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Advances in rapid pathogen detection

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Introduction

Microbiological analysis has progressed considerably in the twenty-first century through the advent of rapid microbiological methods. This is most apparent within the clinical,[1] and pharmaceutical sectors.[2] One area of much needed advancement is with pathology. Here clinical investigators can take samples from the field, from patients or from the environment, and undertake rapid analysis, obtaining results within hours rather than the days or weeks required with conventional methods. Time is often a consequence of slow-growing, difficult to culture, and unculturable microorganisms. Time-to-result is critical when making decisions about patient health. In this editorial, some of the most recent advances with rapid methods for pathogen detection are considered.

Emerging rapid microbiological methods

Rapid (and alternative) microbiological methods are ambiguous terminology, since "rapid" is open to interpretation and requires a new method to be considered relative to an established one. Moreover, rapid methods are invariably presumed to be more accurate, although this is not always the case. Rapid methods cover a spectrum from miniaturized biochemical kits; antibody tests; DNA-based tests; as well as culture-based tests that are modifications of conventional tests, such as the examination of 'micro-colonies' using light scanning.

In terms of assessing pathologies, clinical areas where rapid methods are an advantage include areas where urgent (and correct) decision making is required. Examples include: pneumonia in the immunocompromised; bacteremia; meningitis; soft tissue infection; eye disease; endocarditis and so on.[3]

One such example is with the clinical decision as to which antimicrobial a patient requires in response to a bacterial infection. Administering the incorrect antimicrobial has consequences for the patient, and arguably contributes to the problem of emerging antimicrobial resistance.[4] To allow the clinician to take a sample, obtain a result, and decide on the appropriate antimicrobial inside of one hour, University of Toronto researchers have designed a prototype chip that can rapidly test for antibiotic resistance through phenotyping. The process works by concentrating any bacteria extracted from a patient blood sample into a compact space inside microfluidic wells (two nanoliters in volume). This tony space allows the volume to be increased and, in effective, increases concentration of the starting sample. Inside each well a lattice of microbeads is located. These serve to capture bacteria as a sample is passed through. Captured bacteria accumulate in a well into which a range of antimicrobials are placed together resazurin, which functions as an electrochemical signature molecule through conversion to resorufin.[5]

As an alternative, C-reactive protein (CRP) blood tests have been demonstrated as capable of showing if an infection is bacterial. The method determines the level of C-reactive protein present in the blood; these levels correlate with levels of inflammation present in the body.[6]

In addition to determining the type of organism quantifying the numbers is an additional part of clinical assessment. Classic methods, such as haemocytometer or Neubauer chamber, take time to analyse. A faster method that has been explored is with the use of spectrophotometry to measure the absorbance of a cell suspension. As an example, researchers have reported the success of using this method for assessing numbers of the fungus *Fusarium*, counting values for conidia by concentration.[7]

With viruses, a current area of epidemiological concern is with avian influenza. Here University of Guelph scientists have worked on a novel biosensor that can characterise an influenza virus in around three minutes from blood samples. The technique is based on gold nanoparticles that enable the surface proteins found on viral particles to be detected via nanobiosensor. Here different colours signal the presence of a virus and can sig different types of avian flu. Trials have been successfully undertaken at poultry farms.[8] Biosensors are also being applied to antimicrobial drug discovery. Sensors can be used to screen a range of candidate drugs by obtaining of MIC (Minimum Inhibitory Concentration) values.[9]

In terms of environmental screening, a research group from Ben-Gurion University of the Negev and the Massachusetts Institute of Technology have used qPCR (quantitative polymerase chain reaction) to examine samples of water and soil. With qPCR, screening of pathogens is completed within 24 hours. qPCR looks at the amplification of DNA, through the generation of thousands to millions of copies of a particular DNA sequence, in real time and can detect down to one microbial cell.[10] This allowed a limit of detection of down to one cell to be achieved. In trials, the research group successfully identified a specific strain of *Salmonella* (*S. enterica*), selected from soil samples, and *Pseudomonas aeruginosa* from samples of water.[11] In terms of patient samples, real-time quantitative PCR has been successfully deployed to detect multidrug-resistant tuberculosis within one hour.[12]

With microbial identification, MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time Of Flight) mass spectrometry has emerged as an adjunct test between slower, culture-based phenotypic methods and genotypic methods.[13] With MALDI-TOF it is possible to detect the bacteria in a sample in less than three minutes, with good specificity (depending on the scope of the system database.)

Summary

The development and adoption of rapid microbiological methods comes with several advantages for improvements to the quality of patient care. These are with faster diagnosis and more accurate assessment of pathological conditions. Over the longer-term, these

improved assessments will help to reduce patient health care costs and enable more effective patient management. Thus, it is to such technologies that we should be focusing on in the context of modern microbiology and experimentation.

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Conflicts of interest

Author declares that there is no conflict of interest.

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