LETTER TO THE EDITOR

Current laboratory techniques commonly used for the detection of molecular biomarkers and potential technologies on the horizon

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Madam, Identification of pathogenic mutations and their products by molecular assays are fundamental not only for the accurate diagnosis but the probable prognosis and future counselling. The techniques used range from conventional mapping of chromosomes to recent development of next-generation sequencing (NGS), which has revolutionized the field of molecular diagnostics.

Conventional banding-based chromosomal analysis is a very useful screening tool which provides information about all the chromosomes. Chromosomes arrested in metaphase are examined under the microscope after staining. Various disorders including congenital anomalies, developmental delays and malignant disorders are routinely tested by conventional karyotyping .[1] Whereas, fluorescence in situ hybridization (FISH) utilizes a fluorophore labeled DNA probe that specifically binds to nucleic acid sequence of interest. Although FISH technique is rapid and can be performed on non-dividing (interphase) cells, it can be used only for known genetic aberrations if the probe is available.

The discovery of polymerase chain reaction (PCR) in 1980s was the most significant milestone in history of molecular diagnostics.[2] In a chain reaction, template nucleic acid copies are increased exponentially within hours. Today, PCR has become an essential tool in both research and clinical laboratories. It is widely used for rapid detection of point mutations, microbial genomes and fusions transcripts such as BCR-ABL1 in chronic myeloid leukaemia. In Real-Time PCR, accurate quantification of product during exponential phase of PCR is provided. The increase in fluorescence is measured at every cycle and directly correlates to the amount of PCR product formed.[3]

Nucleic acid sequencing, in contrast to PCR identifies unknown variations in genomic regions of interest. In Sanger sequencing, PCR amplification is followed by capillary electrophoresis. The DNA sequence is read from series of peaks in fluorescence intensity recorded by the detector. Currently, the most widely used technique, capillary electrophoresis based Sanger sequencing is considered gold standard for mutation analysis particularly for rare disorders and the genes that do not have common mutations.

Today, next generation sequencing (NGS) can sequence whole human genome comprising of three billion base pairs in one day. This technology has largely superceded conventional Sanger sequencing particularly in genomic research. It will inevitably become a part of routine clinical practice. The 1000 Genomes Project is an international research effort launched in 2008. It is a detailed catalogue of human genetic variation that can be accessed by molecular pathology laboratories to know the sequence of all human genes.[4]

Although NGS technologies are rapidly gaining their place, currently automated Sanger sequence analysis is the most commonly utilized technique by molecular pathology laboratories for the identification of disease markers. The factors that are important to consider in selection of appropriate assay include volume of patients to be screened and the gene or allele of interest. FISH and PCR continue to be widely utilized techniques for known genetic aberrations.

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