		

(b). MRI with Cystoscopy, DCE, DW and T2W

The study by Deserno et al. (2004) involved 58 BCa patients, which show 96% of sensitivity and 98% of negative predictive values for the Ultrasmall USPIO Ferumoxtran-10 enhanced MRI. Thoeny et al. (2009) assessed 21 patients who have BCa and/or prostate cancer, and have been treated by the USPIO enhanced MRI and DW MRI have been involved, of which the results show 90% of accurate diagnosis rate. Since this agent is not available any more, researchers are trying to investigate whether ferumoxytol-10 could be substituted and legally used as an iron replacement therapy for patients with chronic kidney problems. Table 2.11 summarizes MRI studies included in this review (Bouchelouche et al., 2012).

Table 2.11: MRI studies included in the review (Bouchelouche et al., 2012).

Authors	Year	Method	Sensitivity/Specificity (%)
Barentsz et al. (1996)	1996	DCE & MRI	33 / 92
Beer et al. (2004)	2004	MRI & cystoscopy	91 / 91
Tekes et al. (2005)	2005	T2W & MRI DCE & MRI	79 / 97 55 / 84
Watanabe et al. (2009)	2009	DW & MRI	80 / 79
Abou et al. (2009)	2009	DW & MRI	98 / 92
Takeuchi et al. (2009)	2009	DW & MRI	80 / 94
Avcu et al. (2011)	2011	DW & MRI	100 / 76
Kobayashi et al. (2011)	2011	DW & MRI	92 / 80–90
Abbreviation: DCE-dynamic contrast enhanced; DW-diffusion-weighted; T2W-T(2)-weighted.			

2.6.2 Emerging Methods in Detecting Bladder Cancer

Currently, there is growing interest in the development of novel methods to detect bladder cancer. All early diagnostic methods for clinical use have their own different disadvantages and advantages, thus, the current new emerging methods we have reviewed for detecting bladder cancer are characterized by high sensitivity, specificity, low cost, non-invasive, simple use and repeat-ability, and utilizing their potential and effectiveness.

2.6.2.1 New Generation Optical Diagnostics

Cauberg et al. (2009) suggested that Raman spectroscopy (RS) may empower the estimation of atomic parts of a tissue in a subjective and quantitative manner. It happens when light photons associate with tissue atoms, changing their vibrational vitality states. Another photon is transmitted to represent the vitality distinction between the starting and the last state, bringing about an alternate wavelength of the scattered light. Every atom has remarkable vibrational vitality levels with their relating wavelength shifts. The majority of the moved wavelengths from the diverse atoms in tissue consolidate to shape the Raman range, which is an element of the sub-atomic synthesis of the tissue explored (Figure 2.3).

This atomic synthesis changes if pathologic changes happen, and by this, implies RS can give a target expectation of pathologic determination (Figure 2.4). To help with elucidation of RS, a pseudo-color guide of the tissue examination can be made. Tissue ranges with comparative spectra, and along these lines with comparative atomic piece, are delineated in the same shading, subsequently making a photo practically identical to histopathology (Koljenovic et al., 2005).

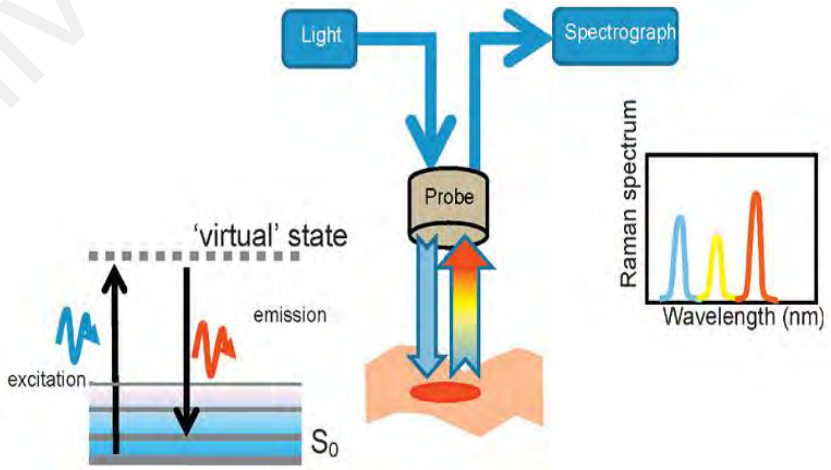


Figure 2.3: Molecular composition technique of the tissue investigated for bladder cancer in Raman spectroscopy (Cauberg et al., 2009).

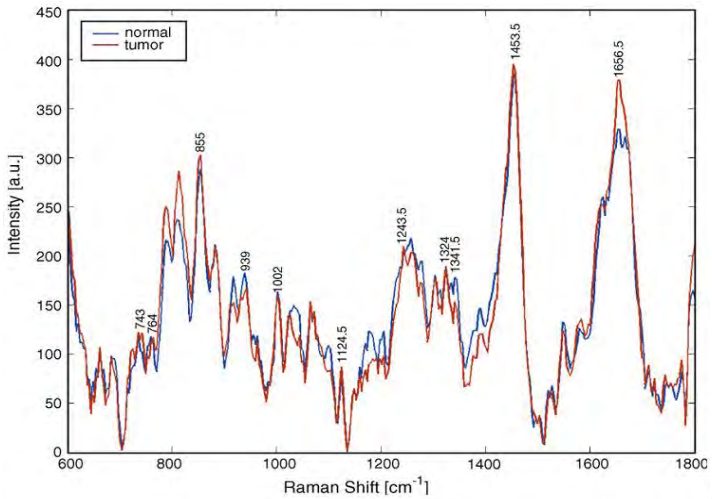


Figure 2.4: The Raman spectra of ordinary urothelium in blue and bladder tumour in red (Cauberg et al., 2009).

2.6.2.2 Diagnosis by High-Volume Raman Spectroscopy

The compact RS framework consists of a 785 nm diode laser (DFB-0785-1000, Sacher-Lasertechnik, Marburg, Germany), fiber-optic test (Emvision LLC, Loxahatchee, Florida), a typically-made spectroscope for the images, charge-coupled gadget camera (PIXIS, Princeton Instruments, Trenton, New Jersey) and a computer.

Briefly, the test has an outer width of 2.1 mm and comprises a focal excitation fiber (400 μm) encompassed by seven accumulation strands (breadth 300 μm). At the last stage of the test, when conveying the fiber, it fuses a band-pass channel, and through exciting light, it can eliminate the Raman waste generated from the fiber conveyance. It puts the long-pass channel or score channel in the clustered filaments to backscatter the square laser back from the tissues. It is able to collect Raman signals that are 2 mm inside the tissue. The Raman sign was gathered in the 400-1800 unearthly locale with otherworldly determination of 4 cm^{-1} (Draga et al., 2010).

When assessing in vivo, RS will not be affected by eco-components. In the first place, near-infrared radiation (NIR) does not cause mutations. Second, Maquelin et al. (2000) demonstrated that Raman estimations of 5 s with a laser yield of 250 mW and a testing

profundity of 500µm caused a temperature rise of 0.8°C. There is no finding showing that tissues are harmed during the histology test. Third, the variety in test tissue weight amid gastrointestinal endoscopy did not bring about critical contrasts in the Raman spectra. It is found that the hemoglobin in blood does not distort the Raman spectrum of tissues. RS has been utilized effectively for bladder disease diagnosis. In the 8-bunch calculation, there is more than 90% of sensitivity and specificity found in Raman microscopy. Raman estimations on bladder tissue tests brought about an analytic precision of 84%. Presently showed in Figure 2.5 that high volume RS could be utilized as a target clinical device for progressing of bladder tumour attack with satisfactory measuring times (Draga et al., 2010).

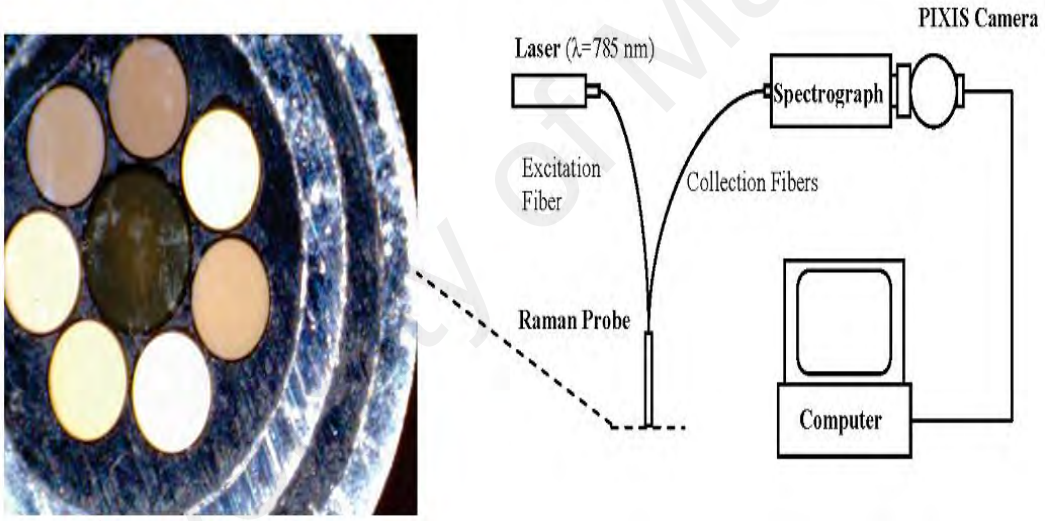


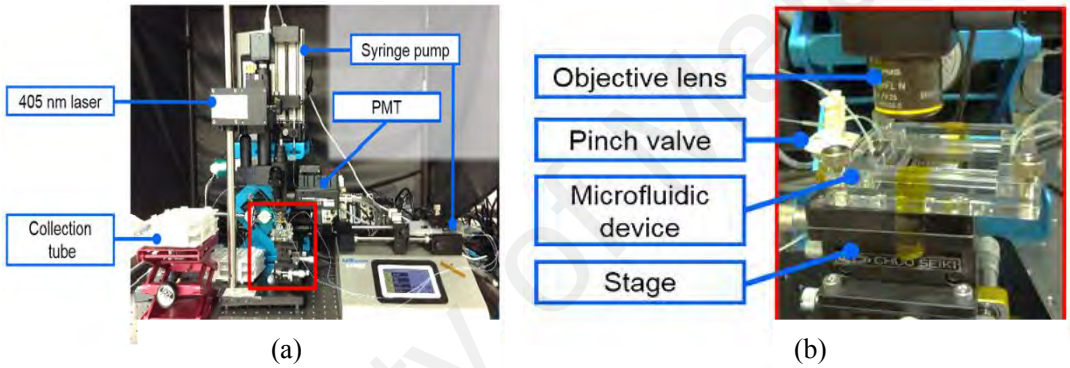
Figure 2.5: (Left) Picture of a Raman probe during a spectrometry test. (Right) Clinical Raman framework chart (Draga et al., 2010).

2.6.2.3 Photoresist-Based Microfluidic Fluorenscent Cell Sorting

Microfluidic fluorescence-initiated cell sorter (FACS) is based on the photoresistive effect. It is being studied on its possibility of being used to detect cancer cells, including from the bladder. FACS is able to distinguish between healthy and malignant bladder cells treated with aminolevulinic acid, which is recognized through fluorescence. The use of an off-chip squeezing valve on the microfluid platform allows the cells to be sorted at the Y-intersection of the micro channel. After collecting the cells, polymerase

chain reaction (PCR) may be performed to study their fine particle quality modification (Takagi et al., 2013).

As shown in Figure 2.6, FACS system has five primary modules (Hirai et al., 2015): (1) a microfluidic gadget for cell sorting; (2) a 405 nm ultraviolet light source for fluorescence excitation; (3) an optical framework comprising mirrors and band-pass channels to focus light; (4) a photosensitive locator, for the most part a photomultiplier tube (PMT); and (5) an off-chip squeeze valve to diminish the many-sided quality and prevent cross-contamination.



(a) (b)

Figure 2.6: (a) Applied outline of the FACS framework. (b) A close-up of the FACS platform and its microfluidic gadget (Hirai et al., 2015).

In order to present this approach, it has used the UMUC3 cell line and PC-3 cell line, in which the former one refers to the propelled high-review human bladder disease cells, and the later one refers to the propelled high-review human prostate malignant cells. In clinical examination, urine from patients who have been diagnosed with bladder cancer is used in the routine clinical practices at Nara Therapeutic College Healing center, and prepared as quickly as time permits (inside of 2-4h) after accumulation. Given the 50M h-ALA (Photocure Inc.), it has conducted the cell lines and urine examinations, of which the temperature is controlled at 37 ºC and the CO_2 concentration is 5%. The whole test lasts 4 hours conducted in a dull field (Wako Immaculate Synthetic Commercial ventures Ltd.), after which all the examples are suspended in PBS (Hirai et al., 2015).

The approach has been used by Hirai et al. (2015) to study the urine of bladder cancer patients with the UMUC3 (urothelial carcinoma) and PC-3 (prostrate cancer) cell lines as positive controls. The authors predict that a fundamentally-enhanced FACS framework will open doors for (1) lab technologists to supplant existing urine cytology routines; (2) early, non-obtrusive growth conclusion; and, (3) differentiation advanced and low-stage disease that is impossible with existing procedures.

2.6.2.4 Expression of Inflammatory Genes

NOD-like Receptor Proteins (NLRP) and the Neuronal Apoptosis Inhibitory Protein (NAIP) are pattern recognition receptors that play key roles in regulating the innate immune response. They cooperate with toll-like receptors to regulate inflammation and apoptosis in immune cells, and also non-immune cells such as epithelial cells. Activation of NLRPs by ligands, such as bacterial antigens and biomolecules produced by cell stress, causes the formation of inflamasomes, which promotes the secretion of IL-1β and IL-18. NLRP3, NLRP4, NLRP9 and NAIP have been observed to be highly expressed in the urine cell sediments of bladder cancer patients. The degree of NLRP expression has been observed to correlate with tumour grade, stage, risk of recurrence and metastasis. Two other indicators for affirming bladder cancer are the over-expression of Cytokeratin 20 (CK20) and anti-apoptotic Surviving gene. The expression of those inflammatory genes have been studied in patients with bladder tumour, patients with bladder irritation (CTR1) and control subjects (CTR0) (Poli et al., 2015). In Table 2.7, the study by (Poli et al., 2015) found that patients with bladder tumour had significantly higher expression of CK20 than CTR1 and CTR0. When comparing between CTR0 and CTR1, the expression of NAIP in high-risk patients was observed to be high. Moreover, the expression of NLRP3, NLRP4, NLRP9 and NAIP in patients with bladder tumour was significantly higher than CTR0. Compared with CTR0, CTR1 has greater expression of

NLRP3 and NLRP9, even though they were not significant. There is no significant difference in the expression of Surviving between all three groups.

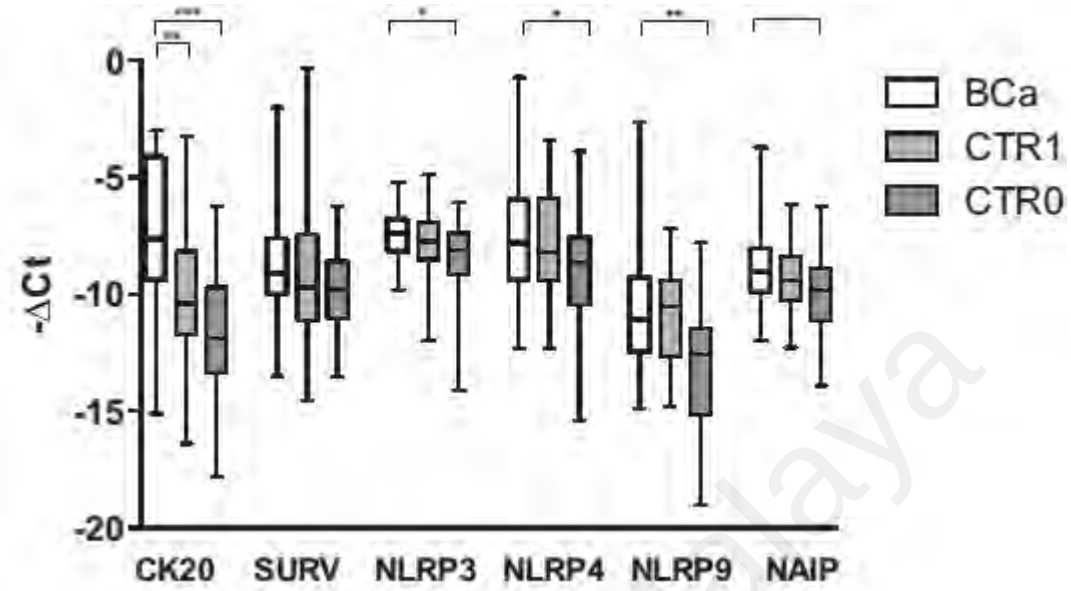


Figure 2.7: Expression of inflammatory genes (-ΔCt) in three groups of subjects (*P<0.05) (Poli et al., 2015).

2.6.2.5 Label-Free DNA Sensor

A DNA sensor that has utilized the silicon micro-resonator is a biomarker used for identifying bladder tumour by testing human urine. DNA biomarker in the urine is an important maker that can analyze and predict the development of bladder disease. To date, numerous hereditary adjustments have been distinguished in urothelial cell carcinomas in the bladder. Specifically, changes of Fibroblast Development Element Receptor 3 (FGFR3) and Harvey RAS (HRAS) gene expression are related to ahead of schedule stage bladder malignancy. The silicon microring resonator is an optical sensor that includes a refraction list, which provides a very fine, unlabeled, constant multiplexing position to bring biomolecules close to the sensor. Another sensor called the nucleic corrosive sensor uses a silicon microring resonator to determine the DNA biomarkers (FGFR3 and HRAS), which is also used for the bladder disease in human urine test. When contrasting or in a test that is nonspecific, the results showed that FGFR3 and HRAS have both completely found their corresponding mutants S249C (codon 249

substituting cysteine for serine) and G13R (codon 13 substituting arginine for glycine) respectively (Shin et al., 2013).

Bladder growth related DNA biomarkers have been recognized for right-on-time location and reconnaissance of tumours (Kim & Bae, 2008; Mitra & Cote, 2009; Proctor et al., 2010). Hereditary modifications of FGFR3 and HRAS are especially connected with second rate tumours (Kompier et al., 2010).

Here, we have proposed an unlabeled DNA biomarker sensor based on the silicon microring resonator for identifying the bladder tumour. It can be seen from the Figure 2.8 that DNA test is immobilized on the surface of silicon. Through using the changes of amine, it is able to capture the coordinating targets which are the single strand DNAs, but they are not completely related to the test.

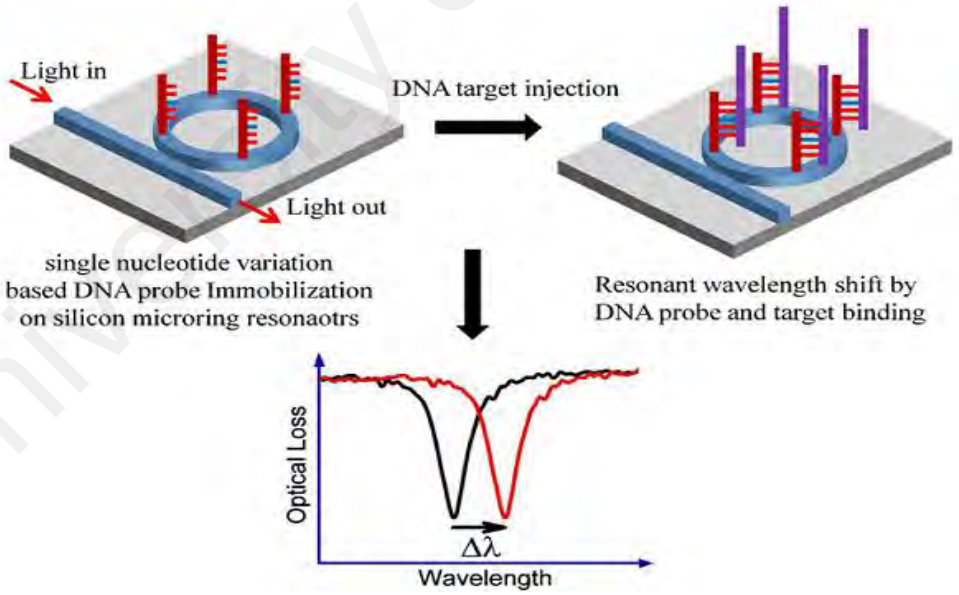


Figure 2.8: Schematic representation of the rule of microring resonators with DNA tests focusing on DNA biomarkers (Fitzgerald et al., 1995; Shin et al., 2013).

According to Shin et al. (2013), they have suggested a strategy that will not leave marks for finding the bladder cancer cells that are associated with the DNA biomarker in the human urine test. There are some clinical approaches that have been reported, suggesting that patients with bladder tumour need very careful care, so that it can be fruitfully

administrated. In order to do so, it also requires early detection, accurate tumour alignment, and long-term observation. FGFR3 and HRAS have been identified specifically in the urine test that is immobilized on the silicon microring sensor. Due to the characteristic point of preference of silicon manufacture and its utilization as an optical sensor in urine, our proposed methodology ought to be valuable for high-throughput examination in clinical biomedical applications and give an exceptionally touchy and particular stage for hereditary investigation in malignancy diagnostics and observation.

2.6.2.6 Polyclonal Antibody from 47 kDa Protein

A study in Indonesia has raised polyclonal antibodies against a protein that can potentially be used as a clinical marker for bladder tumours. The SDS-PAGE gel electrophoresis in Figure 2.10 shows distinct atomic weights of various proteins in bladder tumours compared with normal cells. Regardless of bladder cancer and normal bladder, a protein with a subatomic mass of 122 kDa can be found in their epithelial cells. However, a 69 kDa protein can be found only in the epithelial cells of normal bladders while a 47 kDa protein is seen only in bladder malignancy. The negligible proportion of polyclonal immunizer with antigen is 1:6400 of the counteracting agent and 1:40 of antigen. Thus, this focus was connected to recognize proteins with 47 kDa just in a few malignancy tissues. Therefore, the results will turn out positive for bladder tumour, but negative for prostate, colorectal and breast cancers. The polyclonal counteracting agent created from 47 kDa protein subunit is particular to distinguish bladder tumours and turn into an option biomarker for determining bladder malignancy (Prasetya et al., 2014). Figure 2.9 shows the morphological differences between normal epithelial cells and bladder cancer cells.

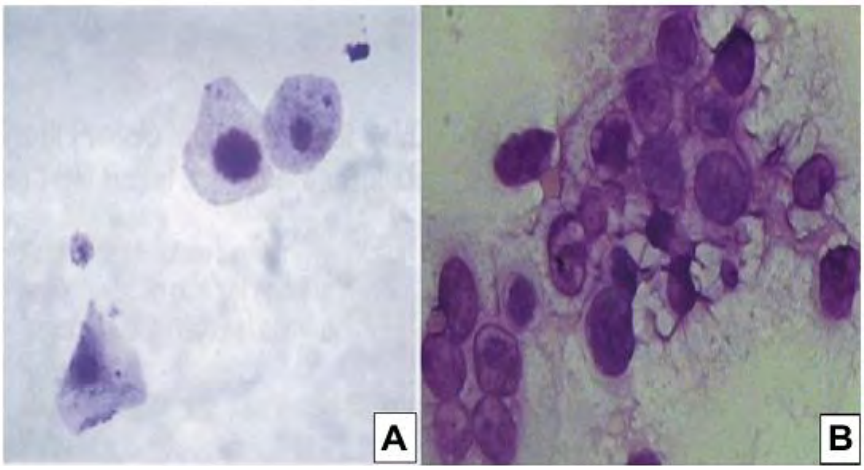


Figure 2.9: Morphology of (A) normal bladder epithelium and (B) bladder cancer (Prasetya et al., 2014).

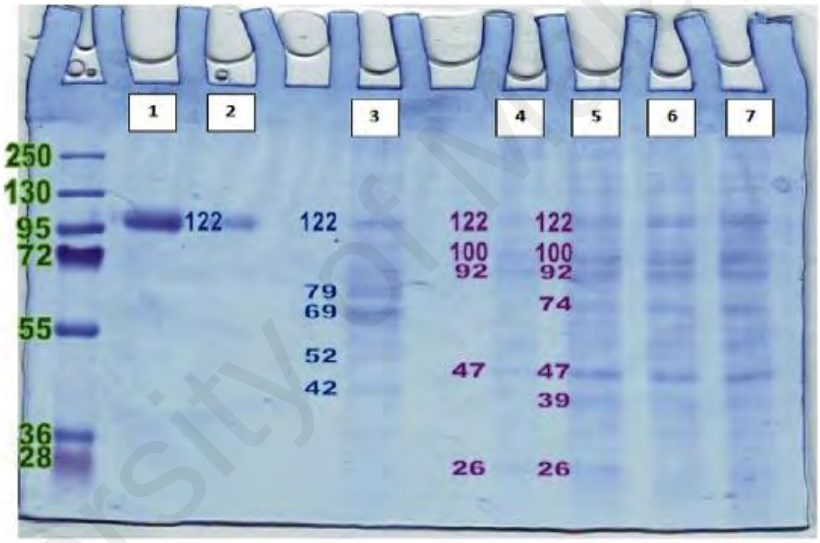


Figure 2.10: SDS-PAGE profile of confined proteins from normal bladder epithelium (well 1-3) and tumours (well 4-7) by Prasetya et al. (2014). A 122 kDa protein is observed in the epithelial cells of ordinary bladders and those with cancer. However, a 69 kDa protein is present only in the epithelials of a normal bladder, while a 47 kDa protein is found only in the epithelial cells of bladder cancer. A polyclonal antibody has been raised against the 47 KDa protein to be used as a biomarker for tumours.

2.7 Summary

This chapter started with the review on dielectric properties. Next, it described the summarization of dielectric property in different biological fluids, such as urine, blood, tissue, cell, etc. Lately, the overview of detection and monitoring method of pregnancy and bladder cancer were presented and summarized. There is no study based on measuring the dielectric property in urinary exfoliated urothelial cell. Various techniques of measurement for diagnostic of bladder cancer and its pro and cons were reviewed and compared with their sensitivity and specificity. These reviews have generated the determination of dielectric properties for hCG and urinary exfoliated epithelial cells, followed by urinary dielectric properties classification for disease diagnosis in later chapters.

CHAPTER 3 METHODOLOGY

3.1 Introduction

Based on the summary of the second Chapter, we have understood how chemistry changes in different fluids can be measured through its dielectric property. Researchers are getting more and more interested in measuring the dielectric properties, thus turning their attention to clinical utilization. In earlier studies, how to measure the dielectric property in urine given different microwave frequency is still limited.

Urine is a solution that is excreted from human body, reflecting the pathophysiological variability inside the body. Urinary hCG measurement is essential to detect and monitor pregnancy for non-invasive approach. There are protein hormones presenting in urine, which can also be used for chartering the hCG. Balarishnan et al. (2015) designed a point-of-care (POC) immunosensor to process hCG utilizing a polysilicon nanogap electrode. They discovered the dielectric properties of the immunosensor with a capacitance (>40nF) for pregnant subjects, and the results showed the dielectric properties in non-pregnant subjects were higher compared with that of pregnant subjects. In order to investigate the dielectric property of the urine of women with pregnancy and without pregnancy, given the microwave frequency from 2 to 3 GHz, Lonappan et al. (2007b) have applied the cavity perturbation to conduct the experiment. It was detected that the reaction of non-pregnant women's urine samples was lower than that of the pregnant women's urine samples and the dielectric constant of non-pregnant women's urine samples was higher than that of the pregnant women's urine samples. In another study, Lonappan (2012) used the variation of hCG in the blood, resulting in changes to dielectric properties. In the frequency range of 2 to 3 GHz, he used the rectangular cavity perturbation technique to analyze the dielectric properties of non-pregnant

women's blood samples as well as pregnant women's blood samples. The reaction of non-pregnant women's blood samples was lower than that of pregnant women's blood samples and the dielectric constant of non-pregnant women's blood samples was also lower than that of pregnant women's blood samples.

However, these studies of dielectric properties for hCG up to 3 GHz were limited and they did not impose temperature control for the investigation. So far, no data has been reported for changes in the dielectric properties of hCG with temperature. Moreover, no measurements have been carried out on the correlation between hCG and dielectric properties. Barry (1986), Ghodgaonkar et al. (1990) and Liao et al. (2003) reported that to measure the complex permittivity of a material, different measurement techniques can be endorsed; the chosen technique may vary, depending on various factors such as the nature of the sample, different temperatures and the frequency range used.

Exfoliated epithelial cell in the urine is an important diagnostic marker of bladder disease. It has been found that many significant changes of cell metabolism are closely related to the chemical changes in the cell membrane, which means that through directly analyzing these electrical changes, it is possible to understand the biological changes in cells (Sun & Morgan, 2010; Valero et al., 2010; Chen et al., 2011). Studies have shown that using the TRIMprob^{TM} system in soft tissues, significant differences were found at 465MHz that can characterize patients who were at risk for bladder cancer and prostate cancer (Bellorofonte et al., 2005; Gervino et al., 2007). In another study, a comparison of impedance data of bladder epithelial cells and corresponding biopsy tissue pathology results, it was found that the resistivity in bladder benign group was lower than that of malignant group, and electrical impedance spectroscopy can be used as a useful technique to identify bladder disease (Keshtkar et al., 2012).

So far, no study has been reported that investigate the dielectric properties of urine in bladder cancer. In addition, there have been no studies measuring the changes in dielectric properties of urinary exfoliated epithelial cells at different temperatures, or any correlation studies between urinary exfoliated epithelial cells and their dielectric constant.

In this chapter, we measured the dielectric properties (dielectric constant and dielectric loss) of the urine of pregnant women, non-pregnant women, healthy person and bladder cancer patients using the microwave technique at frequency between 0.2GHz and 50GHz, and at three different temperatures (25^{o}C, 30^{o}C, 37^{o}C). The correlations between hCG and dielectric properties were conducted and the correlations between urinary exfoliated epithelial cells and dielectric properties were studied as well.

3.2 Materials and Experimental Procedures

3.2.1 Pregnancy Detection

3.2.1.1 Sample Preparation and Collection

This study involved 110 pregnant women and 110 non-pregnant women. All pregnant women and part of the non-pregnant women were admitted to University of Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia. The rest of the non-pregnant women were recruited under the University of Malaya, Kuala Lumpur, Malaysia. All the subjects were informed of the procedures and research purposes, and informed consent was obtained before urine collection. Medical ethics was approved by the Institutional Ethics Review Committee, UMMC, Kuala Lumpur, Malaysia. Ethic approval number is 20161128-4641.

In all subjects, a sample of mid-stream urine was collected properly in a sterile screw-topped container and the container was then closed tightly. The container was labelled with the subject's name and the date of collection. The container of urine was placed in a sealed plastic bag. All urine samples used were as fresh as possible, whereby urine specimens were transported to the laboratory for analyzing mostly within 2-3 hours of collection. Upon collection of the urine, no preservatives were added.

3.2.1.2 Experimental Set-up and Experimental Process

The electrical measurements conducted to analyze dielectric properties used the following devices: a) Agilent E8364C personal network analyzer through Agilent 82357A GPIB interface, handled by Agilent 85070 software (10MHz-50GHz, Agilent Technologies, Santa Clara, Canada); and b) 50GHz open-ended coaxial probe HP85071 with flexible cable designed by Agilent Technologies (2.2mm in diameter, 20cm in length). The experimental set-up in this study is shown in Figure 3.1.

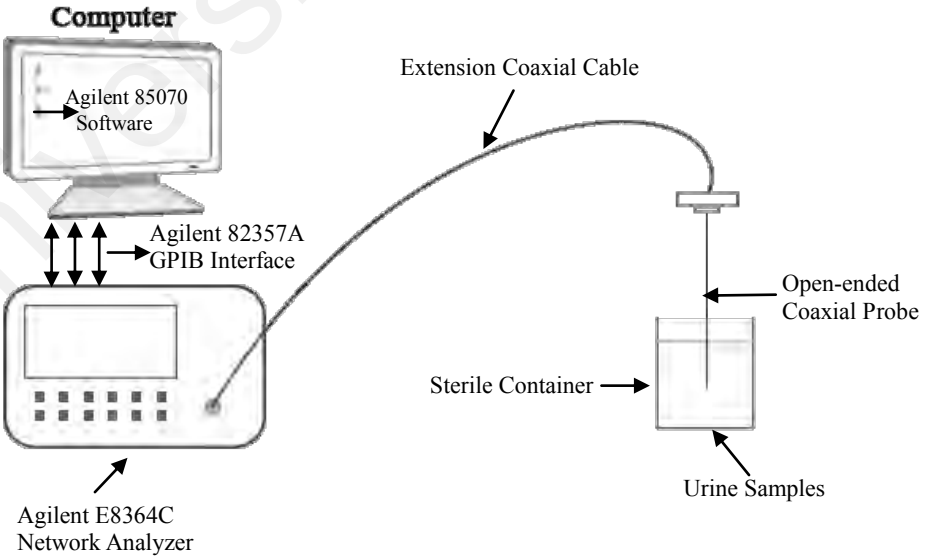


Figure 3.1: Experimental Set-up

Before conducting the tests, the PNA was calibrated with references for air, short circuit and distilled water before measurements took place. The calibration standards for biological solutions to be measured were chosen that have more than 90% water content, so measurements among available reference liquids are required to determine measurement accuracy (Zhadobov et al., 2012). Electronic-calibration (E-Cal) was used for refreshing the calibration. Gabriel and Peyman (2006) has once reported that it is thought that the arbitrary system errors are able to reduce the instability. Agilent is a company that provides vector network used for automatically analyzing the measurements, in which an open-ended coaxial probe is included. Generally, it is used for analyzing a small amount of solution. Compared with the resonant cavity approach, it has provided more frequency targets and wider frequency ranges, but the results provided by resonant cavity approach are more correct (Dube et al., 1988; James et al., 1990). The front of the open-ended coaxial probe was placed in direct contact with the freshly collected urine samples. Through the extension coaxial cable, the vector network analyzer was connected to the other side of the probe. The probe was cleaned with distilled water after it was sterilized using alcohol wipes. During the measurement, the probe was immersed to a depth of more than 2cm with excellent contact between the urine sample and probe surface, while ensuring there were no air bubbles at the end of the probe.

The relative position of the probe in the container maintained through all the liquid measurements to achieve good quality data. Each urine sample was measured three times. The measurements were conducted at 25℃ , 30℃ and 37℃ , respectively. The temperature was controlled using a WNB 7water bath (Memmert WNB7, Duesseldorf, Germany). The range of the temperature fluctuation was ±0.1°C.

3.2.2 Bladder Cancer Detection

3.2.2.1 Sample Preparation and Collection

A total of 70 urine samples were collected from 35 healthy people and 35 patients with bladder cancer (age between 40 and 80 years in both groups). The samples were sent for routine clinical urine chemical variables test and pathological microscopy test. All participants were admitted to UMMC, Kuala Lumpur, Malaysia. These participants were informed of the procedures and purpose of the additional tests, i.e. research, and a signed consent form was obtained before urine collection. Ethical approval was passed by the Institutional Ethics Review Committee of UMMC. The ethical approval number is 2016816-4143.

Mid-stream urine specimens of 60ml (minimum) were collected from each subject in a sterile screw-topped container and the container was then closed tightly. Every container was labeled with the subject's name and the date of collection. Then, the container of fresh urine sample was immediately placed in a sealed plastic bag and stored in a refrigerator at 4℃. All urine samples used were as fresh as possible, whereby urine specimens were transported to the Division of Laboratory Medicine, University Malaya Medical Centre (UMMC), Division of Anatomical Pathology (Faculty of Medicine, University of Malaya) and Electromagnetic Laboratory (Faculty of Engineering, University of Malaya) for analysis within 4 hours of collection. Upon collection of the urine, no preservatives were added.

3.2.2.2 Experimental Set-up and Experimental Process

The experimental setup and process were the same as those in Section 3.2.1.2. For detail information, please refer to Section 3.2.1.2.

3.3 Data Analysis

3.3.1 Pregnancy Detection

The urine samples were sent to the Division of Laboratory Medicine, UMMC for the measurement of clinical chemical variables in terms of bacteria, epithelial cell, glucose, ketone, protein, haemoglobin, crystal and hCG.

Dielectric properties were measured in terms of dielectric constant (Ɛ'), dielectric loss (Ɛ'') and loss tangent (tan $= \frac{\varepsilon''}{\varepsilon'}$), between 0.2GHz and 50GHz with a total of 250 frequency points. Meanwhile, before measuring, a control study (distilled water) was needed to access the accuracy of the open-ended coaxial probe method for measuring dielectric property of urine samples. Microsoft Excel was used to tabulate the raw data. The SPSS Statistics 21.0 software was used to perform the statistical analyses. The data exhibited normally distributed, so independent Samples T-test was used to determine the significant difference in dielectric properties between pregnant women and non-pregnant women. One-way Analysis of variance (ANOVA) statistical test was used to determine the temperature effect on the dielectric properties. Pearson correlation test was used to determine the correlation between hCG with dielectric properties. The significant level was set at $p<0.05$, and all statistical analyses were conducted using SPSS 21 software.

3.3.2 Bladder Cancer Detection

About 20ml of each urine sample was sent to the Division of Laboratory Medicine for the measurement of clinical chemical variables in terms of protein, glucose, ketone, haemoglobin, bacteria, urea, sodium and potassium using Roche Cobas® U601 urine analyzer. Meanwhile, 10ml of each urine samples was sent to Division of Anatomical Pathology laboratory for microscopic examination using the ThinPrep® 2000 method.

Any test subject's urine that tested positive for hematuria and diabetes were excluded from this research.

Dielectric properties were measured in terms of dielectric constant (Ɛ') and dielectric loss (Ɛ'') between 0.2GHz and 50GHz with a total of 250 frequency points. Meanwhile, before measuring, a control study (distilled water) was needed to access the accuracy of the open-ended coaxial probe method for measuring dielectric property of urine samples. SPSS statistic 21.0 software was used to perform the statistical analyses. Non-parametric analysis was used to determine the significant difference in dielectric properties between healthy subjects and bladder cancer subjects since the data was not normal distributed. One-way ANOVA was used to determine the temperature effect on the dielectric properties. The Pearson correlation analysis was used to determine the correlation between urinary exfoliated urothelial cells with dielectric properties. The significance level was set at $p<0.05$ for all the statistical analyses.

3.4 Summary

This chapter presents the experimental set-up, theory and design of the whole study method. We measured the dielectric properties (dielectric constant and dielectric loss) of the urine of pregnant women, non-pregnant women, healthy person and patients with bladder cancer using microwave technique at a frequency of between 0.2GHz and 50GHz, and at three different temperatures ($25^{o}C$, $30^{o}C$, $37^{o}C$). The correlations between hCG and dielectric properties were conducted and the correlations between urinary exfoliated epithelial cells and dielectric properties were studied as well. Sample collection, data processing and procedures are also described in detail.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Introduction

This chapter explains the results and discussions of this research, and compares them with other research. The dielectric properties of urine from pregnant and non-pregnant women differed significantly at 25°C, 30°C and 37°C.

4.2 The results of Dielectric Properties of hCG for Pregnancy

4.2.1 Urine Composition and Measurement Results

In this study, a total of 220 urine samples were collected. However, any subject's urine that showed a presence of positive results (protein, haemoglobin, ketone, glucose, bacteria) was excluded from this study. Thus, only urine samples of 30 pregnant women and 30 non-pregnant women were used in the final analysis. The urine samples were sent to the Division of Laboratory Medicine, UMMC for the measurement of urinary analysis (FEME), the analysis results are shown in Table 4.1. The neutral pH of the urine and absence of bacteria, cells and other biological substances like glucose, protein and hemaglobin indicated that the subjects were healthy with normal renal function and not suffering from diabetes. The significant difference in hCG level ($p<0.05$) indicated that the subjects were undergoing a healthy pregnancy.

Table 4.1: Mean of urine characteristics in pregnant and non-pregnant women

Group / Component	1 (Healthy non-pregnant)	2 (Healthy pregnant)
Sample size (n)	30	30
Mean age (years)	22 ± 1.6	31 ± 4.0
Mean pregnancy period (months)	0	6 ± 1.1
PH	6.5 ± 0.5	7 ± 0.5
Protein (mg/dl)	0	0
Haemoglobin (mg/dl)	0	0
Glucose (mg/dl)	0	0
Ketone (mg/dl)	0	0
Bacteria	Nil	Nil
Epithelial cell	Nil	Nil
Crystal	Nil	Nil
hCG (mIU/mL)	9.4 ± 3.9	5285.4 ± 4571.3

4.2.2 Accuracy and Reproducibility

To ensure reproducibiblity, the same experimental set-up (Agilent E8364C) was used in our study with the previous research by Mun (2016). She had measured the dielectric properties of distilled water for five times at 25 ^{o}C, 30 ^{o}C and 37 ^{o}C, with independent calibration sessions which were proposed by Gabriel and Peyman (2006) and Zhadobov et al. (2012). According to manufacturer Agilent Technologies (2012), the accuracy of the coaxial slim probe was ±0.05, or ±5 % at 23±3^{o}C. To assess technique accuracy, measurements for methanol and 0.1M salt water were performed. However, determining the experimental accuracy of methanol at high temperatures (30 ^{o}C and 37 ^{o}C) was not practical because of its different spectra of dielectric properties than distilled water, especially in the millimeter wave range (Zhadobov et al., 2012). Thus, the measurements for methanol were conducted only at 25 ^{o}C. No significant differences ($p>0.05$) were obtained among the measured data in different calibration sessions at frequencies of 0.2 to 50GHz. The urine measurement reproducibility was the same as those obtained from

the reference liquids. Figure 4.1 and Figure 4.2 show the comparison of dielectric properties with reference values of distilled water extracted from the database of Ellison et al. (1996). Overall, the measured data closely matched the reference data. The maximum deviations compared to the reference data were about 4 %.

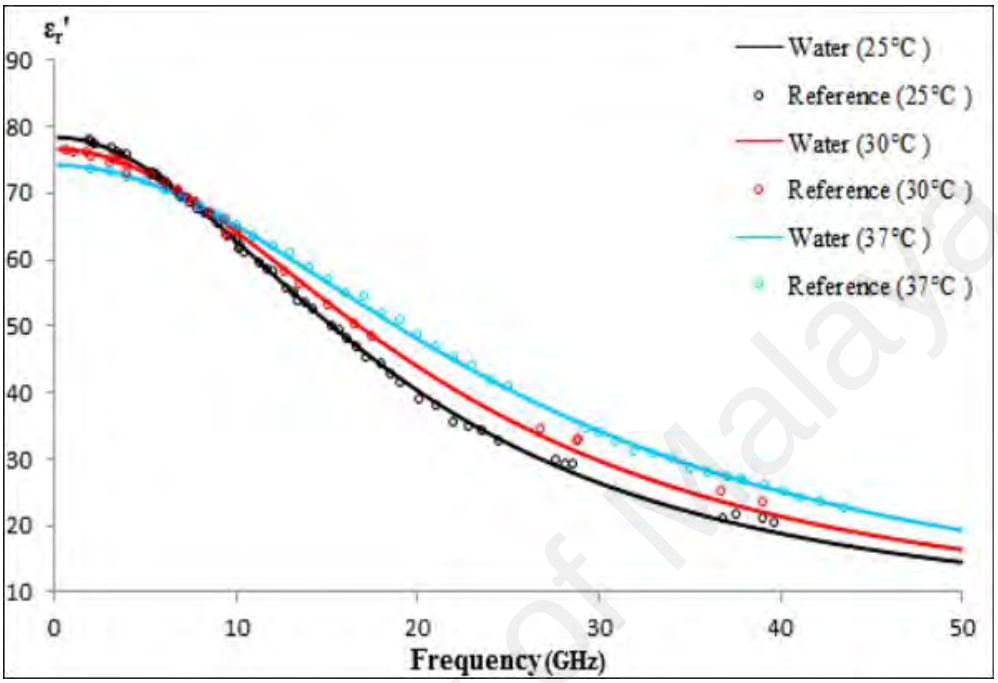


Figure 4.1: Comparison of dielectric constant with reference values of distilled water at 25 °C, 30 °C and 37 °C from 0.2 to 50GHz. (Mun, 2016)

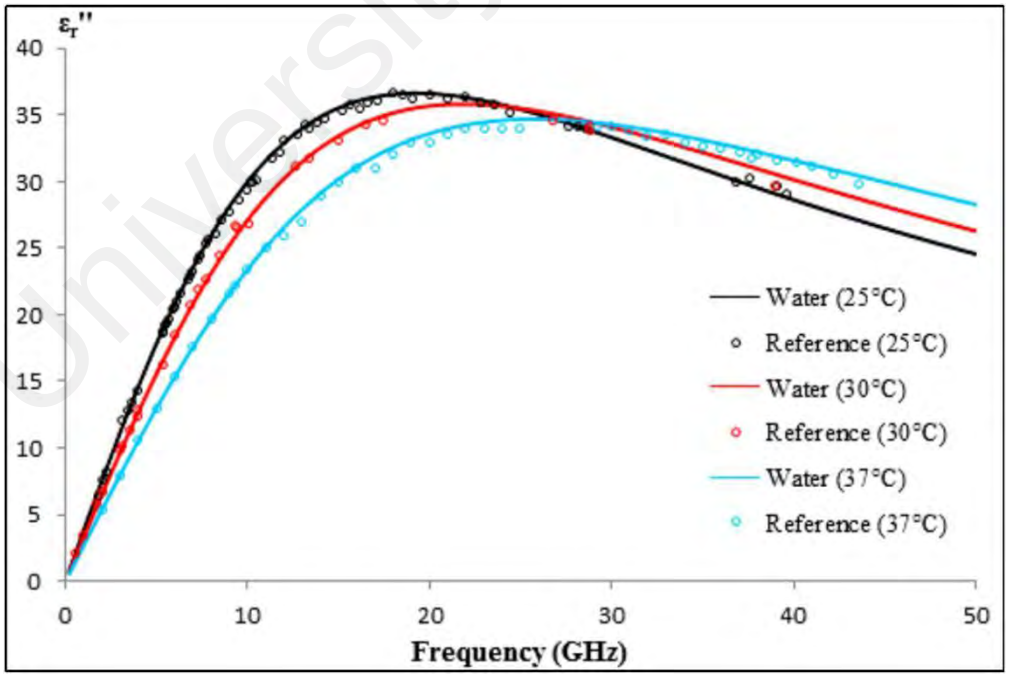


Figure 4.2: Comparison of dielectric loss with reference values of distilled water at 25 °C, 30 °C and 37 °C from 0.2 to 50GHz. (Mun, 2016)

4.2.3 Dielectric Properties of hCG

4.2.3.1 Overview

Figures 4.3 to 4.5 show the changes in Ɛ', Ɛ'' and loss tangent (tan σ) in the urine of pregnant and non-pregnant women when exposed to microwave frequencies of between 200MHz and 50GHz at 25°C, 30°C and 37°C. In Figures 4.3 and 4.5, the Ɛ' and Ɛ'' in all three temperatures were observed to cross each other at 7.4GHz and 26.8GHz, respectively. Initially, the Ɛ' and Ɛ'' seemed to increase to a certain peak value before decreasing. The increase was inversely related to temperature, where the lowest 25°C had the highest Ɛ' and Ɛ'' values. As the values decreased, they would meet at a crossing point and from there onwards, the corresponding temperatures would switch, where urine at 25°C had the lowest Ɛ' and Ɛ'' values, and vice versa for 37°C. The Ɛ' and Ɛ'' values at 30°C remained in between 25°C and 37°C, even after the crossing point. Tan σ constantly increased with the lowest temperature having the highest value.

In general, it was observed that the dielectric constant of the urine of pregnant women was slightly lower than that of non-pregnant women while the dielectric loss and loss tangent of the urine of pregnant women were slightly higher than that of non-pregnant women.

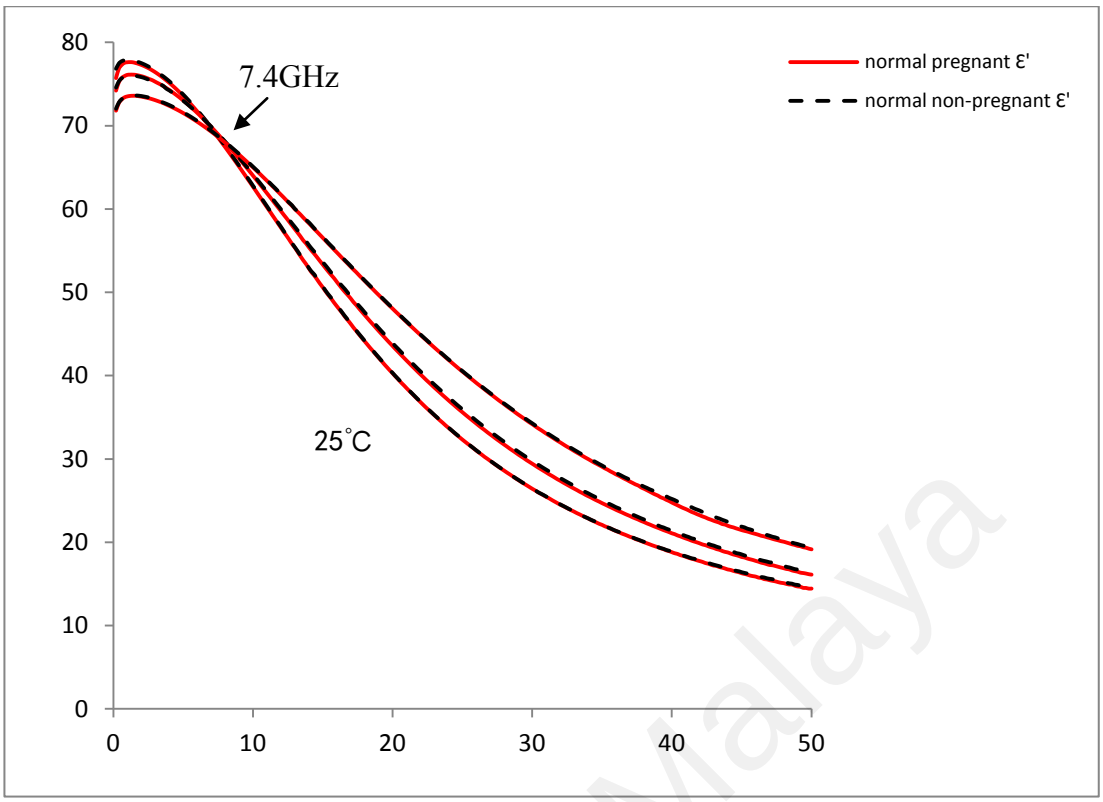


Figure 4.3: Mean dielectric constant (Ɛ') in urine of pregnant and non-pregnant women at 25 °C, 30 °C and 37 °C.

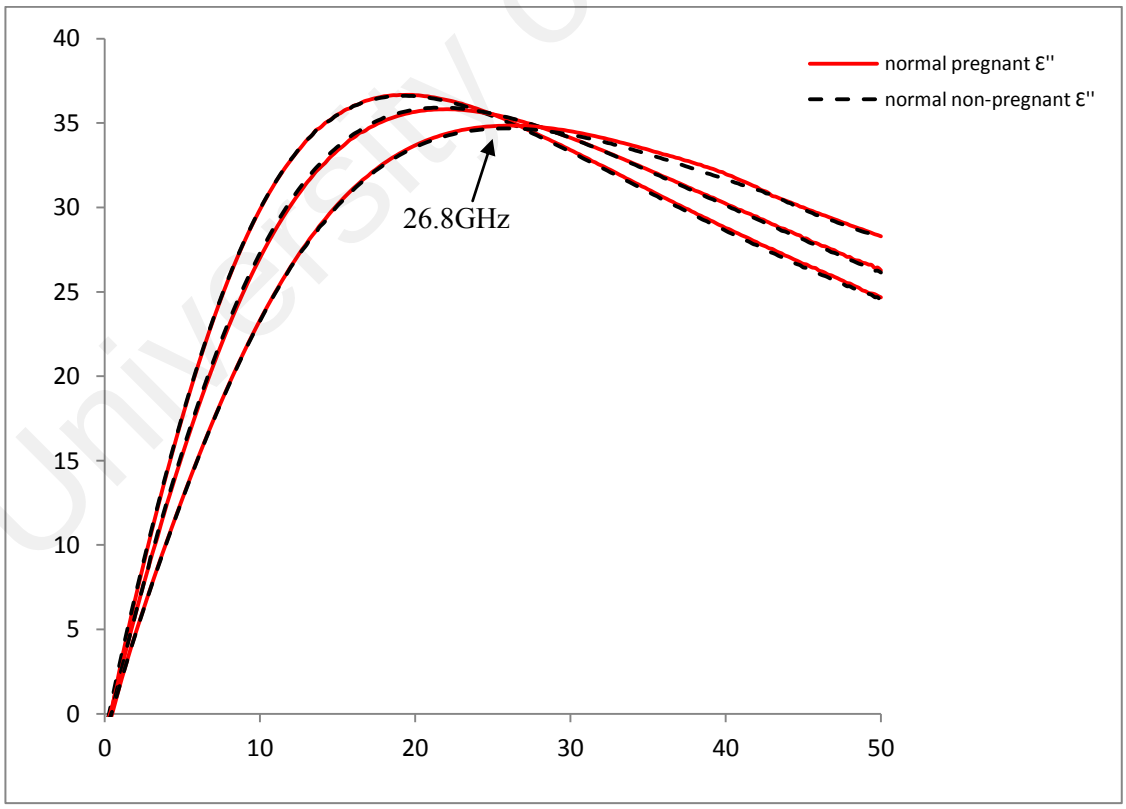


Figure 4.4: Mean dielectric loss (Ɛ") in urine of pregnant and non-pregnant women at 25 °C, 30 °C and 37 °C

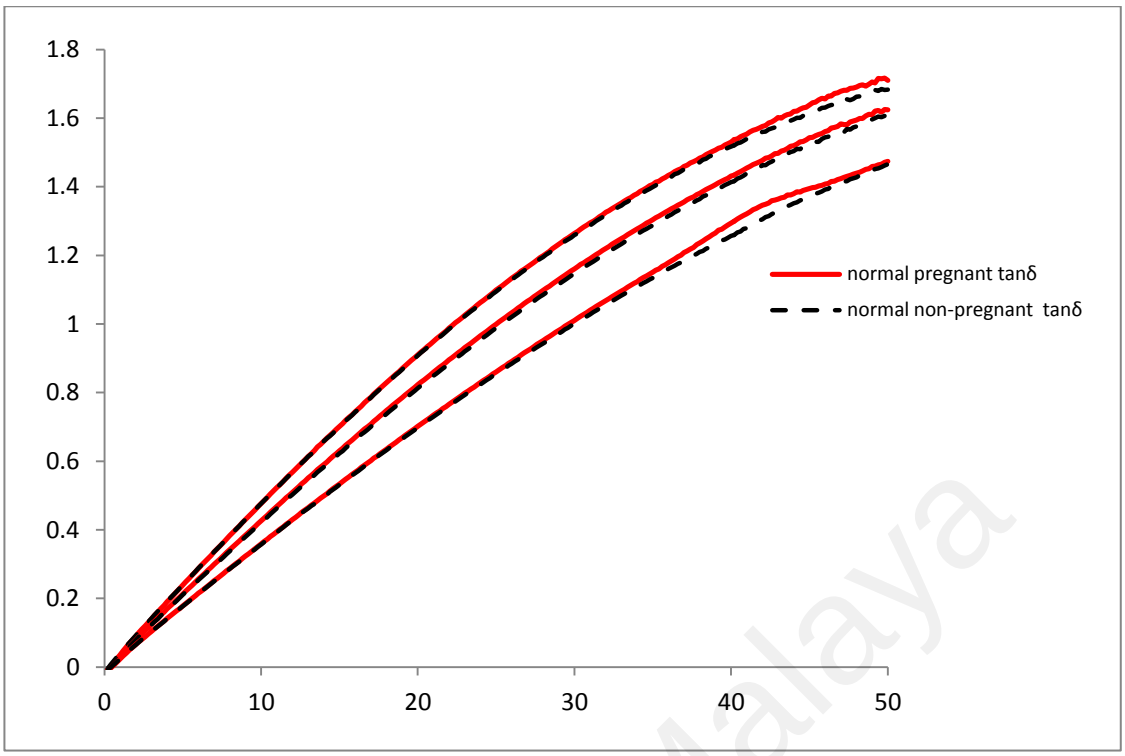


Figure 4.5: Mean loss tangent (tan σ) in urine of pregnant and non-pregnant women at 25 ^{o}C, 30 ^{o}C and 37 ^{o}C

4.2.3.2 Comparison of Dielectric Properties between Pregnant and Non-pregnant Women across Three Temperature

There were significant differences between the Ɛ', Ɛ'' and tan σ values of pregnant and non-pregnant women. As water is the main composition of urine, therefore, the electrical field can use the relaxation process of water. But in pregnant women, who had hCG in their urine, the relaxation process of the water molecules would be altered. As shown in Figure 4.6, the urine of pregnant women had lower Ɛ' values compared with their non-pregnant counterparts. But Figures 4.7 and 4.8 show that Ɛ'' and tan σ values in the urine of pregnant women were higher. The mean relative errors for dielectric properties at three different temperatures are shown in Table 4.2 (a), (b) and (c).

The data exhibited normally distributed, therefore, we used t-test for analysis. The significant difference in dielectric properties between pregnant women and non-pregnant women is indicated by the p-value and t-value. T-value was calculated

from the t-test statistics, p-value can be calculated according to t-value by SPSS software. The highest t-test values for three different temperatures with their respective frequency points are shown in Table 4.3. The Ɛ' in pregnant women's urine was significantly lower than non-pregnant women (P=0.03, 0.026, <0.001 at 25°C, 30°C and 37°C, respectively). However, the Ɛ" (P=0.002, 0.026, 0.001 at 25°C, 30°C and 37°C, respectively) and tan σ (P=0.002, <0.01, <0.01 at 25°C, 30°C and 37°C, respectively) were significantly higher. The reason for this could be explained by the high level of hCG in the urine. However, due to the low t-test values, these significant differences were weak. The reason for this difference can be explained by the presence of hCG that increases in pregnant women's urine.

When the F was at the highest level and the frequency was 23.6GHz, Ɛ" showed great differences between the three temperatures (F=82785.991, $p<0.05$). When F was highest and the frequency was 12.8GHz, Ɛ" showed great differences between the temperatures (F=33051.956, $p<0.05$). When F was at the highest level and the frequency was 16.2GHz, tan σ showed great differences between the temperatures (F=1097.299, $p<0.05$). F number refers to the level of significant difference, and p value refers to the evaluation of F number according to the variability and difference of samples.

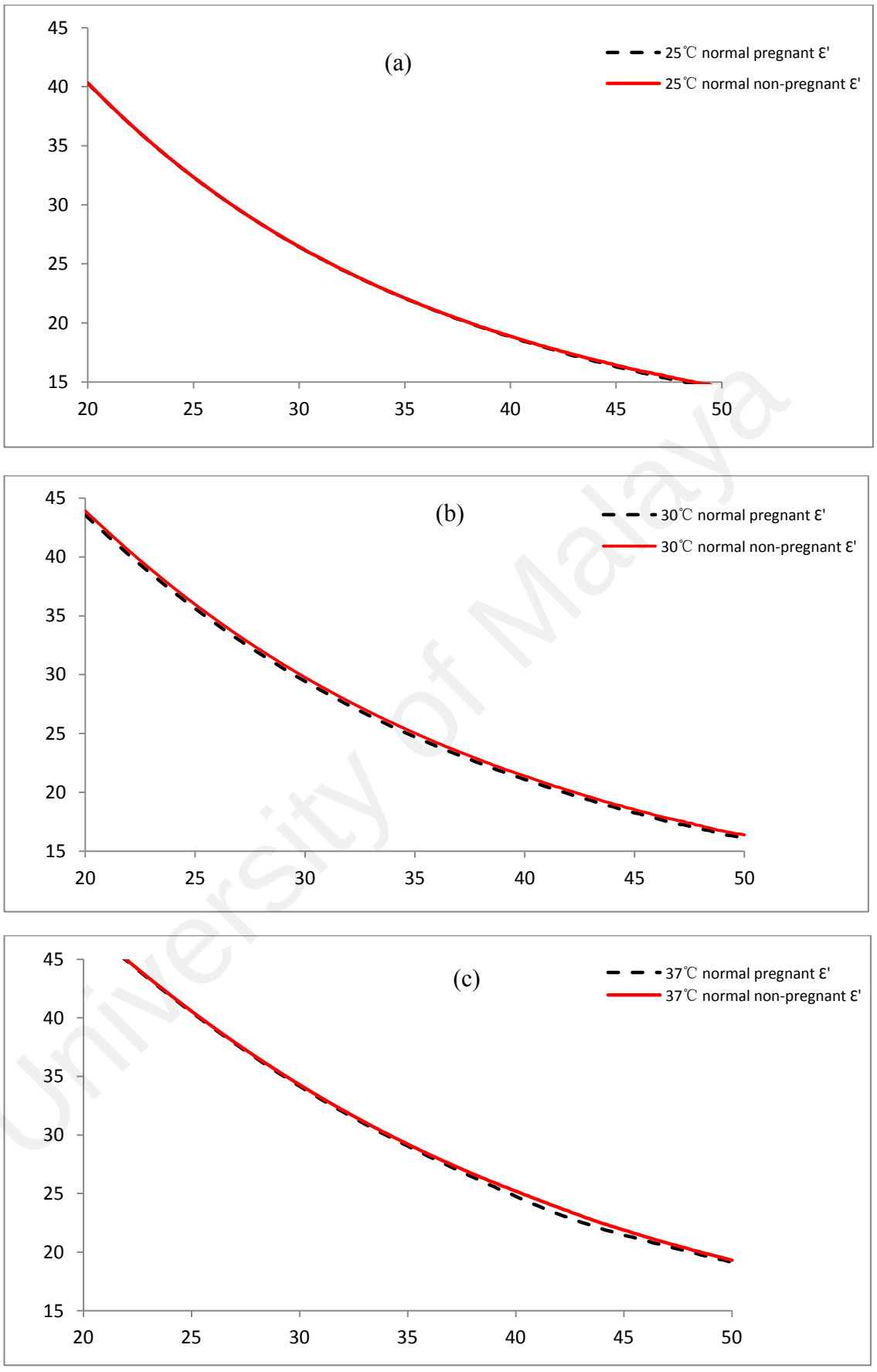


Figure 4.6: Mean dielectric constant in urine samples at (a) 25°C, (b) 30°C and (c) 37°C.

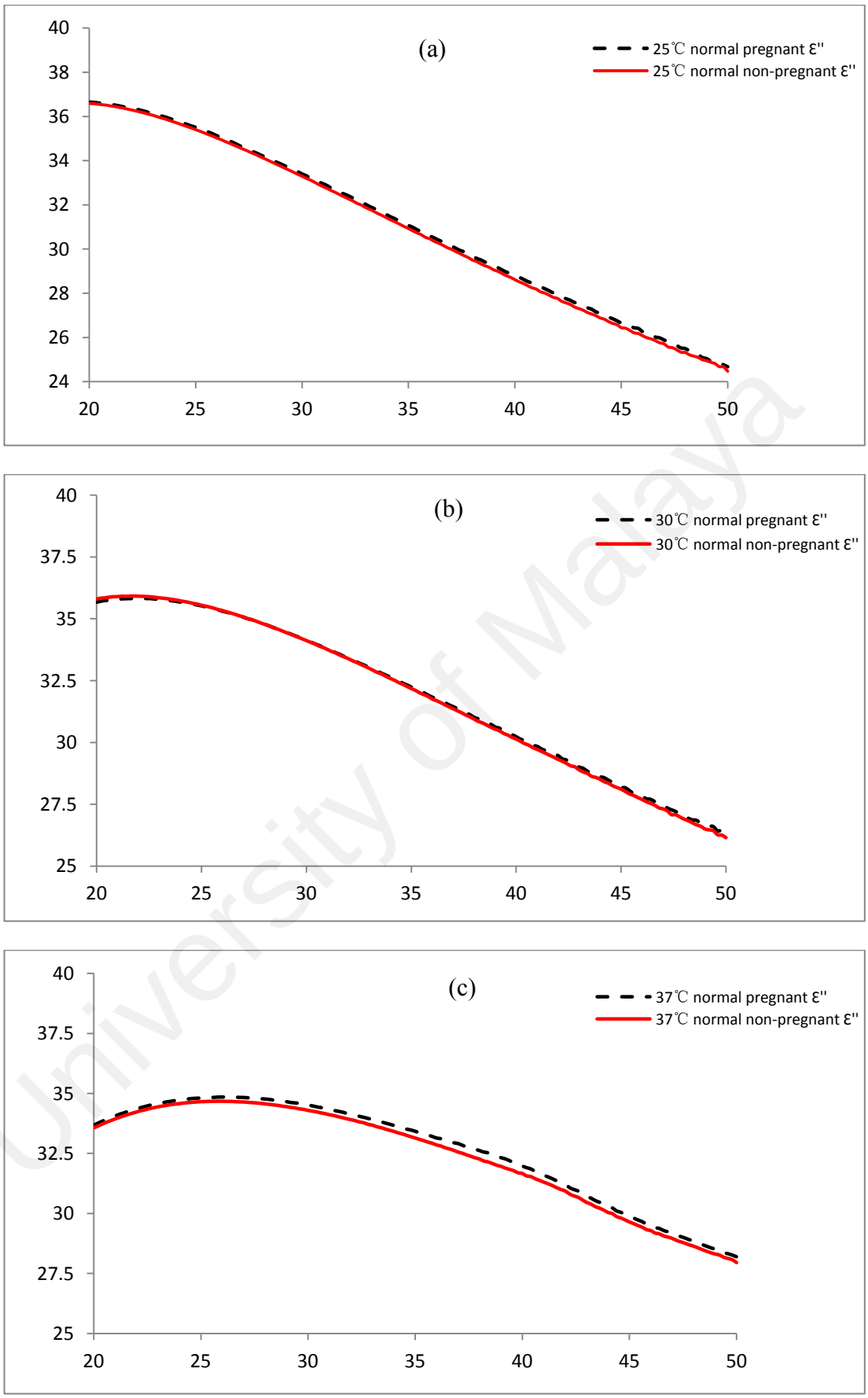


Figure 4.7: Mean dielectric loss in urine samples at (a) 25°C, (b) 30°C and (c) 37°C.

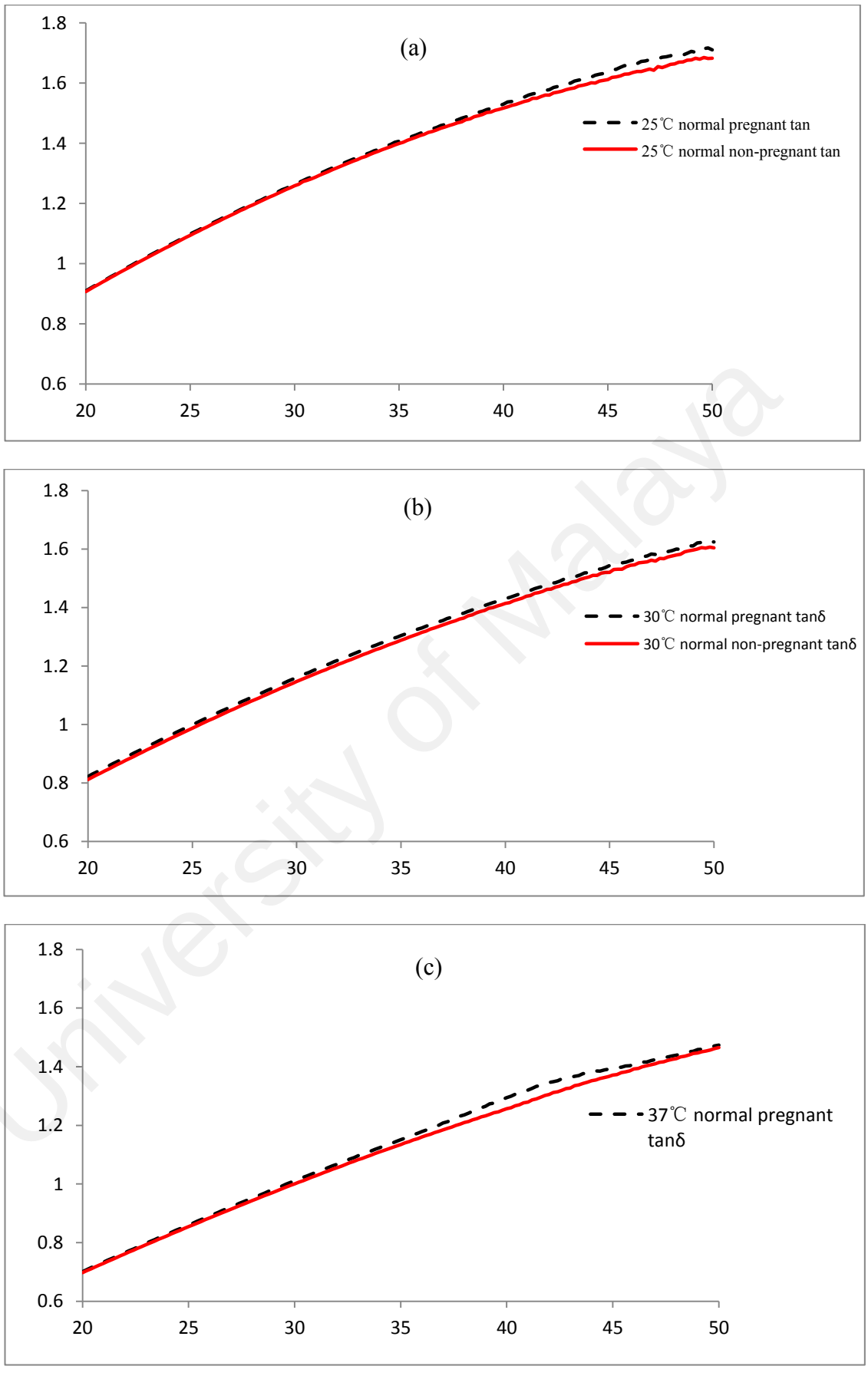


Figure 4.8: Mean loss tangent in urine samples at (a) 25°C, (b) 30°C and (c) 37 °C.

4.2.3.3 Correlation between hCG and Dielectric Properties

The highest Pearson correlation coefficients for Ɛ', Ɛ" and tan σ at three different temperatures are shown in Tables 4.4 to 4.6. Normally, when the r value was between -1 and 1, the Pearson correlation coefficient could be applied, which was used to describe how closely two variables correlated with each other in a linear fashion. Generally the dielectric properties correlated positively with hCG at low frequencies but they correlated negatively with hCG at high frequencies. The correlation coefficients of more than ±0.85 indicate strong correlation which suggested the potential development of a diagnostic tool for pregnancy using the dielectric properties of urine.

Table 4.2(a): Mean relative error for dielectric constant at three different temperatures

		Non-Pregnant			Pregnant		
Temperature (^{o}C)	**Frequency (GHz)**	**Mean**	**±SD**	**Mean relative error**	**Mean**	**±SD**	**Mean relative error**
25	49.4	14.78	0.15	1%	14.53	0.41	2.8%
30	46.8	17.69	0.33	1.9%	17.37	0.68	4%
37	16.4	54.21	0.06	0.1%	54.08	0.16	0.3%

Table 4.3(b): Mean relative error for dielectric loss at three different temperatures

		Non-Pregnant			Pregnant		
Temperature (^{o}C)	**Frequency (GHz)**	**Mean**	**±SD**	**Mean relative error**	**Mean**	**±SD**	**Mean relative error**
25	49.6	24.69	0.16	0.7%	24.87	0.25	1%
30	18	35.14	0.12	0.33%	35.33	0.47	1.3%
37	39.8	31.70	0.13	0.4%	32.09	0.58	1.8%

Table 4.4(c): Mean relative error for loss tangent at three different temperatures

		Non-Pregnant			Pregnant		
Temperature (^{o}C)	**Frequency (GHz)**	**Mean**	**±SD**	**Mean relative error**	**Mean**	**±SD**	**Mean relative error**
25	49.4	1.68	0.02	1.3%	1.72	0.07	4.2%
30	34	1.26	0.01	0.88%	1.28	0.04	3.2%
37	15.8	0.56	0.002	0.4%	0.56	0.003	0.6%

Table 4.5: The microwave frequencies with the highest t-values and corresponding p-value in various dielectric properties of urine for detecting pregnancy

Temperature (^{o}C)	Dielectric constant			Dielectric loss			Loss tangent		
	Frequency (GHz)	t value	p value	Frequency (GHz)	t value	p value	Frequency (GHz)	t value	p value
25	49.4	3.210	0.003	49.6	-3.257	0.002	49.4	-2.665	0.002
30	46.8	2.302	0.026	18	-2.176	0.026	34	-2.166	<0.01
37	16.4	3.912	<0.001	39.8	-3.616	0.001	15.8	-3.811	<0.01

Table 4.6: Highest r value for dielectric constant at three different temperatures in pregnant detection

Dielectric Constant						
Temperature (^{o}C)	**Frequency (GHz)**	**Max +r value**	**p value**	**Frequency (GHz)**	**Max -r value**	**p value**
25	0.6	0.469	0.009	34.2	-0.478	0.008
30	2.4	0.868	<0.001	14.2	-0.849	<0.001
37	4.2	0.48	0.007	34.4	-0.877	<0.001

Table 4.7: Highest r value for dielectric loss at three different temperatures in pregnant detection

Dielectric loss						
Temperature (^{o}C)	**Frequency (GHz)**	**Max +r value**	**p value**	**Frequency (GHz)**	**Max -r value**	**p value**
25	13	0.637	<0.001	49.8	-0.457	0.011
30	5.8/6/6.4	0.866	<0.001	46.2	-0.877	<0.001
37	15	0.856	<0.001	45.8	-0.872	<0.001

Table 4.8: Highest r value for loss tangent at three different temperatures in pregnant detection

Loss Tangent						
Temperature (^{o}C)	**Frequency (GHz)**	**Max +r value**	**p value**	**Frequency (GHz)**	**Max -r value**	**p value**
25	5	0.478	0.008	34.2	-0.868	<0.001
30	5.8/6	0.861	<0.001	26.6	-0.801	<0.001
37	2.8	0.465	0.009	43.4	-0.843	<0.01

4.2.3.4 Accuracy

Sensitivity (also known as true positive rate) refers to the percentage of patients who could be identified by a diagnostic method. It reflects the ability of the method to detect a positive result and a higher sensitivity level indicated a more effective outcome.

Specificity (also known as true negative rate) refers to the percentage of non-patients that the same diagnostic method could identify. It indicated whether the method could correctly identify a person with disease and a higher specificity level indicated a more precise outcome.

Accuracy refers to the ratio of patients to non-patients that a diagnostic method could detect in a population. This ensured that the method produced true positive results while excluding false positives, so that treatment could be correctly applied to those suffering a disease.

The mean value for dielectric constant of urine between non-pregnant subjects and pregnant subjects is 54.214 (f=16.4GHz, 37^{o}C). The range for dielectric constant of urine between non-pregnant subjects and pregnant subjects is between 54.224 (maximum) and 54.204 (minimum). The accuracy of all dielectric properties were illustrated in Tables 4.7 to 4.9. According to Table 4.3, the highest t-value for Ɛ' was 3.912 (f=16.4GHz, $p<0.01$) at 37^{o}C. Thus, we selected all Ɛ' values of pregnant and non-pregnant women at 16.4GHz and 37^{o}C to calculate their accuracy in detecting pregnancy. In Table 4.7, when Ɛ' was 54.22 and 54.204, the highest accuracy rate was 55%.

Table 4.9:Accuracy of dielectric constant in detecting pregnancy

No.	Dielectric Constant Threshold Value	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	54.222	30	76.67	53.33
2	54.22	36.67	73.33	55
3	54.218	36.67	66.67	51.67
4	54.216	36.67	63.33	50
5	54.214	40	63.33	51.67
6	54.212	43.33	63.33	53.33
7	54.21	43.33	56.67	50
8	54.208	43.33	53.33	48.33
9	54.206	53.33	53.33	53.33
10	54.204	56.67	53.33	55
11	54.202	56.67	50	53.33
12	54.2	56.67	46.67	51.67

The mean value for dielectric loss of urine between non-pregnant subjects and pregnant subjects is 31.701 (f=39.8GHz, 37^{o}C). The range for dielectric loss of urine between non-pregnant subjects and pregnant subjects is between 31.715 (maximum) and 31.688 (minimum). According to Table 4.3, the highest t-value for Ɛ" was -3.616 (f=39.8GHz, $p<0.01$) at 37^{o}C. Thus, we selected all Ɛ" values of pregnant and non-pregnant women at 39.8GHz and 37^{o}C to calculate the accuracy in detecting pregnancy. From Table 4.8, when Ɛ" was 31.712, the highest accuracy rate was 46.67%

Table 4.10: Accuracy of dielectric loss in detecting pregnancy

No.	Dielectric loss Threshold Value	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	31.712	53.33	40	46.67
2	31.71	56.67	30	43.33
3	31.708	60	26.67	43.33
4	31.706	60	26.67	43.33
5	31.704	60	26.67	43.33
6	31.702	60	26.67	43.33
7	31.7	60	23.33	41.67
8	31.698	60	23.33	41.67
9	31.696	60	23.33	41.67
10	31.694	60	23.33	41.67
11	31.692	63.33	23.33	43.33
12	31.69	63.33	23.33	43.33

The mean value for loss tangent of urine between non-pregnant subjects and pregnant subjects is 0.625 (f=15.8GHz, 37^{o}C). The range for loss tangent of urine between non-pregnant subjects and pregnant subjects is between 0.686 (maximum) and 0.564 (minimum). According to Table 4.3, the highest t-value for tan σ is -3.811 (f=15.8GHz, $p<0.01$) at 37^{o}C. Thus, we selected all tan σ values of pregnant and non-pregnant subjects at 15.8GHz and 37^{o}C to calculate the accuracy in detecting pregnancy. From Table 4.9, when the loss tangent is 0.556, the highest accuracy rate was 81.67%.

Table 4.11:Accuracy of loss tangent in detecting pregnancy

No.	Loss Tangent Threshold Value	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	0.572	73.33	80	76.67
2	0.57	73.33	80	76.67
3	0.568	73.33	80	76.67
4	0.566	73.33	80	76.67
5	0.564	73.33	80	76.67
6	0.562	73.33	76.67	75
7	0.56	73.33	63.33	68.33
8	0.558	73.33	30	51.67
9	0.556	86.67	76.67	81.67
10	0.554	51.67	50	50.84
11	0.552	51.67	50	50.84
12	0.55	51.67	50	50.84

4.3 The Results of Dielectric Properties of Urinary Exfoliated Epithelial Cells for Bladder Cancer

4.3.1 Urine Composition and Measurement

In this study, a total of 70 urine samples were collected from April 2017 to August 2018. About 20ml of each urine sample was sent to the Division of Laboratory Medicine for the measurement of clinical chemical variables in terms of protein, glucose, ketone, haemoglobin, bacteria, urea, sodium and potassium using Roche Cobas® U601 urine analyzer. The results were shown in Table 4.10. Samples tested positive for hematuria and diabetes were excluded. Thus, only urine samples of 10 healthy subjects and 10 subjects with bladder cancer were used in the final analysis.

Meanwhile, 10ml of each urine samples was sent for microscopic examination using the ThinPrep® 2000 method. The results are stated in Table 4.11. Photomicrographs of urine from healthy and bladder cancer cohorts were shown in Figure 4.9. The number of urinary exfoliated urothelial cells of each bladder cancer patient and stage of bladder cancer are stated in Table 4.12.

Table 4.12: Mean Urine Analysis of Healthy and Bladder Cancer Subjects

Group / Component	1 Normal	2 Bladder Cancer
Sample size (n)	10	10
Age (years)	65 ± 5	55 ± 6
pH	6 ± 0.7	6.5 ± 0.5
Protein (mg/dl)	0	0
Haemoglobin (mg/dl)	0	0
Glucose (mg/dl)	0	0
Ketone (mg/dl)	0	0
Bacteria	Nil	Nil
Urea (mmol/L)	177 ± 19	128 ± 29
Sodium (mmol/L)	85 ± 32	91 ± 31
Potassium (mmol/L)	30 ± 15	22 ± 8
The calculated value is equal to : the mean of each chemical variable of each group (n=10) ± standard deviation.		

Table 4.13: Urine Pathological Microscopy of healthy and bladder cancer subjects

Group / Component	1 Healthy	2 Bladder Cancer
Sample size (n)	10	10
Squamous cells (cells/mL)	5	6
Inflammatory cells (cells/mL)	3	7
Urothelial cells (cells/mL)	0	78
Total cells (cells/mL)	8	91

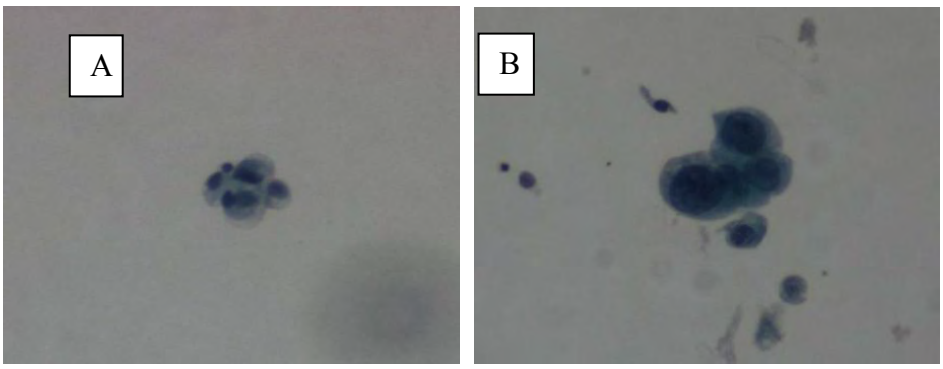


Figure 4.9: Urine ThinPrep® smears (A) Normal cohort: A small cluster of benign transitional cells exhibiting small uniform nucleus and abundant cytoplasm. The urine in the background is clean. [Papanicolaou (Pap) stain, x100 original magnification]; (B) Patient with bladder cancer: urine exhibits a cluster of highly dysplastic cells. These cells have pleomorphic hyperchromatic nucleus and reduced cytoplasm (high nucleo-cytoplasmic ratio, i.e. a feature of malignancy). The background shows scattered inflammatory cells. [Pap stain, x100 original magnification]

Table 4.14: Results of Urinary Exfoliated Urothelial cells

Subjects	Urinary Exfoliated Urothelial Cells (cells/ml)
1	74
2	65
3	80
4	65
5	95
6	80
7	74
8	95
9	80
10	74

4.3.2 Dielectric Properties of Tumour Cells

4.3.2.1 Overview

The results of Ɛ', Ɛ'' and tan σ at three temperatures are shown in Figures 4.10, 4.11 and 4.12, respectively. In all three temperatures, Ɛ' and Ɛ'' values increased with microwave frequency until a peak before decreasing to a crossing point. The crossing points were observed at 7.4GHz and 26.8GHz in Figures 4.10 and 4.11, respectively. Similar to

results of urine from pregnant and non-pregnant women, the highest Ɛ' and Ɛ'' values were at 25℃ while the lowest were at 37°C before the crossing point. After the crossing point, the results were reversed with 37°C having the highest values. A major difference in Ɛ' and Ɛ'' was observed at 25°C in certain frequencies. Generally, in bladder cancer patients, the urine Ɛ' was expected to be slightly higher than healthy subjects while Ɛ'' was slightly lower.

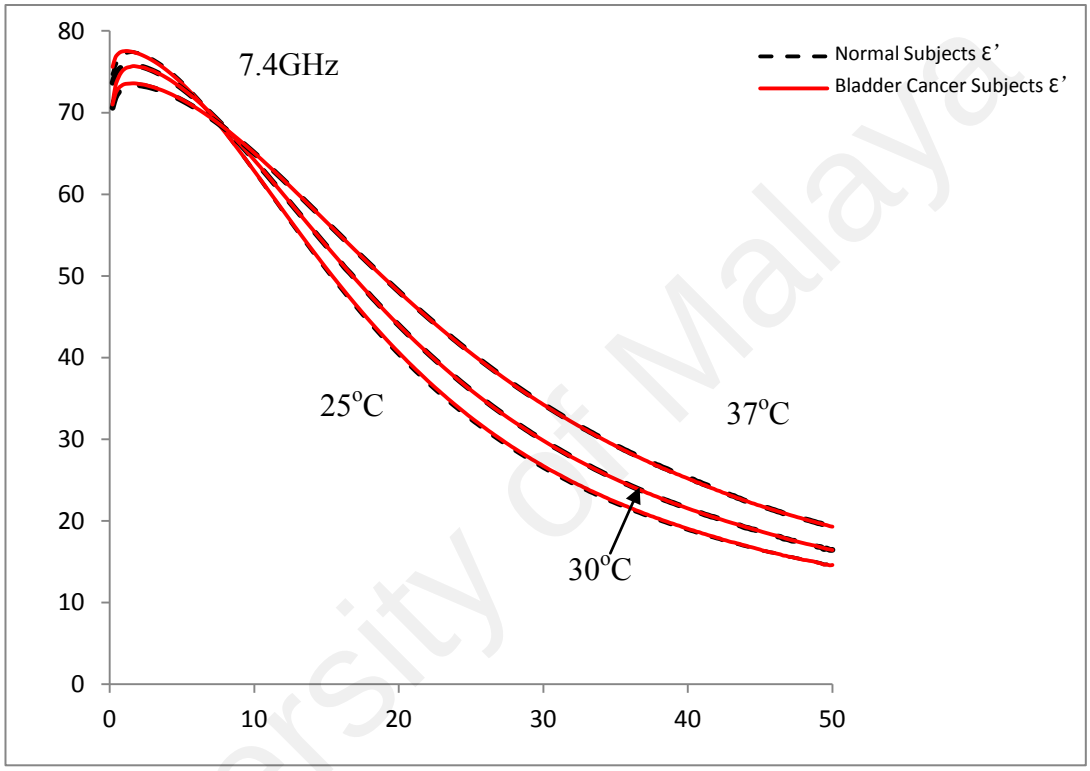


Figure 4.10: Mean dielectric constant (Ɛ') in urine of healthy subjects and subjects with bladder cancer at 25 °C, 30 °C and 37 °C

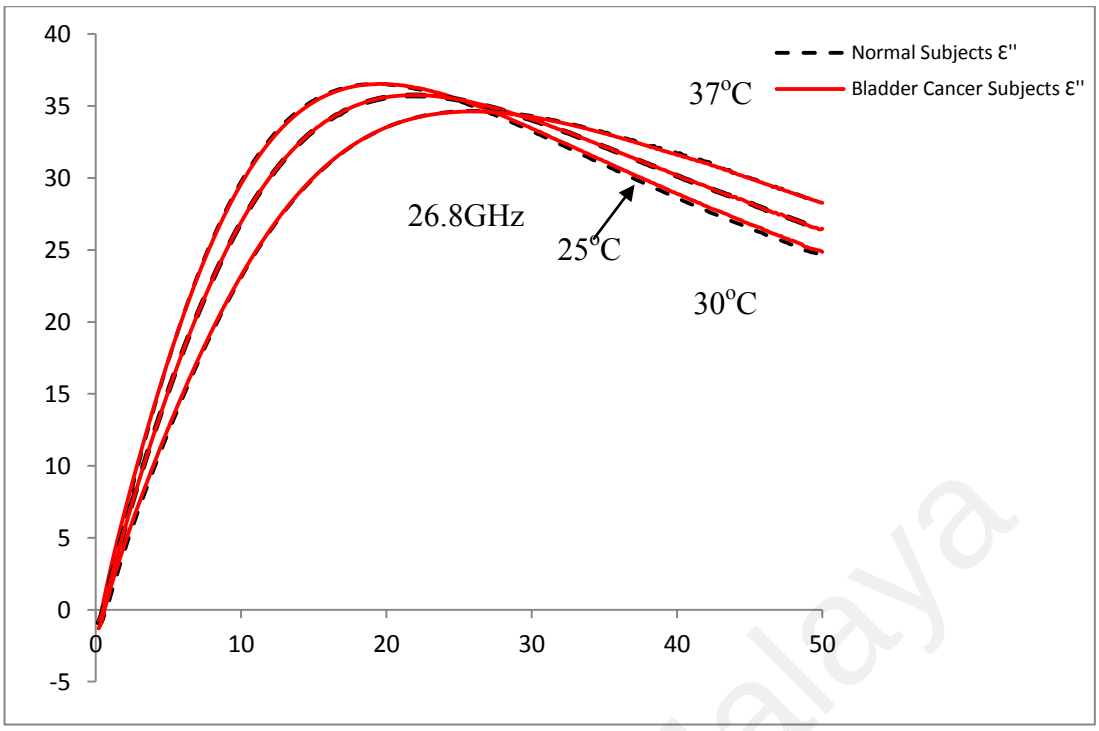


Figure 4.11: Mean dielectric loss (Ɛ") in urine of healthy subjects and subjects with bladder cancer at 25 °C, 30 °C and 37 °C

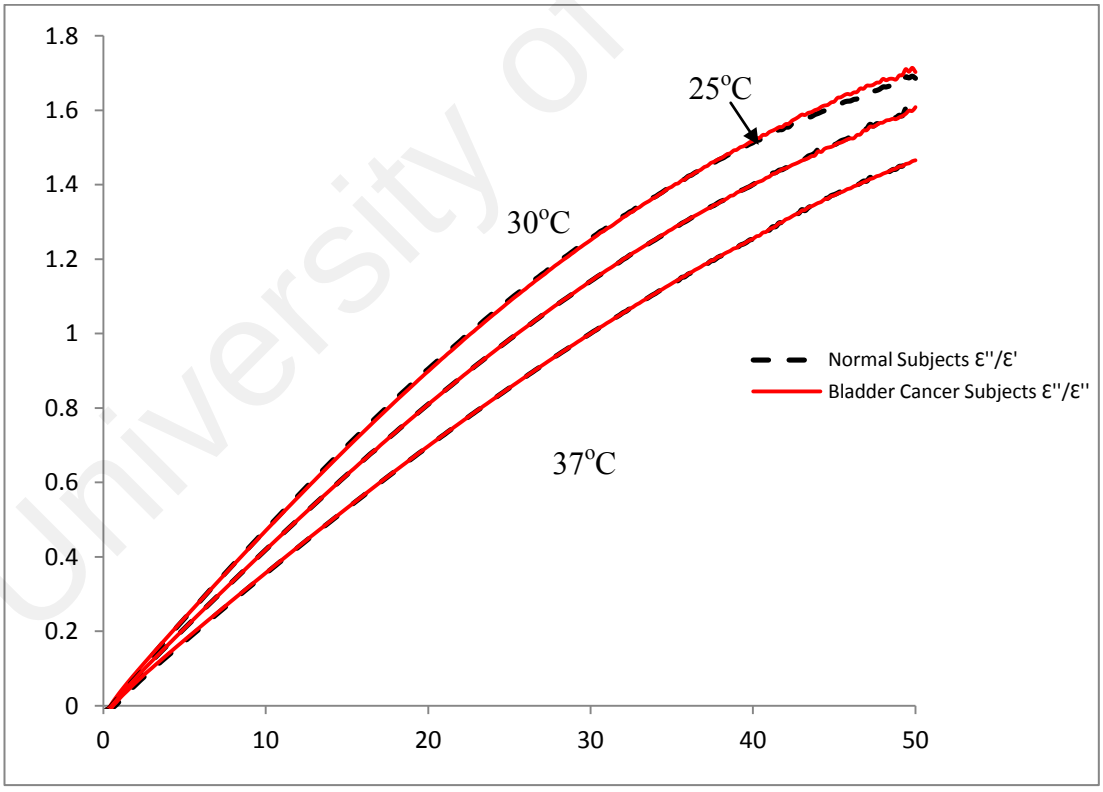
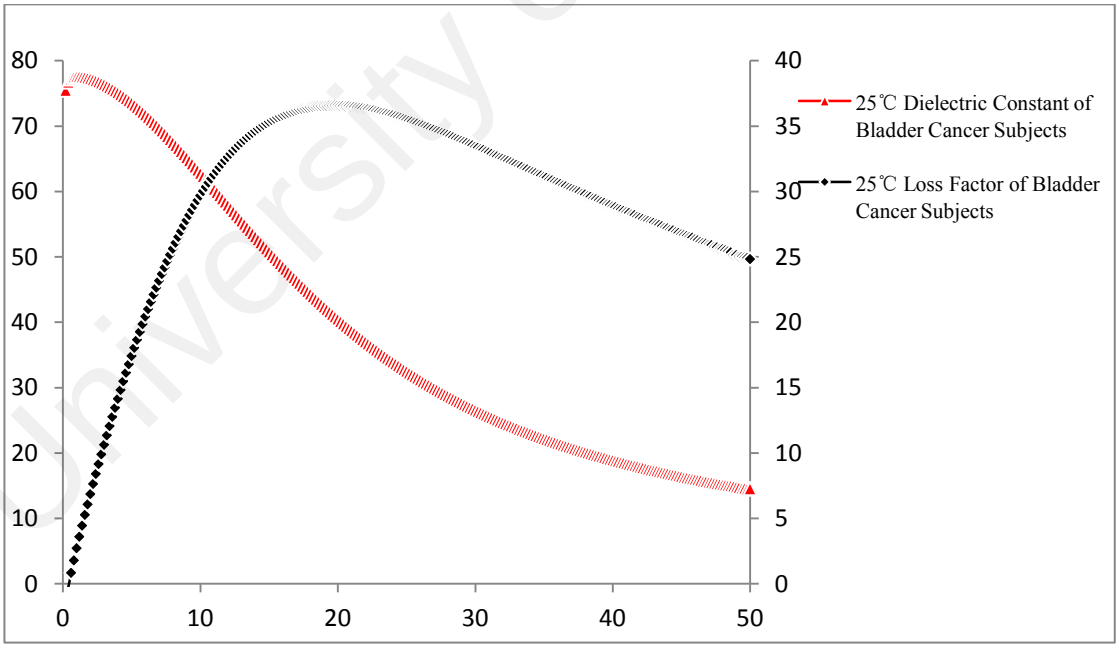
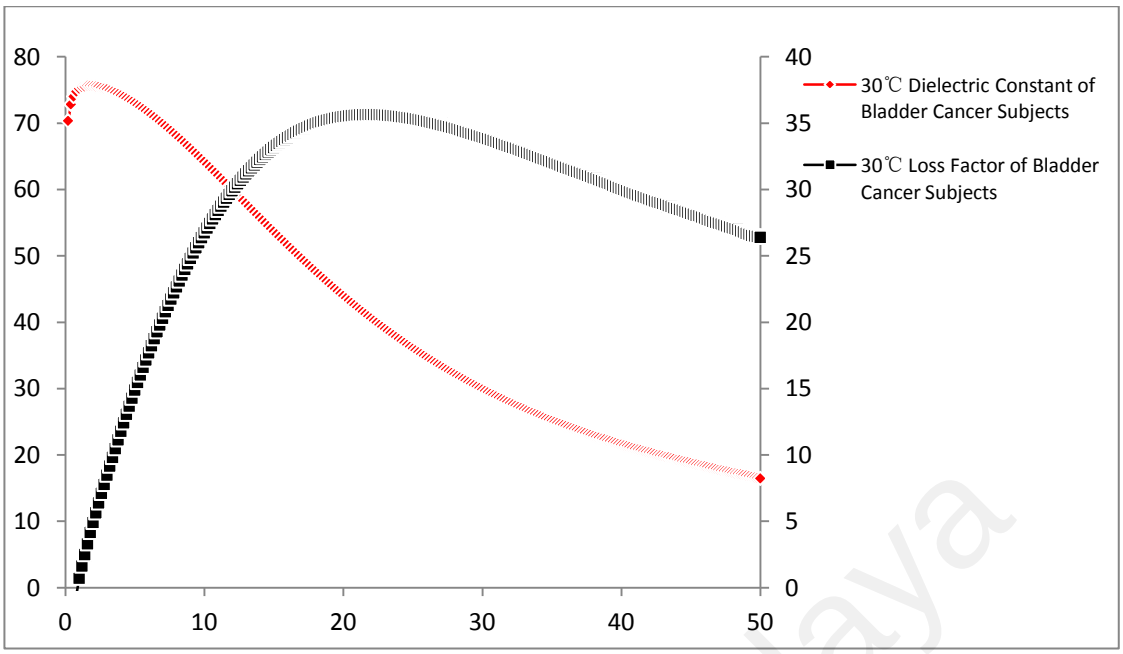


Figure 4.12: Mean loss tangent (tan σ) in urine of healthy subjects and subjects with bladder cancer at 25 °C, 30 °C and 37 °C

Figure 4.13 a, b and c show the Ɛ’ and Ɛ’’ curves in urine of bladder cancer patients when exposed to different microwave frequencies at 25°C, 30°C and 37°C. At low-frequency band (f<1GHz), the low frequency limit of Ɛ’ was high and and Ɛ’’ was low. This could be explained by the composition of urine, which was more than 95% water, and that water had high dielectric properties and particular molecular relaxation characteristics (Clarke et al., 2003). At the middle-frequency band (f=1-20GHz), Ɛ’ began to rapidly decrease whereas Ɛ’’ increased in tandem. This was due to the dielectric relaxation process of water undergoing capacitance charging and discharging (Gregory & Clarke, 2012; Smith et al., 1998). At high-frequency (f>20GHz), the decrease in Ɛ’ became less rapid while Ɛ’’ had reached its peak and was decreasing. This represented a relatively stable process of reduction, which was due to the low insulation and high conductivity characteristics of water in a high frequency field (Chandrau & Bagchi, 2000; Gregory & Clarke, 2012).





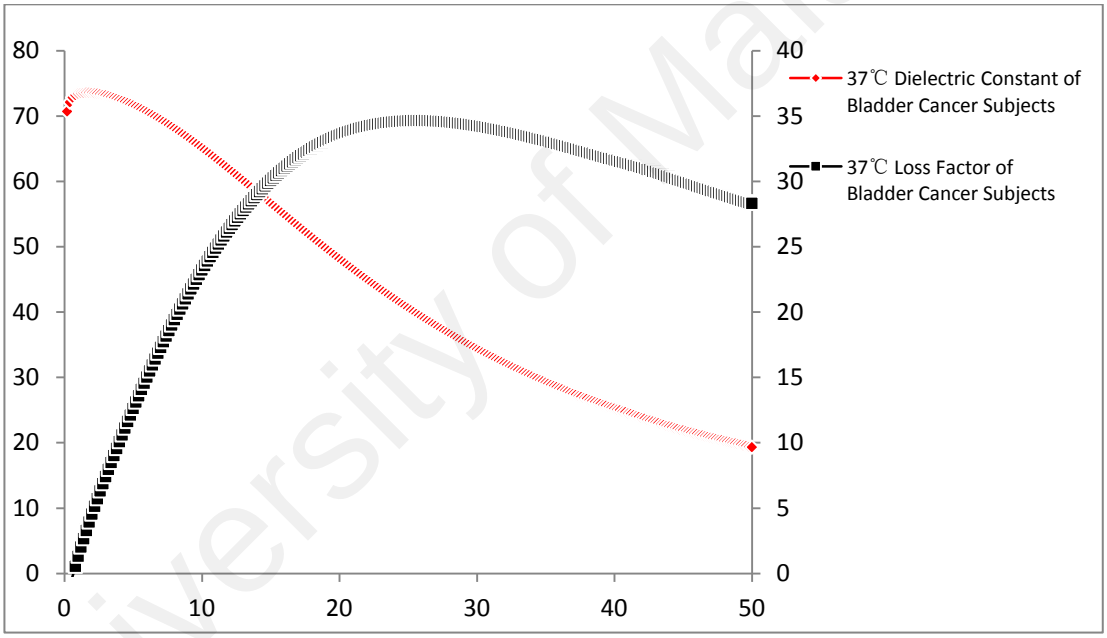


Figure 4.13: The average dielectric constant (Ɛ') and dielectric loss(Ɛ") in urine of 10 bladder cancer patients when exposed to microwave frequency range of 200MHz to 50GHz at 25°C (a), 30℃ (b) and 37°C (c).

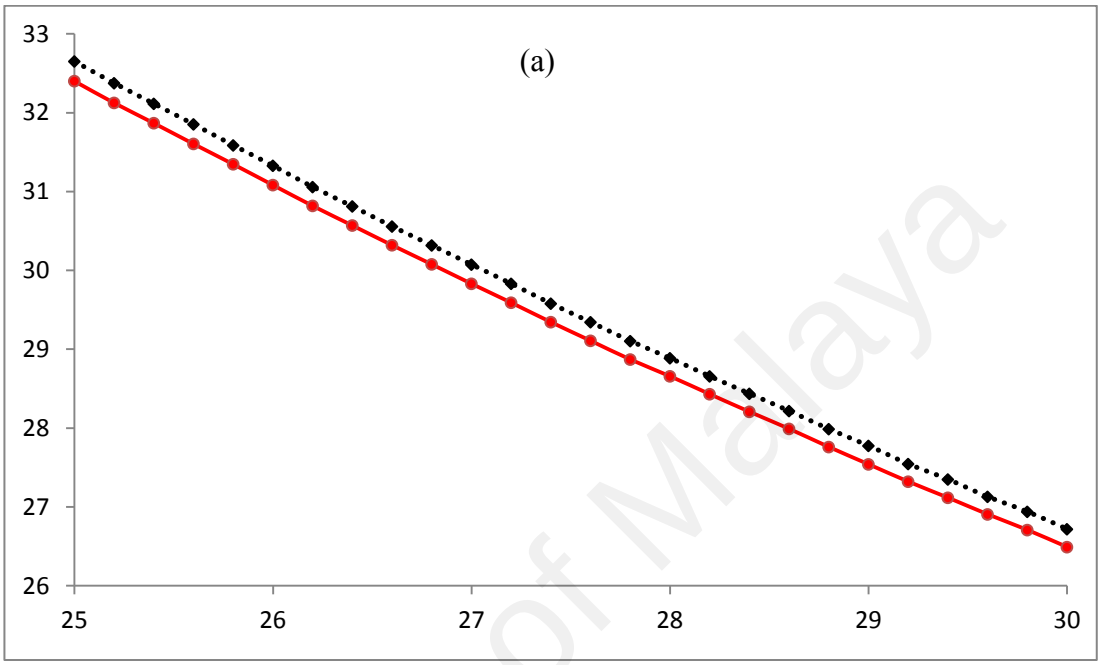
4.3.2.2 Comparison Between Healthy and Bladder Cancer Patients Across Three Temperatures

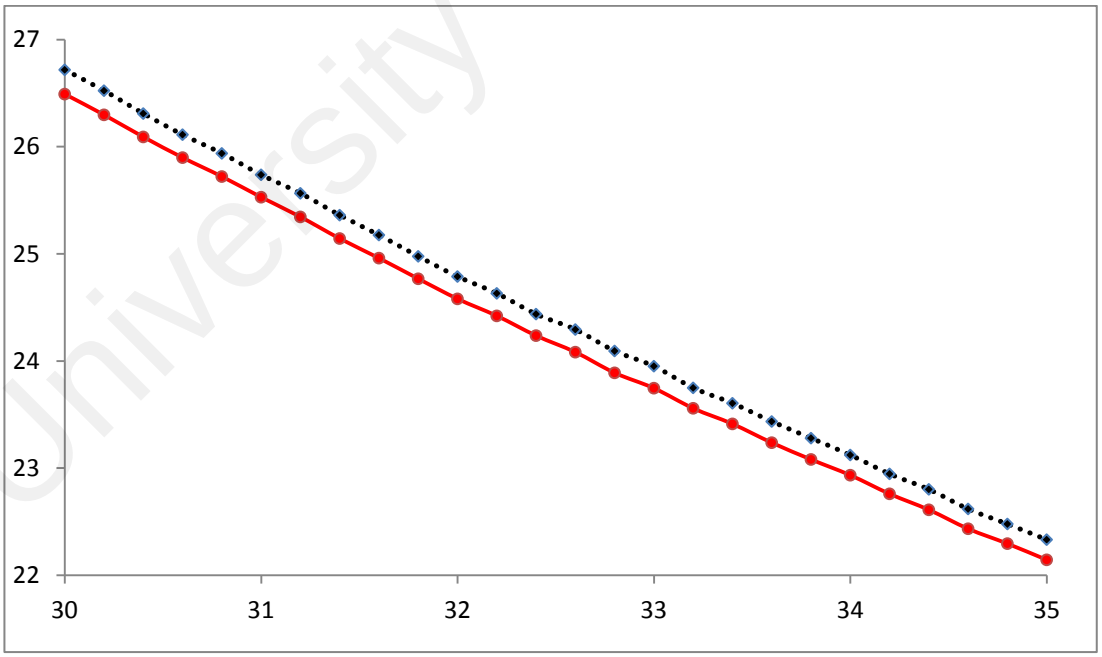
Significant differences in dielectric properties were observed across three temperatures at most frequency points. The relaxation process of water could be used for the electrical field since it is generally known that the vast majority of the component that make up urine is water. A minor difference in terms of Ɛ' and Ɛ'' was observed at 25°C at certain frequencies.

In Figures 4.14 and 4.15, no significant differences could be observed in the dielectric properties between two groups at low (0.2-25GHz) and high (40-50GHz) frequencies. But at the mid-frequency range of between 25GHz and 40GHz, more frequency points were found to have significant differences. The figures showed that the significant differences in Ɛ'' between healthy subjects and bladder cancer patients were more obvious than Ɛ'. With the increase of frequency, the dielectric properties of patients with bladder cancer were higher than that of healthy subjects.

Due to the total subjects in the final analysis of bladder cancer research are small, the data exhibited non-normally distributed. Therefore, we used non-parametric statistic for analysis. As shown in Table 4.13, the highest significant differences (p value) for three different temperatures with their respective frequency points were presented. It was concluded that the dielectric constant (f=21.8GHz, p=0.01, 37^{o}C) of the urine of patients with bladder cancer was statistically significantly lower than that of the healthy subjects while the dielectric loss (f=41.6GHz, p=0.002, 30^{o}C) of the urine of patients with bladder cancer were statistically significantly higher than that of the healthy subjects.

Significant differences in dielectric constant were observed across the temperatures with the highest F number at 35.8GHz ($F=1576.3$, $p<0.01$). Significant differences in dielectric loss were observed across the temperatures as well with the highest F number at 21GHz ($F=1759.1$, $p<0.01$).





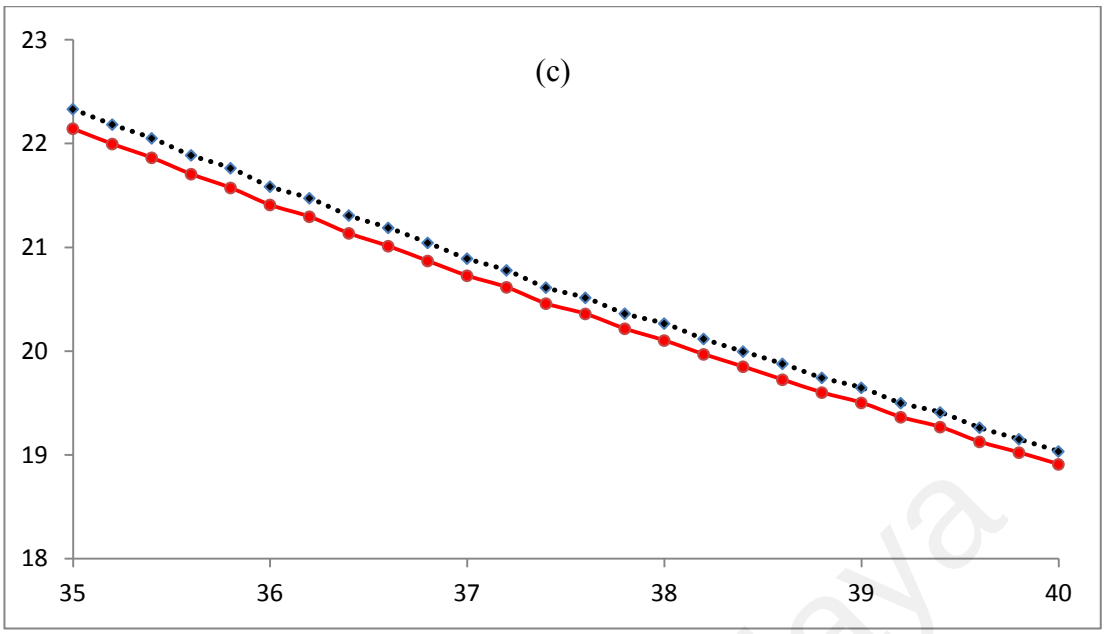
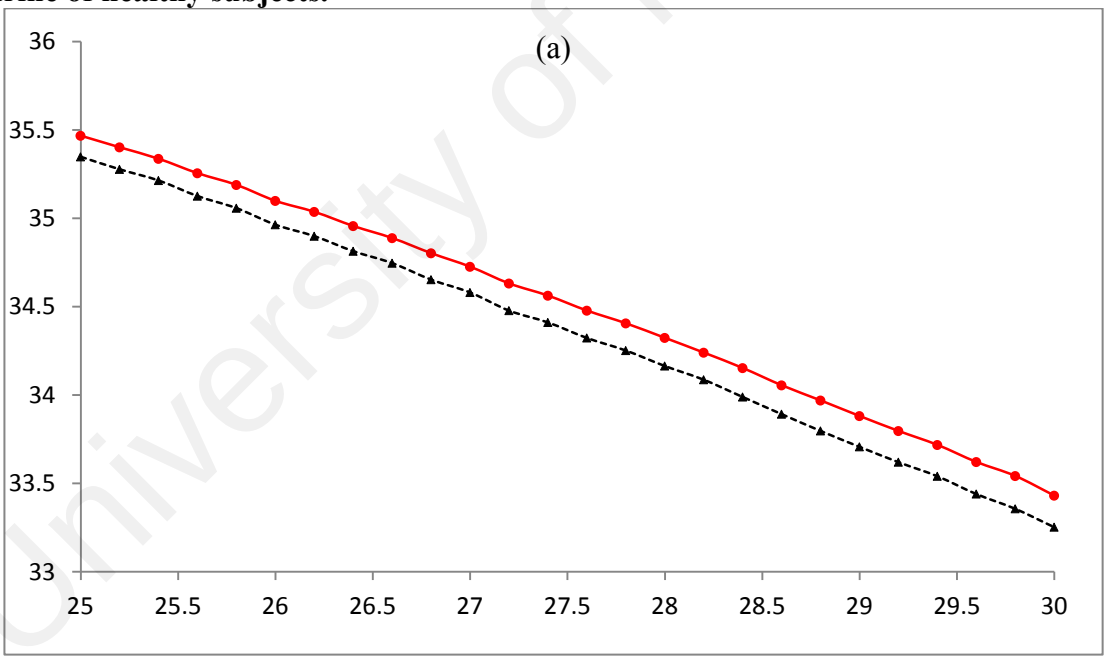
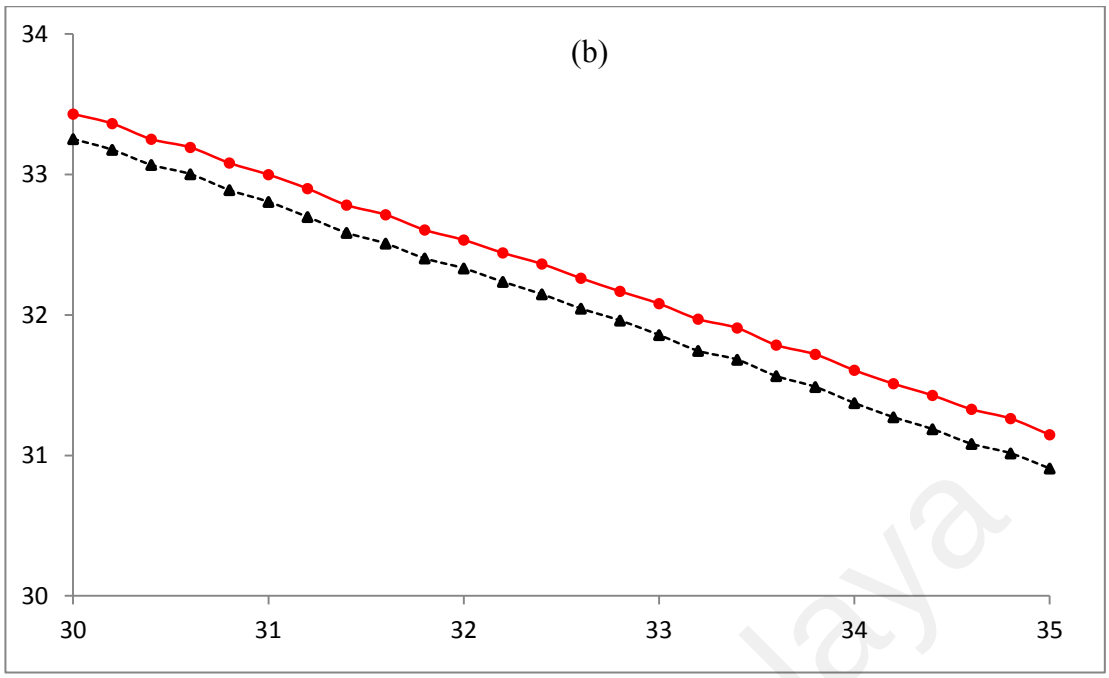


Figure 4.14: Dielectric constant in urine of healthy subjects and bladder cancer patients measured at 25℃ in microwave frequencies of (a) 25-30GHz, (b) 30-35GHz and (c) 35-40GHz. Black dotted line represents dielectric constant of urine of bladder cancer patients, red solid line represents dielectric constant of urine of healthy subjects.





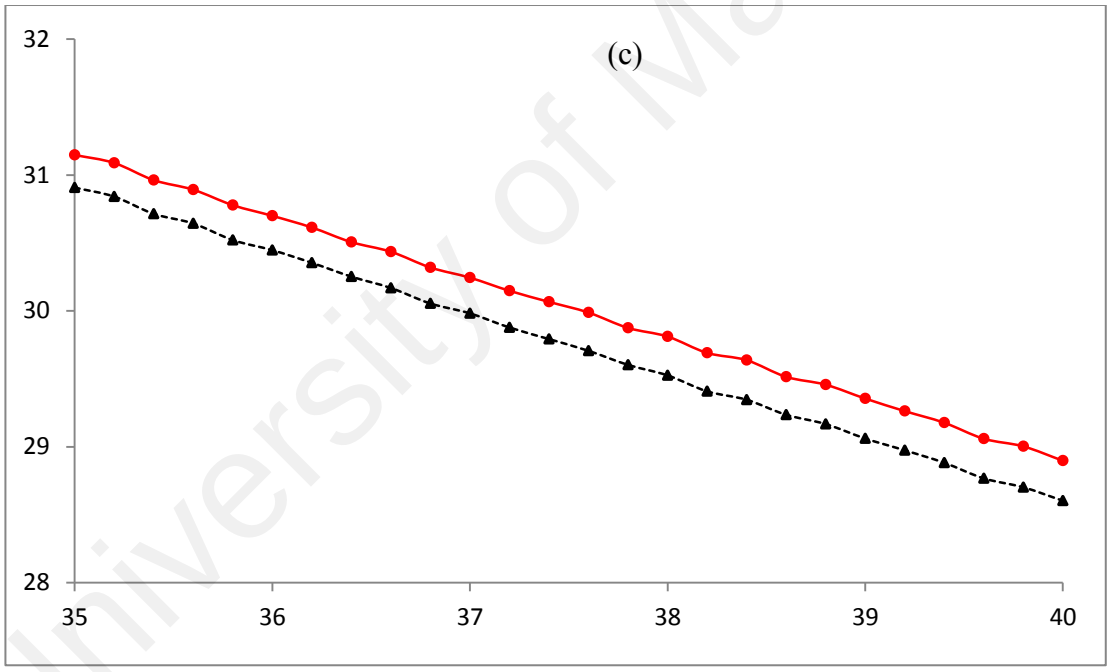


Figure 4.15: Dielectric loss in urine of healthy subjects and bladder cancer patients measured at 25℃ at (a) 25-30GHz, (b) 30-35GHz and (c) 35-40GHz microwave frequencies. Black dotted line represents dielectric constant of urine of bladder cancer patients, red solid line represents dielectric constant of urine of healthy subjects.

4.3.2.3 Correlation of Urinary Exfoliated Urothelial Cells and Dielectric Properties

The highest Pearson correlation coefficients for dielectric properties across three temperatures are shown in Table 4.14. At 25°C, it was observed that Ɛ' had negative correlation with the number of cancerous cells (r=-0.662, p=0.03), whereas, Ɛ" had positive correlation with the number of cancerous cells (r=0.664, p=0.02).

In Figure 4.16 the linear graph for potential prediction between 65-85cells/ml in Ɛ' was presented as a negative correlation (30°C, 25.4GHz, r=-0.63), whereas potential prediction between 55-80cells/ml for Ɛ" was presented as a positive correlation (30°C, 40.6GHz, r=0.664). Direct lines indicated potential for prediction, thus, more data were need to validate in the range of 55 to 88cells/ml.

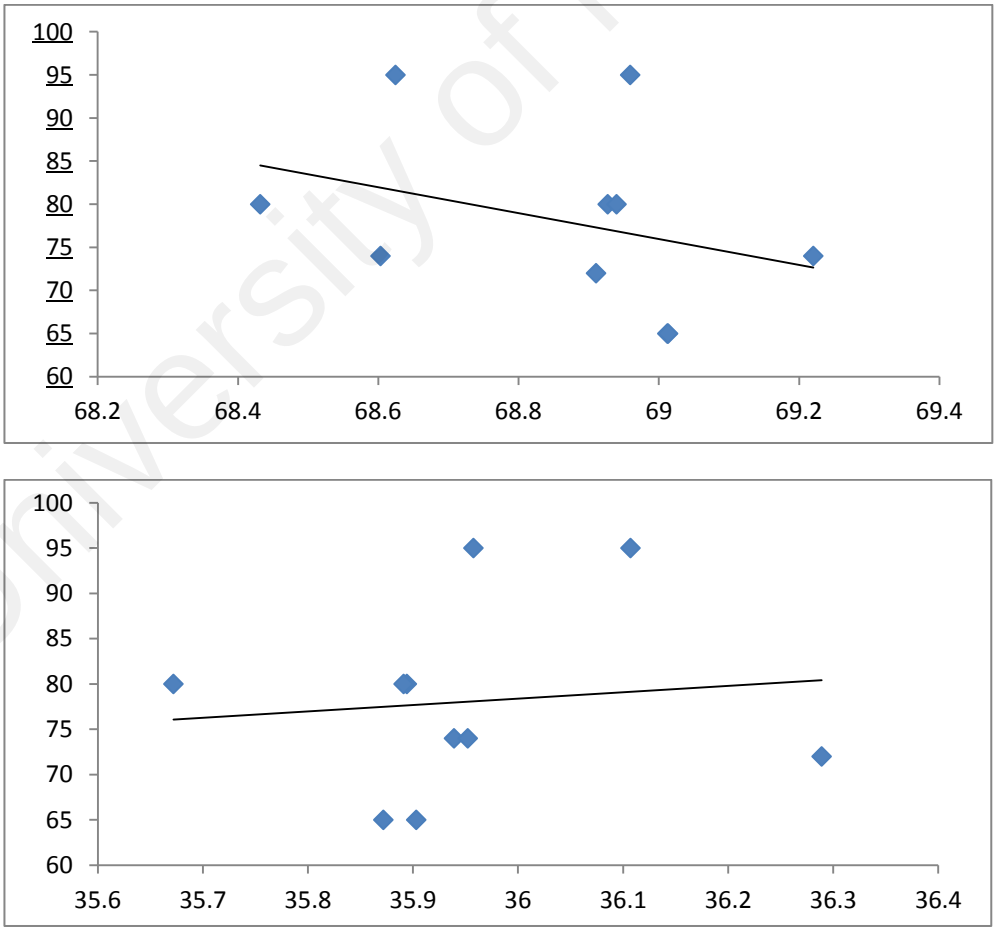


Figure 4.16: Potential prediction in dielectric constant (above) and dielectric loss at 25°C

Table 4.15: Highest significant differences (p value) for dielectric constant and dielectric loss at three different temperatures in bladder cancer detection

Dielectric constant			Dielectric loss	
Temperature (^{o}C)	**Frequency (GHz)**	**p value**	**Frequency (GHz)**	**p value**
25	6.2	0.05	25.2	0.007
30	49.4	0.05	41.6	0.002
37	21.8	0.01	41.2	0.003

Table 4.16: Highest r value for dielectric constant and dielectric loss at three different temperatures in bladder cancer detection

Dielectric constant				Dielectric loss		
Temperature (^{o}C)	**Frequency (GHz)**	**Maximum r value**	**p value**	**Frequency (GHz)**	**Maximum r value**	**p value**
25	5	-0.662	0.03	40.6	0.664	0.02
30	25.4	-0.63	0.04	49	0.598	<0.05
37	3.4	-0.597	0.07	41.6	0.4359	<0.05

4.3.2.4 Accuracy

Sensitivity (also known as true positive rate) refers to the percentage of patients who could be identified by a diagnostic method. It reflects the ability of the method to detect a positive result and a higher sensitivity level indicated a more effective outcome.

Specificity (also known as true negative rate) refers to the percentage of non-patients that the same diagnostic method could identify. It indicated whether the method could correctly identify a person with disease and a higher specificity level indicated a more precise outcome.

Accuracy refers to the ratio of patients to non-patients that a diagnostic method could detect in a population. This ensured that the method produced true positive results while excluding false positives, so that treatment could be correctly applied to those suffering a disease.

The mean value for dielectric constant of urine between healthy subjects and subjects with bladder cancer is 73.792 (f=5GHz, 25^{o}C). The range for dielectric constant of urine between healthy subjects and subjects with bladder cancer is between 73.808 (maximum) and 73.776 (minimum). According to Table 4.13, the highest t-value for dielectric constant was -0.662 (f=5GHz, p=0.03) at 25^{o}C. Thus, we selected all values of bladder cancer subjects and healthy subjects at 5GHz and 25^{o}C to calculate the accuracy. The accuracy of dielectric properties were illustrated in Tables 4.15 and 4.16. From Table 4.15, when the dielectric constant was 73.76, the highest accuracy rate was 65%.

Table 4.17: Accuracy of dielectric constant for bladder cancer

No.	Dielectric Constant Threshold Value	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	73.8	40	80	60
2	73.795	40	80	60
3	73.79	40	80	60
4	73.785	40	80	60
5	73.78	50	60	55
6	73.775	50	60	55
7	73.77	50	50	50
8	73.765	50	50	50
9	73.76	80	50	65
10	73.755	70	40	55

The mean value for dielectric loss of urine between healthy subjects and subjects with bladder cancer is 28.31 (f=40.6GHz, 25^{o}C). The range for dielectric loss of urine between healthy subjects and subjects with bladder cancer is between 28.36 (maximum) and 28.26 (minimum). In Table 4.13, the highest t-value for Ɛ" was 0.664 (f=40.6GHz, p=0.02) at 25^{o}C. Thus, all Ɛ" values at 40.6GHz and 25^{o}C were selected to calculate accuracy. Table 4.16 shows that when Ɛ" was 28.27, the highest accuracy rate was 75%.

Table 4.18: Accuracy of dielectric loss for bladder cancer

No.	Dielectric loss Threshold Value	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	28.36	70	60	65
2	28.35	70	60	65
3	28.34	70	60	65
4	28.33	80	60	70
5	28.32	80	60	70
6	28.31	80	60	70
7	28.3	80	60	70
8	28.29	80	60	70
9	28.28	80	60	70
10	28.27	80	70	75

4.4 Discussion

4.4.1 Discussion on the Results of Pregnancy Detection

4.4.1.1 Effect of Urine Composition

According to Tables 4.1, the results of urine analysis showed that there are several elements might affect urine dielectric properties, e.g. PH, protein, haemoglobin, glucose, ketone, bacteria, epithelial cell, crystal and hCG. No statistical difference was reported between healthy non-pregnant subjects and healthy pregnant subjects for PH, protein, haemoglobin, glucose, ketone, bacteria, epithelial cell and crystal ($p>0.05$). The absence of elements and neutral pH of the urine samples indicated that the dielectric properties of are not distorted by other pathological conditions. This ensures that the dielectric properties in the samples were influenced only by hCG, which was the gold standard in detecting pregnancy. The hCG level was significantly different between non-pregnant and pregnant women ($p<0.05$)..

4.4.1.2 Effect of Temperature on Dielectric Properties

Significant differences in dielectric properties were observed in the three temperatures at most frequency points. As water was the largest composition of urine, therefore, the electrical field could use the relaxation process of water. Wentholt et al. (2008) showed that the dielectric properties of pure water could vary with temperature due to intramolecular hydrogen bonding between water molecules. Ellison (2007) pointed out that when the temperature was at 25^{o}C and 30^{o}C, the f_r of pure water was 18.56GHz and 21.65GHz, respectively. Mun et al. (2015) compared the relaxation of pure water with normal urine from Ellison (2007), and observed a similar data.

Lonappan et al. (2007b) reported that at 2 to 3 GHz, reported that at 2 to 3GHz, electrical conductivity in urine samples of non-pregnant women would be lower than

pregnant women and Ɛ' would be higher. In this study, we found a similar trend of dielectric properties. However, the value of Ɛ' reported by Lonappan et al. (2007b) was much lower, and this difference could be due to different measurement methods, temperature, quantitative limitation and time control.

As shown in Figure 4.6, the Ɛ' in urine of pregnant women was lower than non-pregnant women. From Figure 4.7 and Figure 4.8, it could be seen that Ɛ'' and tan σ in the urine of non-pregnant women were lower than pregnant women. Table 4.2 showed the mean relative errors in terms of dielectric properties in pregnant and non-pregnant women, given under three different temperatures, of which the significant differences were indicated by the P-values. From Table 4.3, it could be deduced that Ɛ' in the urine of pregnant women ($t=3.912$, $f=16.4$GHz, $p<0.05$, 37^{o}C) was significantly lower than non-pregnant women, while Ɛ'' ($t=-3.616$, $f=39.8$GHz, $p<0.05$, 37^{o}C) and tan σ ($t=-3.210$, $f=34$GHz, $p<0.05$, 37^{o}C) were significantly higher.

However, due to the low t values, these significant differences could be considered "weak". The reason for this could be explained by the high level of hCG in the pregnant women's urine. Lonappan (2012) observed that hCG could change the dielectric properties of blood and urine. He reported obvious changes in Ɛ' and electrical conductivity in blood and urine samples exposed to a microwave frequency of between 2 and 3GHz. He explained that this was due to the high level of hCG in the blood and urine of pregnant women, which was in line with this study.

Significant differences in dielectric constant were observed across the temperatures with the highest F number at 23.6GHz ($F=82785.991$, $p<0.05$). Significant differences in dielectric loss were observed across the temperatures with the highest F number at

12.8GHz (F=33051.956, p<0.05). Significant differences in Ɛ" were observed across the temperatures as well with the highest F number at 16.2GHz (F=1097.299, p<0.05). F number indicate the levels of significant difference, the p-value is used to evaluate F number based on the sample variations and differences. Ellison (2007) observed that the impact of temperature on dielectric properties might be due to the molecular movement and hydrogen bonds in urine when the temperature changes. It could be observed that the relaxation time was shorter. Therefore, when the temperature became higher, the solution viscosity would become weaker because ion mobility within the solution was increasing, which meant that the solution's viscosity would become negatively correlated with relaxation frequency.

4.4.1.3 Effect of hCG on Dielectric Properties

The highest Pearson correlation coefficients for Ɛ', Ɛ" and tan σ at three different temperatures were stated in Tables 4.4 to 4.6. The dielectric properties decreased with temperature before the crossing point and they increased with temperature after the point. Generally the dielectric properties correlated positively with hCG at low frequencies but they would become negatively correlated at high frequencies. Generally the dielectric properties correlated positively with hCG at low frequencies but they correlated negatively with hCG at high frequencies. The correlation coefficients of more than ±0.85 indicate strong correlation, which suggested the potential development dielectric properties in urine as a tool to detect pregnancy.

Due to the limited research in dielectric properties of hCG, we propose for a comparative experiment to verify our conclusion. We experimented with urine voided in the morning and collected in two containers, with one being sealed and stored at 4 ^{o}C and the other left open at room temperature. After five hours, bacterias were detected in the “open” sample. In a repeat experiment, Ɛ', Ɛ" and tan σ of the exposed urine sample

(with bacteria) were found to be different from the sealed sample. This result was consistent with our previous conclusions.

4.4.2 Discussion on the Results of Bladder Cancer Detection

4.4.2.1 Effect of Urine Composition

Similar to the pregnancy detection experiment, the urine analysis and microscopy results in Tables 4.10 to 4.12 found no elements in the samples of healthy subjects and bladder cancer patients. The levels of pH, urea, sodium and potassium were not statistically different between subjects. No statistical difference was reported between healthy subjects and patients with bladder cancer for urea ($t=0.092$, $p>0.05$), sodium ($t=0.15$, $p>0.05$), potassium ($t=0.916$, $p>0.05$), PH ($t=-0.643$, $p>0.05$), squamous cells ($t=1.04$, $p>0.05$) and inflammatory cells ($t=-2.316$, $p>0.05$). However, a significant difference was observed in urinary exfoliated urothelial cell number ($t=-23.447$, $p<0.001$), with bladder cancer patients shedding more abnormal cells in their urine.

4.4.2.2 Effect of Temperature on Dielectric Properties

Significant differences in dielectric constant were observed across temperatures with the highest F value at 35.8GHz ($F=1576.3$, $p<0.01$). Significant differences in Ɛ" were observed across the temperatures as well with the highest F value at 21GHz ($F=1759.1$, $p<0.01$). We have not yet determined why the 35.8GHz in Ɛ' and 21GHz in Ɛ" were more significant in bladder cancer patients than other frequency points. It was possible that the other frequencies could be used to detect other body organs or diseases. Temperature could alter the dielectric properties of urine by affecting molecular movement and hydrogen bonds of water (Ellison, 2007). The relaxation time was shortened. When the temperature increased, the viscosity of the solution would decrease due to more molecular movement and ion mobility. Therefore, when viscosity was

affected, the relaxation frequency of molecules would become inversely proportional (Ellison, 2007; Roberts et al., 1993). However, this study found that with the increase of temperature, the random movement of molecules could have weakened the effect of cancerous cells. Thus, this could explain the significant differences that mostly occured in urine samples tested at 25°C.

In Table 4.10, the highest significant differences (p-value) for three different temperatures with their respective frequency points were presented. The reason for the significant differences could be explained by the presence of urinary exfoliated urothelial cells that were increased in the urine of bladder cancer patients, and cancerous cells have lower electrical impedance and electrical membrane potential. The dielectric properties of cancer cells were related to the properties of their cell wall, the area where the charge was generated. There was “leaky” dielectric fields in these cells, thus any changes can influence the dielectric properties of cells. The kinematic viscosity of human urine was essential to accurate modeling of heat transfer and fluid mechanics during hyperthermia treatment of bladder cancer, and it was temperature-dependent and always higher (approximately 10%) than pure water (within 20-42°C). Meanwhile, the age, gender, glucosuria, ketonuria, urinary tract infection and haematuria did not affect the kinematic viscosity of human urine (Brant et al., 2013).

Due to the lack of tissue properties reports, the detection of bladder disease has been dealt with an estimated "effective thermal conductivity" parameter that tried to imitate the integrative effect of conductivity and convection within the bladder wall and bladder urine (Yuan et al., 2012). Previous studies had also used urothelial cells to detect pre-malignant urothelial lesions and urothelial carcinoma (Christoph et al., 2004; Fakhry et al., 2012).

4.4.2.3 Effect of Urinary Exfoliated Epithelial Cells on Dielectric Properties

Urinary exfoliated urothelial cells were closely related to bladder cancerous disease (Halling et al., 2002; Ishiwata et al., 2001). To use FISH to assay urinary exfoliated urothelial cells for detecting bladder cancer in voided urine had been successfully investigated and the urine markers could also predict recurrence (Ishiwata et al., 2001). In addition, some current methods (such as the UroVysion kit , telomerase test, BTA stat test and hemoglobin dipstick test) were used for detecting urothelial carcinoma in urine, and each method had different sensitivity and specificity (Halling et al., 2002). Therefore, further research work was needed to investigate the role of urinary exfoliated urothelial cells in diagnosing, treating and monitoring bladder cancer.

Due to the limited research in dielectric properties of urinary exfoliated urothelial cells, we propose a comparative experiment to verify our conclusion. We experimented with a person's morning urine in two containers, one of the containers was stored in the refrigerator at 4 ^{o}C, and the other one was left open without the lid at room temperature. After five hours, the "open" urine sample produced some bacteria. During the repeat experiment, we found that the dielectric constant and dielectric loss of the "open" urine sample (with bacteria) were different with hat of the "closed" urine sample. This result was consistent with our previous conclusions.

The highest Pearson correlation coefficients for Ɛ' and Ɛ" at three different temperatures were stated in Tables 4.14. The dielectric properties decreased with temperature before the crossing point and they increased with temperature after the point. Meanwhile, the dielectric constant correlated negatively with urinary exfoliated urothelial cells at low frequencies while dielectric loss correlated positively at high frequencies.

4.4.3 Discussion on the Results of Accuracy and Reproducibility

Arai et al. (1995) reported that dielectric properties measured at temperatures higher than room temperature produced lower accuracy due to technical problems in thermal expansion and temperature gradient of the coaxial probe. To overcome this, the coaxial slim probe was heated to 30 ^{o}C and 37 ^{o}C in a water bath before measurements were taken. This increased the reproducibility of measurements to more than 90%. Hence, measurement accuracy might differ between two different techniques, cavity perturbation and open-ended coaxial probe technique. Meanwhile, less comparable data was observed at high frequencies for dielectric properties of methanol. The measurements of methanol were mostly obtained at frequencies below 10GHz as the dielectric properties of methanol, especially in millimeter waves, were considered too unreliable from the technique used in this study (Kaatze, 2007). The appropriate method for measuring dielectric properties of biological solutions with approximately 0.1M salt content had been validated by using the open-ended coaxial probe technique with accuracy of more than 95%.

4.5 Summary

This chapter discussed the results of this study. The urine of pregnant women were observed to have significantly lower Ɛ' than non-pregnant women at 25°C, 30°C and 37°C but only at certain frequencies ($p<0.05$, mean relative error<0.48%), Ɛ" ($p<0.05$, mean relative error<0.56%) and tan σ ($p<0.05$, mean relative error<0.71%) were significantly higher in pregnant women at the three temperatures but also only at certain frequencies. The dielectric properties decreased with temperature before the crossing point and increased again with temperature after the crossing point. Generally the dielectric properties correlated positively with hCG at low frequencies but negatively at

high frequencies. The correlation coefficients of more than ±0.85 indicated strong correlation, which suggested the potential development of a diagnostic tool for pregnancy detection. The highest accuracy achieved in this study was 81.67% (f=15.8GHz, σ=0.556, 37^{o}C).

Statistically significant differences in Ɛ' and Ɛ'' were observed between the urine samples from healthy people and bladder cancer patients across all three temperatures. A high level of correlation was observed between urinary exfoliated urothelial cells and the dielectric properties of the urine at 25°C. The highest accuracy for bladder cancer was 75% (f=40.6GHz, Ɛ'=28.27, 25^{o}C).

Then we compared and discussed our results with other papers. The results of this study suggested that it was possible to detect bladder cancer based on the dielectric properties of urine.

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Dielectric properties of the urine of pregnant women, non-pregnant women, subjects with bladder cancer and healthy people were determined at Microwave frequencies between 0.2GHz and 50GHz at 25^{o}C, 30^{o}C and 37^{o}C.

In the study of pregnancy, the Ɛ' and Ɛ'' were observed to be significantly different between the urine samples of the two groups across all three temperatures. The dielectric constant was generally lower in pregnant women compared with non-pregnant women. The dielectric loss and loss tangent was higher in pregnant women compared with non-pregnant women. Dielectric properties decreased with temperature before the crossing point and increased with temperature after the crossing point. Statistically significant differences in the dielectric constant and the dielectric loss were observed between pregnant and non-pregnant women. A high level of correlation was observed between hCG and the dielectric properties of the urine. The dielectric properties correlated positively with hCG at low frequencies and correlated negatively with hCG at high frequencies. They correlated positively with hCG levels at low frequencies (r_{max}=0.868, f=2.4GHz) but negatively at high frequencies (r_{max}=-0.877, f=46.2GHz).

In cases of bladder cancer, the dielectric properties were generally lower in healthy subjects compared with patients with bladder cancer, especially at 25°C. Dielectric properties decreased with temperature before the crossing point and increased with temperature after the crossing point. Statistically significant differences in the dielectric constant and the dielectric loss were observed between healthy subjects and patients with bladder cancer. Statistically significant differences in Ɛ' and Ɛ'' were observed

between healthy subjects and patients ($P<0.05$). A high level of correlation was observed between urinary exfoliated urothelial cells and the dielectric properties of the urine at 25°C. The Ɛ' correlated negatively with urinary exfoliated urothelial cells (r_{max}=-0.662, f=5GHz) while Ɛ'' correlated positively with urinary exfoliated urothelial cells (r_{max}=0.664, f=40.6GHz).

No obvious in value of significant differences between pregnant women and non pregnant women, between healthy subjects and bladder cancer can be observed in graph between the groups. Furthermore, there is 5% error in the dielectric probe measurement. This error is higher than the percentage of difference between the groups. For the time being, this limits the application of the dielectric measurement for the diagnosis purpose unless, the percentage difference is much higher compared to the measurement error.

5.2 Limitation of the Study

The study of dielectric properties of urine in related to pregnancy is limited while the study of dielectric properties of urine for bladder cancer is new. In our study, the urine samples of bladder cancer patients were limited. Because of the distance between UMMC and our laboratory, urine cannot be measured immediately, which may lead to a few bacteria entering the urine container. Meanwhile, the error of the measurement has not been minimized.

5.3 Recommendations

Due to the limited bladder cancer urine samples and temperature control, more subjects and more temperature settings should be considered in future studies. At the current stage, the differences in urinary dielectric properties of hCG and urinary exfoliated cells are insufficient to be used as a diagnostic tool. In future studies, more samples will be required, and cross-comparison of dielectric properties may be needed to determine the

validation of differences between different groups.

Currently, hospitals still cannot apply dielectric spectroscopy to diagnose or monitor diseases by measuring the differences in the dielectric properties of hCG in urine and exfoliated epithelial urine cells. It probably requires using a cross-comparison of dielectric approaches to determine the differences among test subjects. When collecting and measuring the urine, it is essential that they are carefully handled, thus avoiding contamination that would influence the test results. In future, when studying the dielectric properties in urine, more samples from women who are pregnant and from patients who have bladder tumour will be required, and at the same time, a greater frequency range will also be required to improve measurement.

Besides, it is also suggested to conduct studies on how the dielectric properties of hCG and exfoliated epithelial urine cells correlate with each other. The range of measurements that use dielectric properties can be wider, for example it can be used for measuring the dielectric properties of oral tumour, leukemia, prostate tumour, etc. Such diseases are determined by testing biological fluids. As a significant parameter, for clinical utilization, the dielectric property can be used for determining how the biomaterials variate in human bodily fluids.

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