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# Rapid microbiological diagnostics in medicine using electromigration techniques

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ABSTRACT

This review article focuses on the utilization of the electromigration techniques in determination of microorganisms and shows the applicational possibilities of these techniques mainly for medical purpose. First it describes the origin of bacterial surface charge and the impact of functional groups on bacterial properties measured by hyphenated techniques, such as: Fourier Transform Infrared (FTIR), X-ray photoelectron spectroscopy (XPC), NMR spectroscopy ($^{13}$C NMR) and intact cell matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (IC MALDI-TOF MS). In the next stage this article describes present key points in the determination of microbes by capillary electrophoresis (different approaches – capillary wall modification). Special attention will be put to medical aspect, such as: surgical site infection (SSI), infection of blood, cerebrospinal fluid in tuberculosis and determination of bacteria responsible for urinary tract infections. The algorithm of diagnostic strategy and some future directions will be also presented.



**Contents**



## 1. Introduction

The chemical compounds which are present in the environment more often cause bad effects on health. The most serious ones are tumors and various mutations on the cellular level. Those compounds, from the analytical point of view, can serve the function of biomarkers, constituting measurable changes in the organism's cells and biochemical processes occurring therein. The challenge of the XXI century is therefore searching for effective and reliable methods of identification of biomarkers as well as understanding bodily functions, which occur in the living organisms on the molecular level.

From a historical point of view Antonie Van Leeuwenhoek was one of the first to observe microorganisms, using microscopes of his own design. Later Lazzaro Spallanzani found that boiling broth would sterilize it, killing any microorganisms in it. He also found that new microorganisms could only settle in a broth if the broth was exposed to air. In 1876, Robert Koch he found that the blood of cattle which were infected with anthrax always had large numbers of *Bacillus anthracis*. For a decade until 1903 he investigated the nature of tuberculosis, and methods of treatment in hospital in Slawentzitz in Upper Silesia in Sławięcice district of Kedzierzyn-Kozle in Poland. Koch found that he could transmit anthrax from one animal to another by taking a small sample of blood from the infected animal and injecting it into a healthy one, and this caused

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the healthy animal to become sick. He also found that he could grow the bacteria in a nutrient broth, then inject it into a healthy animal, and cause illness. Based on these experiments, he proposed criteria for establishing a causal link between microorganism and disease and these are now known as Koch's postulates. These postulates cannot be applied in all cases, they do retain historical importance to the development of scientific thought and are still being used today. As a result of his research on tuberculosis, Koch received the Nobel Prize in physiology or medicine in 1905 [1].

Up to now many kinds of bacteria are not only used to create food products (yogurts, vinegar, cheese) but also they are used in, for instance, molecular biology for manipulation of growing proteins, such as insulin. On the other hand, many kinds of bacteria can cause very dangerous diseases. Those bacteria are called pathogenic. Accurate and definitive bacterial determination, including identification and pathogen detection, is essential for correct disease diagnosis and treatment of infection. Bacterial identification is used in a wide variety of applications including: microbial forensics, criminal investigations, bio-terrorism threats and environmental studies. Many different methods have been established for fast detection and identification of harmful microorganisms: different staining procedures, specific antibodies, polymerase chain reaction (PCR) or DNA-typing and finally MALDI-TOF MS/MS analysis. In general, these methods rely on phenotypic identification of the causative organism using Gram staining, culture and biochemical methods. Unfortunately they have two major disadvantages. First of all, they can only be used for organisms that can be cultivated in vitro which is highly time-consuming. Secondly, some strains exhibit unique biochemical characteristics that do not fit into patterns that have been used as a characteristic of any known genus and species.

In recent years separation science has became one of the most useful scientific tools. Separation science works in many different applications, such as: analytical chemistry, biochemistry, biotechnology, forensic science, food science, clinical and neuron science, medical research and production and also pharmaceutical science. Certainly, different samples and different complicate matrices are characterized by different separation techniques [2,3].

Capillary zone electrophoresis, is a relatively new technique used for the separation and analysis of various chemical compounds. It is a method which is increasingly being used by analysts use for chemical and biochemical analyzes. Capillary electrophoresis has many advantages such as: high efficiency and the rate of separation, the use of cheap and durable capillaries, small amount of samples and low consumption of reagents. By using a capillary electrophoresis it is possible to analyze ionic polar compounds, polar non-ionic and non-ionic and also small molecules (inorganic ions, organic acids, amino acids, hydrocarbons, drugs, steroids and chiral compounds) as well as large molecules such as proteins, hormones, nucleic acids, and even living cells [2]. A lot of different techniques used in modern bioanalytical laboratory are connected with electrophoretic phenomenon which is defined as the motion of dispersed particles relative to a fluid under the influence of an uniform electric field (Fig. 1) [2].

Electrophoresis should be used in many branches of science mainly for the following reasons:

1. Non chromatographic mechanism provides additional information;
2. It requires small sample – low cost of reagents;
3. In the case of proteins, peptides, nucleic acids or chiral compounds determinations this technique is less time-consuming and much cheaper.

Clinicians usually make an diagnosis of infection with an intermittent examination observing changes in temperature, blood pressure, smell and sight. Depending on the severity of the infected wound and infectious agents, this may cause multiple organ dysfunction, failure of body systems and ultimately death. For the fast diagnosis culture of microorganisms or molecular analysis is perform but these detection methods are time-consuming up to three days) and unreliable for identification of pathogens in up to 50% of patients. From this reason a rapid, inexpensive, definitive fast screening tests capable of ruling out infection and identifying the

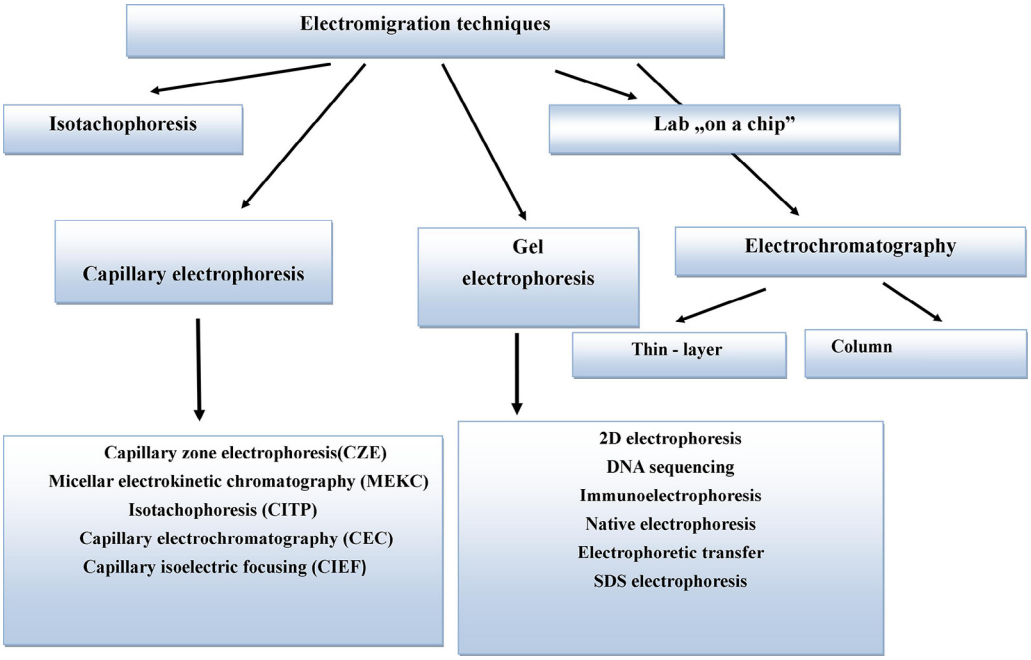


**Fig. 1.** Electromigration techniques division.