

Measurement of Cancer Biomarkers by Flow Cytometry and Molecular Technology: A Rapidly Expanding Area for ACM Global Central Laboratory.

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Biomarkers are distinctive biological properties that can be detected and measured in parts of the body like the blood and/or tissue. They may indicate either normal or diseased processes in the body. Early in 1998, a study group from the NIH suggested a biomarker could be defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes (predictive biomarker), pathogenic processes (diagnostic biomarker), or pharmacologic responses to a therapeutic intervention (responsive or prognostic biomarker)”. Several malignant disease biomarkers such as P53 gene and MMPs had been identified for cancer patients. More recently, molecular biomarkers are generating heightened interest in relevance with clinical oncology; including the role of K-ras in colorectal cancer and other EGFR-related tumors. Therefore, measuring a sample from solid tumor for its K-ras status (eg. normal vs. mutant type) unquestionably helps to identify prognostic benefit in those patients who received targeted anti-tumor therapy, such as cetuximab (Erbixux). In order to use a biomarker for diagnostics, the sample material must be easy to obtain and the detection method must be accurate and easy to perform. Regarding the discovery of molecular biomarkers, flow cytometry and molecular genetics are most commonly used as technologies in the identification of cancer biomarkers in conjunction with more expensive genomic and proteomic platforms for biomarker assay techniques.



FLOW CYTOMETRY [FC]

Flow cytometry is a powerful, rapid and cost-effective technique for the identification and monitoring hematopoietic neoplasms as well as other tumors. Flow cytometers are outstanding instruments that together with specific monoclonal antibodies and fluorochrome reagents, allow us to quantitate biomarkers (antigens) of the surface, cytoplasm, and DNA content of thousands of cells suspended in fluid to determine their lineage and to estimate their potential biologic and pathologic behaviors. These measurements can be made on a per-cell basis at rates of up to 10,000 cells per second. The ability to perform multiparametric analysis (10- or more than 10-color) on an individual cellular basis is unique feature of this technique and offers distinct advantages comparing with immunohistochemistry. Usually, leukemia/lymphoma cells and other tumor cells from biopsy samples are identified by an abnormal gain and loss from normal antigen expression patterns, as named as immunophenotyping. The outbreak of acquired immunodeficiency syndrome (AIDS) incidentally accelerated the acceptance of flow cytometers as routine laboratory instruments because of the tremendous demands for testing the helper: suppressor T-cell ratio as a screening technique in the early epidemic of AIDS. FC has the advantage of being more efficient, sensitive, accurate, and reproducible than manual techniques. With a flow cytometer, multiple specimens can be simultaneously processed with a panel of 10 or more monoclonal antibodies, and tests can be completed within several hours. The current simplification of this automated instrument makes it feasible to use as a routine laboratory procedure, not only in large medical centers but also in medium-sized hospitals and professional reference laboratories.

Multiplex bead (Luminex) assay represents an approach to simultaneously analyze multiple, soluble, cell-free ligands, such as antibodies, cytokines and growth factors, in patient's fluid, serum or plasma. Comparing with ELISA, the major advantages of Luminex assays are that they can measure as many as 100 ligands in a small volume (as little as 50 mL) of fluid at the same time in a rapid and cost-effective manner. Now, Luminex assays are making their way into clinical laboratories; those assays are not only applied to quantitation of cytokines/growth factors, but have also been adapted to a wide variety of clinical needs such as autoimmune antibody determination, HLA typing, pathogen detection and tumor biomarker testing. In the clinic laboratory, implementation of Luminex assays will ultimately lead to substantial cost and time saving.

MOLECULAR DIAGNOSTIC TECHNIQUES

Analysis of nucleic acid (DNA and RNA) in patient samples form the foundation of laboratory medicine so called molecular diagnostics or DNA/RNA-based diagnostic technology, the most rapidly growing area of laboratory medicine. These methods are widely used to assist in the diagnosis and monitoring of many genetic, infectious, and malignant forms of hematologic disease and other types of cancers. The DNA/RNA-based diagnostic technological methods most commonly implemented in clinical settings are Southern blot analysis, polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), DNA sequencing, and array technology for gene profiling. DNA/RNA-based technology is another powerful new tool for laboratory diagnosis. Tremendous advancements in molecular testing are prospected for the coming years. Using those new, highly sensitive methodologies pathologists can identify diseasing-genes and their proteins in minute quantities of tissues and/or body fluids. Many of those techniques have been developed to the stage where they are employed as "high-throughput" processes that enable to analyze single patient sample for multiple diseased-gene and/or disease-proteins. Some of these processes have already become completely automated. Advances in test methodology and platform design have made the transfer of this technology to the academic medical center and reference laboratory possible. Of course, increasing numbers of diseased gene probe-based kits are being approved by the food and drug Administration (FDA) for use in clinic laboratories, and more than half of those are kits for evaluating tumor-specific translocations.

At ACM Global Central Laboratory, flow cytometry was first initiated when partnering in a global clinical trial. Since that time flow cytometry and molecular testing offerings have expanded 500%. Investigators can now monitor patient response to viral infections along with using esoteric markers as exploratory endpoints in today's clinical trials.

Along with molecular, flow cytometry, multiplex and safety capabilities across both our US and UK facilities, ACM Global Central Lab has the unique services of a strong team of pathologists experienced in performing primary histopathology services with the capability of offering discrete teams to perform secondary reads and final adjudication interpretations. This is achieved by providing standardized reportable data to facilitate regulatory submissions.