QUALITATIVE AND QUANTITATIVE DETECTION FOR BACTERIA AND BACTERIAL ENDOTOXIN USING LASER AND MICROWAVE SPECTROSCOPY

By

Muhammad Mahmoud Sayed Elsayeh

A Thesis Submitted to the
Faculty of Engineering at Cairo University
in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY
in
Biomedical Engineering and Systems

FACULTY OF ENGINEERING, CAIRO UNIVERSITY
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Title of Thesis:
Qualitative and Quantitative Detection for Bacteria and Bacterial Endotoxin using LASER and Microwave Spectroscopy

Key Words:
Rapid Microbial detection; Rapid Pyrogen Detection; Microwave Spectroscopy; Dielectric Spectroscopy; Ultra Wide Band; Cepstrum Analysis; Raman Spectrscopy;

Summary:

This work presents two novel methods, the first method uses electromagnetic waves in the Ultra Wide Band (UWB) region of the microwave spectrum to detect and identify bacteria and bacterial endotoxins. The developed method is based on the properties of interaction between organic materials and electromagnetic waves. The interaction is measured quantitatively and qualitatively. The scattered parameters of sample network are measured and cepstrum coefficients are estimated for the analysis of the scattered parameters signals energies.

The second method based on Raman spectroscopy to detect and identify bacteria and bacterial endotoxin. It uses the frequency properties of Raman scattering through the interaction between organic materials and electromagnetic waves. The scattered intensities are measured and wave number converted into frequency, then the cepstral coefficients are extracted for both the detection and identification. The methodology depends on normalization of Fourier transformed cepstral signal to extract their classification features.

Numerical simulation and practical experiments' results proved effective identification and detection of bacteria bacterial endotoxin even with concentrations as low as 0.0003 Endotoxin unit (EU)/ml and 1 Colony Forming Unit (CFU)/ml using signal processing based enhancement techniques for both methods.

Acknowledgment

First of all, I say “praise be to Allah” who was beside me in this step of this thesis.

Words cannot express my deepest gratitude and appreciation to my parents and family for their sincere support, care, and encouragement.

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Especial thanks and gratitude to my wife and my son “Adam”.

Dedication

This thesis is dedicated to my mother.

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Nomenclature

Abbreviation	Description
ATP	Adenosine Tri Phosphate
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CE-LIF	Capillary Electrophoresis - Laser Induced Fluorescence
CGE	Capillary Gel Electrophoresis
CIEF	Capillary Isoelectric Focusing
CZE	Capillary Zone Electrophoresis
DRA	Dielectric Resonant Antenna
EKC	Electrokinetic Chromatography
ELISA	Enzyme Linked ImmunoSorbent Assay
FDTD	Finite-Difference Time-Domain
FE	Finite Element
FIT	Finite Integration Technique
FTICR-MS	Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy
GC-MS	Gas chromatography –Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
ITP	Isotachophoresis
LAL	Limulus Amebocyte Lysate
MALDI-TOF-MS	Matrix – Assisted Laser Desorption Ionization –Time of Flight Mass spectroscopy
MECC or MEKC	MicellarElectrokinetic Capillary Chromatography
MEEKC	Micro Emulsion Electrokinetic Chromatography
MoM	Method of Moments
NACE	Non-Aqueous Capillary Electrophoresis
PCR	Polymerase Chain Reaction
RPT	Rabbit Pyrogen Test
TSEE	Total Signal Energy Expansion
UWB	Ultra Wide Band

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Abstract

Sepsis is a global health problem that causes risk of death. In the developing world, about 60 to 80 % of death cases are caused by Sepsis. Rapid methods for detecting its causes, represent one of the major factors that may reduce Sepsis risks. Such methods can provide microbial detection and identification which is critical to determine the right treatment for the patient. Microbial and Pyrogen detection is important for quality control system to ensure the absence of pathogens and Pyrogens in the manufacturing of both medical and food products.

This work presents two novel methods, the first method uses electromagnetic waves in the Ultra Wide Band (UWB) region of the microwave spectrum to detect and identify bacteria and bacterial endotoxins. The developed method is based on the properties of interaction between organic materials and electromagnetic waves. The interaction is measured quantitatively and qualitatively. The scattered parameters of sample network are measured and cepstrum coefficients are estimated for the analysis of the scattered parameters signals energies.

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Chapter 1 : Introduction

1.1. Introduction

Sepsis and Endotoxaemia are a global health problem that causes higher risk of death. Bacterial infection is a root cause of sepsis and could lead to Endotoxameia if it is a gram negative Bacteria. In developing world 60-80% of sepsis diagnosed patients lost their lives. The absence of an automatic system for bacteria detection and identification that provides a rapid method for bacteria and bacterial endotoxin detection complicate the choosing of suitable clinical therapeutic procedures and actions. Such a system may represent essential tool for a quality control system in pharmaceutical industry of intravenous injection products.

There are several methods for bacteria identification and classification such as gram staining, culturing and biochemical methods. Another known method such as polymerase chain reaction (PCR) used to amplify short DNA fragments (Primers) that recognize sequences of genes that encode essential molecule. In case of bacteria detection and identification, the PCR method depends on the primers of DNA sequences of bacteria genes [1]. PCR needs sophisticated equipment, several components and reagents. PCR testing takes greater than 15 minutes.

Another drawback is the difficulty to identify and detect mixed containment. One of the new and instrumental based rapid method is the adenosine triphosphate (ATP) bioluminescence. It depends on special sample preparation utilizes specific enzyme combination and surfactant. The enzyme breaks down microbial ATP and produce visible light which measured the microbial presence. One of main drawback is the non-microbial ATP reaction and false positive indication. It is time consuming method requiring 24-48 hours to be completed. There are many other instrumental based rapid methods for microbial detection [2]. However, they are rapid, they have drawbacks such as time consuming, lower specifity, false identification and quantification.

On the other hand, there are several endotoxins' assays such as Thiobarbituric acid – assay, Rabbit Pyrogen Test(RPT), Human blood test , Endotoxin Activity Assay and the well-known (gel clotting or photometric) Limulus Amebocyte Lysate (LAL) assay. Different methods and instrumentation, used in detection of endotoxin, already exist such as Capillary electrophoresis, Laser Induced Fluorescence (CE-LIF), Gas chromatography –Mass Spectroscopy (GC-MS), Matrix – Assisted Laser Desorption Ionization –Time of Flight Mass spectroscopy(MALDI-TOF-MS), Ion trap mass spectroscopy and Fourier Transform Ion Cyclotron Resonance Mass Spectroscopy [3-5]. However, since using the above mentioned instrumental methods, is very artful and complicated. This due to the fact that as these methods need samples' preparations and derivatization as preparatory step for instrument to detect the LPS. As a result, these methods are time consuming which is not preferred especially in case of severe sepsis that cause death in less than an hour. Moreover, they need complex procedures to ensure accurate measurements and have expensive implementations.