Microbial Diagnosis – An Update

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PLENARY LECTURE

Microbial diagnosis requires the joint effort of the clinician and Medical microbiologist. Conventional methods of diagnosis, such as Gram's staining to culture methods, are widely used all over the world because they are sensitive and inexpensive. The microbiology laboratory offers advice concerning the differential diagnosis, choice of specimens, as well as the optimal stains and cultures to facilitate diagnosis. Additionally, the rapid interpretation of Gram-stained smears provides useful, occasionally lifesaving, information relative to the etiologic diagnosis and empiric antimicrobial therapy. The microbiology laboratory also provides further interpretation of culture and antimicrobial testing results that allow the clinical service to focus on the most critical data. The accurate diagnosis of infection is essential for effective treatment, high quality surveillance, control of outbreaks and epidemics and to successful prevention of infectious diseases.

This talk would throw light on over-view of the diagnosis starting from normal Microbiological laboratory techniques to the recent advances in Diagnostic Microbiology. The conventional method of diagnosis tends to be labor and resource intensive and require considerable expertise. However these isolates also require further characterization by molecular techniques to confirm identification.

Serological diagnosis have also played a vital role in the diagnosis of infectious diseases where culturing of the micro organisms are difficult. Immunochromatographic methods (Rapid tests like tridot, Pregnancy tests, HBsAg tests) have replaced the ELISA systems which is time consuming. Western

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Blotting method is used as a confirmatory test for HIV has helped the clinicians for diagnosis of the infections.

Molecular Microbiology has emerged as the leading field in clinical microbiology laboratory and created new opportunities for laboratory diagnosis to increase patient care. It helps in disease prognosis and monitoring the response to treatment. Even though cultures have long been the 'Gold standard' for infectious diseases, now it has been replaced by molecular methods due to the rapid and accurate diagnosis at the genome level. Hepatitis C, Enteroviral meningitis, Herpes simplex viruses, Chlamydia trachomatis are some examples where molecular methods are the new gold standards. Molecular methods are more advantageous in situations where conventional methods are slow, insensitive, expensive or not available.

Molecular methods range from Southern blot, Hybridization, etc to Polymerase chain reaction (PCR) and sequencing. Recent Developments in PCR has come up with real time PCR and Multiplex PCR which provides rapid diagnosis. These techniques have wide application for the detection of bacteria, fungi and toxins from patient samples and identification from culture. This is particularly useful for organisms that are difficult to cultivate, like Mycobacterium tuberculosis and Chlamydia trachomatis.

Molecular microbiological research is now entering into an era of 'big science' called as "Micro array". The classical Southern and Northern blotting approaches for the detection of specific DNA and mRNA species provided the technological basis for microarray hybridization with fluorescently labelled cDNA. The idea of depositing multiple DNA spots representing different genes onto a solid surface with the help of robotics to achieve high spotting densities of DNA on glass slides was innovative and facilitates the construction of microarrays containing up to 50000 genes on a single microscope slide. This allows a single hybridization to be performed against multiple replicates of a single bacterial genome, or against copies of several unrelated genomes on a single glass slide. The development that has facilitated the reproducible comparison of gene expression between two samples, and hence between experiments, is dual fluorescent labeling. Simultaneous hybridization of two cDNA populations labelled with the fluorescent dyes Cy3 and Cy5 allows accurate assessment of relative levels of gene expression.

Even though microarray technology is in its infancy, this would prove to be an useful and productive tool in future for the diagnosis of infectious diseases.

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