# A Review of Advanced Medical Laboratory Methods and Techniques.

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## Abstract

Technology goods determine the clinical laboratory's analytical capability. Every generation of analytical equipment and techniques that have been accessible in the laboratory since the Auto Analyzer in 1957 has been more automated (or mechanized), prolific, sensitive, specific, and adaptable than the one before it. There was a lot of leeway in the selection and assembly of the analytical systems utilized in a clinical laboratory between 1957 and the middle of the 1970s. For instance, an instrument may be chosen and bought from a single vendor, and the seller could supply the reagents and procedures or they could be acquired from other sources. A new generation of analytical systems, with all the instrumental, methodological, and reagent components combined into a single self-consumed unit, accrues from the numerous technological advancements over the past 15 years. These initiatives, and which have become recognized as "closed," are highly automated, and they now handle many of the analytical tasks that formerly needed labor or human intervention. The way that the clinical laboratory of the future is manned, furnished, set up, and run will be greatly impacted by the availability of these new systems that reduce the need for human touch in the analytical process. This study examines a number of domains where notable technological breakthroughs have occurred and talks about the influence these technologies are having, or will likely have, on the clinical laboratory.

**Keywords**: Deoxyribonucleic acid DNA, Liquid chromatography-mass spectrometry LCMS/MS, Transluminal angioplasty TLA

## 1. Introduction

In order to provide safe, appropriate, and effective patient care, laboratory medicine plays a crucial role in the healthcare industry by giving medical practitioners reliable data to guide disease prevention, risk assessment, diagnosis, prognosis, treatment, and monitoring of patients, clinical judgement. Significant technical developments in laboratory medicine during last couple of years have considerably improved clinical laboratory monitoring and diagnoses methods, thus raising the standard of patient care[1]. Consequently, the healthcare industry now recognizes the importance that The aforementioned advances in technology have brought to laboratory medicine. Clinical chemistry, hematology, and microbiology testing has historically been incredibly time-consuming for laboratory personnel, who were in charge of the frequently numerous procedures required in the analytical process, from getting biological specimen to be disposed of eventually. However, the use of primarily automated analyzers by clinical chemistry and hematology laboratories in recent decades has significantly reduced expenses associated with laboratory testing as well as laboratory-induced errors and increased testing efficiency [2-4]. A new era of Additionally, laboratory medicine has been been made possible by notable analytical developments in the fields of genetics and genomics, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and microfluidics that have coincided with growing laboratory automation. In fact, the discipline of transcriptomics and genomics has undergone a revolution thanks to the advancement and evolution of next-generation and single-cell sequencing, which has made whole-genome DNA and RNA sequencing more affordable and feasible [5]. Current developments in

NMR and MS technology define proteomics and metabolomics and extend their applicability across multiple therapeutic fields [6]. Furthermore, a number of laboratory instruments, such as lab-on-a-chip technology, have easily benefited from the innovative application of microfluidic technology. Furthermore, the culture of innovation in laboratory medicine technologies and cutting-edge AI may open the door to a personalized and precise medical era, greatly enhancing the vital role that labs play in the delivery of healthcare. The most recent developments in laboratory medicine technology will be covered in this overview, together with their clinical uses, present difficulties, and potential future paths. We will also discuss the potential for precision medicine in the future, which will be based on sophisticated analytical methods, as well as the necessity of modern bioinformatics.

*1.1. Importance of Advanced Medical Laboratory Methods.*

Automation has defined the modern era of laboratory medicine. The Auto Analyzer [7], which conducted continuous flow analysis, and the Robot Chemist [8], which automated conventional human analytical stages, were the devices that first popularized the idea of laboratory automation. The field of laboratory medicine has undergone significant transformation with the introduction of stand-alone analyzers in subsequent generations. These devices have enhanced efficiency, increased throughput, expanded assay menus, and decreased mistakes [1-4]. In order to address labor-intensive and time-consuming tasks in both the pre-analytical and post-analytical phases—such as specimen storage and archiving—more automation was included. These tasks included sample identification, sorting, and centrifugation. The result of these technical developments was total laboratory automation (TLA), which consists of multiple parallel-operating instruments connected by artificial intelligence (AI) and a robotic track

system to link all phases of the analytical process. Marzinke has previously discussed how centralized TLA systems are perceived in depth [9].

### *1.2. Evolution of medical laboratory Techniques*

Though all current hematology and analyzers for clinical chemistry are highly automated, very few TLA systems have been created. One automated core laboratory tool that can handle sample processing, analysis, and storage on its own is the Cobas® (Roche Diagnostics). This device can also be used with in vitro diagnostic specimen tubes to carry out sample sorting, de-capping, quality control, aliquoting, and recapping when paired with one or more connection modules on a track system. Similar to this, Abbott Diagnostics just unveiled the Accelerator, a highly automated pre-analytic system that can be linked to a core laboratory system with many instruments. One benefit of the Accelerator is that it is "open," which makes it easier to integrate it with other systems and enhances the consolidation of testing across different laboratory disciplines. Additional commercially available TLA systems include Siemens Healthcare's Aptio® Automation, Beckman's Power Express Clinical Automation system, Thermo Fisher's TCAutomationTM, and Ortho Biomedical's VITROS® Automation Solutions. It has been exhibited that these centralized methods lessen human error and enhance analyzer performance [10–15]. Furthermore, TLA's introduction to microbiology and the ensuing revolution in this domain have been produced possible by recent developments in the digitalization of culture plate pictures and the capacity to electronically read incubated plates [4, 22, 24, 25]. Clinical laboratories now use two commercially available microbiological automation systems: WASPLab® (Copan Diagnostics Inc.) and KiestraTM (Beckton Diagnostics). Both instruments are capable of using different levels of

automation, ranging from TLA to front-end processing only (automatic plating) [4, 14, 15, 16]. Because clinical microbiology involves a lot of manual labor, increasing automation has many advantages. These perks include reduce costs [15,] more technicians can be freed up to perform more skilled tasks (like microscopy, plate interpretation, and antimicrobial susceptibility testing) [14,15], increased efficiency (measured using LEAN or other benchmarks) [17,18], shorter turnaround times (TAT), better performance [19], and consolidation of laboratory space [20].

## 2. Molecular Diagnostics

Omics research today covers the domains of genomes, transcriptomics, metabolomics, and proteomics, spanning the range of molecular biology thanks to recent advances in biotechnology throughout the preceding decades [21]. Every technique targets a different kind of molecule by utilizing particular analytical methods, which ultimately produce a systemic profile of a biological system [21].

### *2.1. Polymerase Chain Reaction (PCR)*

In medical and biological research labs, polymerase chain reaction (PCR) is widely employed for a range of applications, including genetic fingerprint recognition, infectious illness diagnosis, genetic cloning, paternity testing, and DNA computing. PCR was created in 1983 by Kary Mullis and is currently a widely used and significant technique having numerous uses in biological and medical research facilities. These include the diagnosis of genetic illnesses, the discovery of genetic fingerprints (used in forensic sciences and paternity testing), the detection and diagnosis of infectious diseases, and DNA cloning for sequencing, DNA-based phylogeny, or functional research of genes. Numerous human diseases can be diagnosed with PCR, which is also useful across a broad range of studies and analyses [22]. A few of these are spoken about below.

i. Diseases caused by infections Hepatitis, syphilis, HIV, CMV, mycoplasma, pneumonia, cancer, and fungal and protozoal diseases, among others.
ii. Cancer diagnosis, particularly for lymphomas and leukemia
iii. Paternity testing and genetic fingerprinting
iv. The most advanced cancer research technique at the moment is PCR, which is already widely utilized to diagnose cancer early on in cases like leukemia and lymphomas. At a sensitivity that is ten thousand times more than that of other techniques, translocation-specific malignant cells can be found directly on genomic DNA samples using PCR assays.

*2.2. Next Generation Sequencing (NGS)*

From the perspective of their basic enzymatic foundations, the majority of so-called "massively parallel" or "next generation" sequencing techniques and tools have deep philosophical ties to date ties to Sanger sequencing, as will be delineated here. The main distinction is that enormously parallel sequencing techniques Don't split apart Sanger sequencing differs from enzymatic nucleotide incorporation from sequence ladder separation and data collecting. Instead, NGS devices execute the enzymology and data collecting in a stepwise and coordinated manner, making it possible to concurrently generate sequence data from tens of thousands to billions of templates. Thus, the phrase "massively parallel" describes this increased ability to generate data, which has led to substantial advancements in DNA sequencing and its uses ever since it was first introduced. A range of detection methods and enzymes that support the relevant instrument platform to gather information while adhering to enzymatic synthesis on a template. Additional common concepts for these platforms are as follows: [23] the genome or another

‘’DNA/RNA’’ material that is to be sequenced physically sheared before sequencing; [24] creation of a chaining "library" by attaching universal, platform-specific converters (synthetic oligonucleotides with a predetermined sequence) at both ends of the pieces of the template to be sequenced; and [23] surface-level template amplification through means of hybridization of library fragments to covalently bonded sequence-complementary oligonucleotides to the artificial adaptors. The templates are equipped after fragment amplification because of distinct sequences found in the synthetic adapter, It provides 30 hydroxide for free for on-instrument data detection in conjunction with enzyme augmentation on the templates. Nucleotide amalgamation step data can be allocated to a single template thanks to the on-surface amplification, which also fixes the X-Y coordinate for each template. In the end, SBS is restricted by the length of the sequence read, (or "read length") because SBS techniques are sleuthing nucleotide embodiment from a population of augmented template molecules at each X–Y coordinate, leading to strengthen in noise across sequential incorporation and imaging cycles. Additionally, different different backgrounds improved signal-to-noise ratios have been achieved through changes to enzymology, nucleotide chemistry, or synthesis, also more perceptive detectors eventually allowing for longer reads. Sanger read lengths are still longer than SBS decipher lengths. This has affected the scrutiny of the sequencing data, as will be discussed in the section on SBS data's analytical aspects noise contribute cumulatively at each step in the embodiment reactions.

*2.3. Microarray Analysis.*

Analyzing the levels of gene expression can reveal information about the molecular variations between various cells or tissue samples moreover the chronological alterations that occur in stimulated cells. By measuring the

hybridization of mRNA to thousands of immobilized genes on a glass surface (the "chip"), Microarrays gauge the degrees of gene expression in parallel and offer a sensitive, high-throughput method that is well-suited to exploit the sequence and clones generated in genome sequencing efforts. From reverse Northern blots on filters identified by radioactive probes to a highly sophisticated sector comprising miniaturized synthesis, multi-color fluorescence labelling, and database-driven sample and data management, microarray technology has progressed. Whole genomes have been examined recently [26], and certain gene families have been researched [27]. Microarray amalgamation, sample drawing up, and gene selection, array combinations, recognition, and data scrutiny are the standard procedures in microarray analyses, with the necessary controls needed for each. This investigation considers every step of a microarray analysis (notice that we refer to the genes on the chip as "targets," the complicated sample in solution as the "probe," and each individual DNA spot on a microarray as a "element" in this review). The use of cDNA clones on microarrays eliminates the requirement for costly, upfront DNA sequencing of uninteresting clones. cDNA arrays have an advantage over oligonucleotide arrays in that they may be more sensitive to variations in hybridization brought about by gene polymorphisms and have access to more target sequence for hybridization. Before creating the targets, the cDNA library might be normalized using a somewhat gentle subtraction technique. This has the benefit of decreasing the representation of the more prevalent genes, hence enabling a wider range of genes to be examined using a microarray. Based on the IMAGE sequencing effort, Incyte (Palo Alto, CA) has created a set of four microarrays with 40,000 clones (28). While this method yields the most information possible, 10–30% of clones may be misidentified due to clone

confusion throughout the sequencing and microarray development processes. This implies that in order to confirm the identity of any intriguing clones, more sequencing is required. Sequence-vented cDNA microarrays are being produced with great care, with a greater focus on ensuring clone identity during the sequencing and microarray production processes.

*2.4. Digital PCR*

Digital PCR offers previously unheard-of opportunities for Molecular Genetic examination in cancer research and serves for illustrative purposes of the potency of PCR. Courtesy of a single DNA template and minimally diluted samples, the technique produces amplicons that come exclusively from a single template, that has the ability to detected using different fluorophores or sequenced to distinguish between different alleles (e.g., paternal vs. maternal alleles or wild type vs. mutant). To enable statistical analysis of the PCR result, digital PCR converts the exponential, analogue signals from conventional PCR into linear, digital signals. Mutant allele computation and allelic asymmetry identification in clinical specimens have been consummate by the use of digital PCR, a promising genetic diagnostic technique for cancer detection. This article's goal is to equip an panorama of digital PCR's fundamentals and practical uses in cancer research and molecular cancer detection. [29]

**3. Immunohistochemistry (IHC) and Immunofluorescence (IF)**

In both immunohistochemistry and immunofluorescence, an antibody is attached to a target cellular or tissue antigen, and the bound result is subsequently visualized using fluorescence or a 3, 3½-diaminobenzidine (DAB) chromogen detection technique. IHC and IF are very advantageous diagnostic gadgets as well as a way to direct particular therapies that

target a particular antigen on cell/tissue samples, as there are an increasing number of available antibodies against cellular epitopes. Depending on the kind of specimen being studied, the level of sensitivity needed, and the budget, a number of IHC and IF staining techniques can be used. Here is a simple "generic" approach to IHC, IF, direct, and indirect protein and other antigen localization. [30].

*3.1. Principle of Immunohistochemistry*

Because of their tumor selectivity and propensity to react with the tumor under investigation, antibodies are chosen for immunohistochemical testing. One of several detection techniques is used to identify positive reactions (tumor antigen-antibody binding) in tissue sections subsequent their treated with the potential antibodies. The most touchy ones use a secondary antibody that is conjugated to or coupled to an enzyme marker and is reactive against the original antibody [31]. Because it permits the attachment of a comparatively large number of enzyme molecules, such peroxidase, at the antigen site, this method frequently exhibits high sensitivity. The choice of a precipitating chromogen, often aminoethylcarbazole (red) or diaminobenzidine (brown), with which the enzyme reacts, determines the color of the reaction.

*3.2. Applications of immunohistochemistry*

In recent years, the focus in diagnostic immunohistochemistry (IHC) has switched from figuring out how cancerous tumors derive their cells to finding prognostic markers that may be useful in therapy selection.[32]. The surgical pathologist can benefit from a variety of highly specific immunostains that have been developed as a result of growing interest in immunohistochemical staining for both diagnostic and investigative purposes.[33]The following steps are part of the IHC technique:

i. Sections of tissue similar to those on poly-lysine-coated slides must be deparaffinized in

order for aqueous solutions to pass through.

ii. Endogenous enzyme quenching (which prevents IHC reagents from reacting with them and producing false-positive results). Among these endogenous enzymes are biotin, alkaline phosphatases, and peroxidases. Usually, 3% H20zor combined with free avidin does this.

iii. Recovery of antigens.

iv. Preventing binding locations that aren't specified.

v. Primary antibody binding.

vi. Secondary antibody binding that is biotinylated. vii. Peroxidases-ant peroxidases, avidin-biotin conjugates, peroxidases complexes, or the more recently used two-step polymer labelling method are examples of detection techniques.

vii. Chromogen substrate addition; often, Diaminobenzidine is used. Removing moisture, covering, and counterstaining the slide. Various immunohistochemical staining methods can be employed to identify and illustrate tissue antigens.

viii. Chromogen substrate addition, often Diaminobenzidine molecule.

ix. Covering, drying, and counterstaining the slide [34]

Various immunohistochemical staining methods can be employed to identify and illustrate tissue antigens.

*3.3. Immunofluorescence Techniques*

Using fluorescent-labeled antibodies, immunofluorescence is a potent approach for detecting certain target antigens. It is extensively utilized in both scientific studies and clinical settings. Important ideas on the usage of antibodies in immunofluorescence and their use in the diagnosis of dermatological disorders are covered in this article. Immunofluorescence is a microscope-based method that is used in

clinical settings to identify autoantibody–antigen complexes and diagnose certain cutaneous disorders.

Depending on the clinical situation, methods such as salt-split skin, indirect immunofluorescence, and direct immunofluorescence are used. Patients' skin is subjected to direct immunofluorescence, which uses fluorophore-labeled antibodies that attach to the pathogenic autoantibody–antigen complexes in the skin directly. The main purpose of indirect immunofluorescence methods in dermatology is to identify pathogenic autoantibodies that are circulating.

**4. Mass Spectrometry in Clinical Laboratories.**

At the start of the twenty-first century, mass spectrometer use in clinical laboratories is growing significantly [35].The remarkable advancements in mass spectrometry during the past ten years have fiddle a significant role in this expansion [36]. The 11th Sanibel Conference on Mass Spectrometry et al, "Mass Spectrometry in the Clinical Diagnosis of Disease," offered an exhaustive synopsis of the significance of MS in medicine. Every conference attendee concurred that mass spectrometry is going to play a bigger and bigger part in clinical chemistry. MS is no longer the intricate and time-consuming instrument reserved for highly skilled mass spectrometrists. This is a powerful, adaptable, and easily available technology what is advantageous for resolving analytical and research issues beyond a wide extent of scientific fields.

*4.1. Introduction to Mass Spectrometry*

Mass spectrometry [37] is quickly moving from specialized testing to a wider range of applications and offers specific capabilities in the clinical laboratory. Major historical effects of multiple sclerosis include the verification of immunoassay-positive drug screens the discovery of inborn metabolic abnormalities and the evaluation of steroid hormones [38]. In recent times,

MS has significantly reduced the amount of time needed for microbiological identifications. The analytical specificity of MS serves as a foundation for this transformation, which is being propelled by ongoing advancements in analytical platforms. Different principles underpin the conclusive identification of molecules ranging in size from tens of Daltons (small molecules) to hundreds of thousands of Daltons (biomolecules). For instance, LCMS/MS is currently used to identify tiny compounds. The identifications made employ this approach are stationed on a count of distinct features, such as parent ion ratios, retention times, and fragment ion ratios. Samples are directly pumped into the mass spectrometer for neonatal screening, and identifications are made based on certain transitions of precursor and product ions. Identifications in the microbiology lab are based on ion patterns produced by laser ablation of microbial proteins [39].

*4.2. Applications of Mass Spectrometry in clinical Diagnostics.*

One of the main drivers of Mass Spectrometry gradual transformation of laboratory medicine practice is its increased sensitivity and specificity in analysis. For example, measurements of testosterone and other sex steroids have been reported, where MS is the recommended technique. This move is aided by professional organization guidelines (e.g., urology now, and endocrinology originally) that mandate by means of Mass Spectrometry. For the purpose of identifying and treating several illnesses (such as infertility, certain malignancies, virilization, and polycystic ovarian syndrome), measuring testosterone in children and adults of both sexes is crucial [40]. Another clinical example where MS has been demonstrated to provide better assay quality than conventional immunoassays is the detection of thyroglobulin. Thyroglobulin is used to assess therapy efficacy and the risk of

thyroid cancer recurrence. Ant thyroglobulin autoantibodies, however, have the potential to interfere with immunoassays and produce artificially low thyroglobulin readings. The interference of ant thyroglobulin autoantibodies can be removed by LC-MS/MS measurement of the thyroglobulin-specific peptides using tryptic digestion with peptide-specific immunocapture. Additionally, it's thought that the MS-based approach eliminates interferences from heterophilic antibodies, which might lead to erroneously elevated thyroglobulin readings in immunoassays.

*4.3. Challenges and Future Perspectives.*

In conclusion, new technological developments continue to propel laboratory medicine forward by enhancing the laboratory's capacity for monitoring, diagnosis, prognosis, and prevention. The automation of core laboratory analyzers has advanced significantly, and data to date shows numerous important advantages for the laboratory setting, such as better clinical workflow, lower TAT and cost, and enhanced overall efficiency, provided that personnel receive the necessary training and instruction. Modern developments in analytical methods, including as micro technology, NMR, genetic sequencing, and mass spectrometry, have also shaped the field of laboratory medicine in the present and expanded its involvement in the delivery of healthcare. Our progress towards this future patient-centered approach has been greatly supported by advancements in omics and single-cell micro technology, and tremendous progress has already been made in the field of oncology. However, in order to fulfil this promise, sophisticated AI is still needed. Future research should evaluate factors for clinical deployment and aim to broaden the clinical uses of AI and precision medicine across disciplines in the setting of laboratory medicine.

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