Gastrointestinal Microbial-Detector Biosensor (GMDB) For Detection of Multiple Microorganisms in a Single Test; A Hypothesis

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Abstract

Gastrointestinal microbial-detector biosensor (GMDB) is a proposed design of a new biosensor for detecting multiple microbial targets in microbial flora of gastrointestinal tract. A novel immunoassay for the detection of gastrointestinal pathogens and saprophytes is described in this study. GMDB would reveal the qualitative spectrum of gastrointestinal (GI) microorganisms. The approach has the potential for miniaturization of immunoassays. The proposed procedure is that GMDB would be swallowed by the patient; in its pathway throughout gastrointestinal tract, the target microorganisms would adhere specifically to their polyclonal antibodies. Patients would collect GMDB after bowel movement. GMDB would float on surface of toilet water since it has a built in air vesicle which makes it light floating and easy to visualize. The patient will rinse and hand it to laboratory for further processing. A set of specific conjugated fluorescent monoclonal antibodies is applied to GMDB, incubated for 30 min at 37 C, rinsed and then simply evaluated for fluorescent antibody-antigen reactions by use of a fluorescent light microscope. Qualification of GMDB immunoassays is achieved by measuring the presence of specific fluorescence labels. Positive readings are indicative of the spectrum of microorganism antigens present in GI tract of the tested patient. The assay is able to accurately detect specific antigens at levels similar to those detected by a conventional ELISA. These advances should prove useful in the future development of a miniaturized immunoassay system for use in GI microbial diagnostics. Extended applicability of GMDB would include multiple detection of microorganisms in other types of environments as food, soil, agricultural produce, air and water. GMDB may also offer a solution for some microbial detection challenges in probable biological warfare and bioterrorism activities in the future.

Key words: Gastrointestinal microbial-detector biosensor, Immunoassay, Fluorescent antibody-antigen labeling

1. Background

Despite a plethora of constructions covering a diverse range of detection devices for microrganisms, genuine applications are rare. Conventional methods for detection and identification of GI microorganisms are not designed to detect wide range of GI microorganisms in one single test. In spite of providing quantitative and qualitative information of the number and the nature of the target microorganisms, the tests are cost effective and time consuming. Such approaches make use of morphological evaluation of the microorganisms, biochemical screening, serological confirmation, selective enrichment, or assessment of the ability of microorganisms to grow in various types of defined media under specific conditions. It is, however, acknowledged that certain bacterial strains in certain conditions may become viable but non-culturable, rendering cultivation approaches of little value.

2. Limitations of conventional methods for detection of GI microorganisms

The intestinal tract of humans contains a complex bacterial ecosystem, usually referred to as the normal flora, consisting mainly of obligatory anaerobic bacteria [3,6,14,18,19]. The composition of the normal flora plays an important role in human health and disease through its involvement in nutrition, pathogenesis, and immunology of the host. The normal flora provides colonization resistance and might stimulate immune responses to potentially pathogenic bacteria [4,5,7,8,15].

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To understand these phenomena and to be able to evaluate the effect of treatments aimed at modulating the normal flora, it is important to have accurate means to enumerate the various microbial populations. Conventional methods for determining the flora composition rely on the cultivation of bacteria on anaerobic selective media. However, many bacteria are difficult to culture or are unculturable [2,13,16,17], and often media are not truly specific or are too selective for certain bacteria [19]. A large proportion of the bacteria encountered in feces are strict anaerobes, often non-sporulating [8]. Although it is relatively easy to obtain a total viable count, enumerating individual bacterial species by culturing methods is laborious and time consuming. Drawbacks associated with culture-based techniques are exacerbated in anaerobic habitats. Selective media are not available for most of the strict anaerobes and several hundred isolates from each fecal specimen should be identified for reliable statistics. Following their individual isolation, end-product analysis of fermentation in pure cultures is essential for reliable identification. Due to slow growth, the identification of a single anaerobe will generally take up some two weeks. As a consequence, studies on population dynamics of the intestinal microflora are often limited in the number of subjects or the number of bacterial species investigated, thus limiting the statistical reliability of results [3,15,16].

3. Hypothesis

The **aim** of this hypothesis is to detect multiple microbial targets in gastrointestinal tract. Proposed **structural constituents** of GMDB consist of: 1) Antibody array platform (AAP). AAP bears fixed polyclonal antibodies of a wide range of GI microorganisms in stratified specified arrays on a tiny bed surface (e.g. a transparent cellophane strip of 2x5 mm). Stratified array is a ladder of specific polyclonal antibodies arranged in sequential order, 2) Small plastic air vesicle (PAV, diameter of about 5mm) attached to AAP, 3) Gelatinous protective capsule which encapsulate AAP and PAV. **Procedure** using GMBD consists of the following steps: 1) GMDB would be swallowed by the patient, 2) Gelatinous protective capsule dissolves in stomach, 3) AAP is exposed to anchor microbial antigens as it passes throughout gastrointestinal tract, the target microorganisms would adhere specifically to their polyclonal antibodies, 4) Patient would collect GMDB after bowel movement; because of PAV, GMDB floats and easy to visualize on toilet water, 5) Patient will rinse and hand it to laboratory for further processing, 6) A set of specific conjugated fluorescent monoclonal antibodies is applied to GMDB, incubated for 30 min at 37° C, rinsed and then simply evaluated for fluorescent antibody-antigen reactions by use of a fluorescent light microscope, 7) **Qualification** of GMDB immunoassays is achieved by measuring the presence of specific fluorescent labels on the ladder. Positive readings are indicative of the spectrum of microorganisms present in GI tract of the tested patient. The assay is able to accurately detect specific antigens at levels similar to those detected by a conventional ELISA.

4. Clinical significance

GMDB will bear the ability to detect multiple microorganisms antigens present in microbial flora of gastrointestinal tract in a single test. This approach permits the qualitative elucidation of the abundance of microbial species and how their presence interacts with diet, health or disease. As confidence in the GMDB immunoassays and its detection ability increase, the adoption will be evolutionary. GMDB immunoassays would also be excellent tools for measuring microorganisms in vast types of environments as food, soil, agricultural produce, air and water. GMDB may also offer a fast, simple, cheap, and accurate solution for some microbial detection challenges in probable biological warfare and bioterrorism activities in the future; and this is where the future challenges for GMDB resolution will be. Furthermore, GMDB has a quality potential for equipment miniaturization due to its small size. Such integration and miniaturization also offer a genuine tool in fundamental science, as the constructions are revealed in studying microorganisms in complex environments.

5. Future testing

Considering the application of GMDB, further studies are needed to confirm its applicability in clinical cases. The effectiveness should be verified firstly by animal experiments. GMDB technology is to be increasingly improved for analysis of the complex intestinal ecosystem and contribute to a better understanding of the interaction between host and microbes in the intestinal tract. Throughout the process of optimization, this application should enhance our understanding of processes and indeed to identify opportunities and pitfalls in applying GMDB methods to the study of community structure and dynamics of microorganisms in other types of environments as food, soil, agricultural produce, air and water. Consequently, further optimization of the GMDB performance in terms of sensitivity, speed of the response, the choice of targets to detect, and analyses detection limit should be influenced and improved.

These remarks cover the basis of the evolution of the 'complete GMDB engineering' and the validation of the procedures and represent the bridge between GMDB design and application.

6. Conflicts of interest

I declare that I have no financial and personal relationships with other people or organizations that can inappropriately influence my work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, the article entitled ''Gastrointestinal microbial-detector biosensor (GMDB) for detection of multiple microorganisms in a single test; a hypothesis".

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