ELECTRO-OPTICAL TECHNIQUE FOR DETECTION AND IDENTIFICATION OF BIOLOGICAL AGENTS

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Abstract: A novel, portable Electro-Optical (EO) Biosensor (CELAN) for the detection of extremely low concentrations of microorganisms in real-time (<25 minutes) with immediate on-the-spot interpretation of the results has been developed. The EO biosensor consists of three major subsystems: (1) a fluid-handling subsystem, (2) electro-optical device and (3) computer hardware and software with a graphical user menu. The method is based on measurement of suspended cells polarizability before and after their interaction with selective labels. Monoclonal antibodies, phages, and gold particles have been used as markers. Live vaccine strains of Bacillus anthracis, strain STI, and Francisella tularensis were used as test pathogen agents. Mathematical data processing, includes accumulation of the data, their filtration and comparison of two functions and Frequency Dependence of Anisotropy Polarisability (FDPA) before and after biospecific cell-antibody interactions have been optimized.

1. INTRODUCTION

Current scientific technologies offer a wide range of methods for selective detection and identification of microorganisms. However, most of them require complicated chemical, biochemical and immunological manipulations or are time-consuming. Physical methods including optical techniques are more convenient, but still far from the sensitivity and selectivity required. Our contribution to this subject is directed towards developing an electro-optical approach, which relies on the measurement of AC electrokinetic effects. The theoretical and experimental aspects of AC electrokinetic effects have been developed in several laboratories. The AC
electrokinetic effect depends on dielectric properties of bioparticles and their suspending media, and on the frequency of applied electrical field. When a bioparticle (bacterium or virus) is exposed to an external electric field, it becomes electrically polarized. As a result of this the action of an applied electric field induces electrical charges to appear at the boundary between the particle and the surrounding medium. These field-induced charges provide a large electric dipole moment of the bioparticle, which arises from the induced charges that accumulate at the interface of the bioparticle. In general, permanent dipoles are randomly oriented, but if an external electric field is applied, they will be reorienting statistically. Induced dipoles will have the direction of the applied field. If a direct current (D.C.) electric field of uniform intensity is replaced by one of alternating current (A.C.), then because of the particle's inertia the electrophoretic effect becomes small for frequencies above around 1 kHz. However, dipole moments associated with Maxwell-Wagner interfacial polarizations can exert their influence up to frequencies of 50 MHz and beyond. The cell polarization and temporal orientation of dipoles, with respect to the applied field, depends on the dielectric properties of the suspending medium and the bioparticles.

The electro-optical technique includes a number of interrelated processes [1-5]: 1) action of electric field on the suspended bioparticles; 2) generation of induced charges on the boundaries of cell structures and cell surface; 3) creation of the driving torque; 4) cell transition into oriented state, which leads to the anisotropy of optical properties of a cell suspension. Such effects induce the fluctuations of light scattering and relevant variations in optical density. The changes of optical density can be detected by optical techniques, thus allowing the rapid and accurate detection of target bacteria in aqueous solutions. Such effects are birefringence, dichroism, induced changes of light scattering and relevant variations in optical density [6-8].

2. SELECTIVE ANALYSIS OF CELLS BY ELECTRO-OPTICAL TECHNIQUE

The method is based on measuring electrophysical parameters of cells, in particular Frequency Dependence of Anisotropy Polarisability (FDAP), when exposed to a selective factor [9]:

- antibodies
- selective nutrient medium
- lysogenic selective factor