International Journal of Health Technology and Innovation
An Official Journal of **Kalam Institute of Health Technology** | ISSN - **2583-8547**
REVIEW ARTICLE

Evolution of *In-vitro* Diagnostics Methods

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Received: 15th January, 2024; Revised: 10th February, 2024; Accepted: 04th March, 2024; Available Online: 05th April, 2024

ABSTRACT

In-vitro Diagnostics or IVD tests and their methods have evolved significantly over the past decades with each segment witnessing advancements to enhance accuracy, precision and efficiency.

In the clinical chemistry segment, this transformation has been characterized by a shift from the traditional chemical methods to the advanced enzymatic methods improving test specificity, reducing sample/reagent volumes and facilitating automation. Similarly, in the immunology segment, this progression included a transitioning from RIA, with its radioactive hazards, to ELISA and CLIA technologies, offering safer and more sensitive quantitative determinations.

Complete blood counts and hematology methods have leapfrogged from the manual counting of cells to 6 part- differential fully automated analysers. These analysers can report all the 5 types of WBCs separately and classify immature granulocytes (IG) as a sixth subpopulation of the WBCs. The transition in hematology illustrates exceptional advancements in precision and efficiency.

In microbiology, the adoption of automated culture systems and RT PCR methods has marked a significant surge towards molecular diagnostics. RT PCR has revolutionized the way laboratories diagnose human microbial infections. RT PCR is fast emerging as the preferred method for the detection of a large number of infectious agents. It offers the benefits of high sensitivity, accuracy and fast diagnosis in addition to its high throughout and quantification.

The evolution of methods and techniques in IVD, reflects a continuous journey towards innovation, with each advancement bringing us closer to more efficient, accurate, and accessible diagnostics.

Keywords: RIA: Radio Immuno Assay; ELISA: Enzyme Linked Immuno Sorbent Assay; CLIA: Chemiluminescence Immuno Assays; RT PCR: Real Time Polymerase Chain Reaction

International Journal of Health Technology and Innovation (2024)

How to cite this article: Kohli V. Evolution of *In-vitro* Diagnostics Methods. International Journal of Health Technology and Innovation. 2024;3(1):1-4.

Doi: 10.60142/ijhti.v3i01.01

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In- vitro Diagnostics or IVD tests constitute a crucial component of healthcare across the globe. The IVD tests are performed outside the body of the patient, in pathology laboratories, hospitals and blood banks.

The word '*vitro'* is derived from the Latin word 'vitreous' meaning glass. Since test tubes were traditionally made of glass and these tests, that were mostly conducted in test tubes, came to be known as *in- vitro* diagnostics tests.

IVDs encompass tests that could be segmented into clinical chemistry, immunology, hematology, microbiology, molecular diagnostics, point of care testing (POCT) , coagulation, urine analysis and others. Clinical chemistry, immunology, hematology and microbiology are the four leading segments of IVD.

IVD tests and their methodologies have evolved significantly over the past decades with each segment witnessing advancements to enhance accuracy, precision and efficiency.

Clinical Chemistry

Clinical chemistry is the largest segment of *in-vitro* diagnostics and consists of tests that are based on biochemical reactions. They are conducted on body fluids like blood, serum/plasma, urine etc. for the determination of organ function tests.

In the 1980s, a majority of the clinical chemistry tests were based on chemical methods, e.g.:

- o-Toluidine method for glucose.
- Liebermann-Burchard method for cholesterol.

These chemical methods were fraught with limitations, including low specificity, extensive manual intervention and

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prolonged processing times.

As a consequence, these methods could not deliver the desirable precision, accuracy, linearity and thus reliability. These methods could not be automated and had to be read on manual colorimeters and semi-automated photometers. The 1990s heralded a paradigm shift with the introduction of enzymatic methods enhancing precision, specificity and enabling automation. Enzymatic methods for a number of important clinical chemistry parameters like cholesterol, SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), triglycerides, urea, uric acid etc. were introduced during this period. Glucose was the first parameter to be conducted by an enzymatic method, e.g. Glucose oxidase method for glucose; Cholesterol oxidase method for cholesterol; Uricase method for uric acid.

This leap forward significantly reduced sample volume requirements and human error, laying the foundation for faster and more reliable diagnostics.

These methods fulfilled the need of the clinical chemistry lab to such an extent that there has not been any exceptional transformation in this segment since then. Only incremental improvements have been made in these methods rendering them more user friendly and efficient. These tests now require single, liquid stable, ready to use reagents, in very small quantities for a majority of the parameters.

This transformation in clinical chemistry from rudimentary chemical methods to sophisticated enzymatic assays marks a significant milestone in IVD's history.

It is pertinent to note, however, that some of the critical clinical chemistry parameters like creatinine and bilirubin along with albumin, total protein etc. are still being widely conducted by the chemical methods and therefore still suffer from a few limitations.

The clinical chemistry segment also witnessed the introduction of 'closed systems' that conducted the tests using a unique, thin-film dry slide technology.

Figure -1 : The shift from 1980s' chemical methods to the 1990s' enzymatic methodologies represented a transformative phase, improving test specificity, reducing reagent use and facilitating automation.

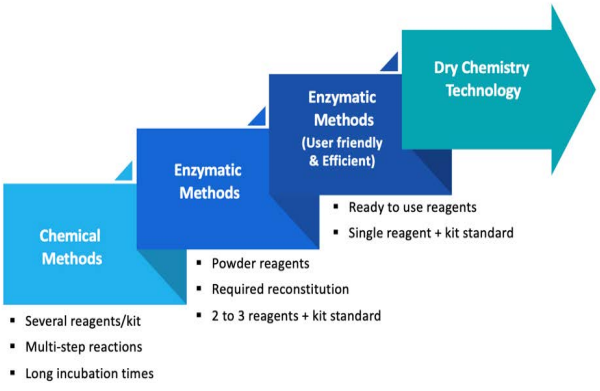

Figure 1: Evolution of Testing Methods in Clinical Chemistry

Immunology

Immunology is the second largest segment of *in-vitro* diagnostics and embodies methods that are based on antigen and antibody reactions.

In the 1980's, the immunology methods primarily in use were the latex based agglutination tests wherein the end result was read manually with the naked eye e.g.: latex agglutination slide tests for the detection of human Chorionic Gonadotropin (hCG), Hepatitis B surface Antigen (HBsAg), Anti-Streptolysin-O (ASO), Rheumatoid Factors (RF), C-Reactive Protein (CRP) and rota virus etc. This method lacked the desired sensitivity and specificity yielding false negative and positive results. These methods were further limited by their subjective interpretation and lack of precision.

The development of immunochromatography and the introduction of immunoassays based on colloidal gold technology in the 1990s represented significant advancements in terms of sensitivity and specificity, allowing for rapid, point-of-care testing.

The Radio Immuno Assay (RIA) technique was first introduced in 1959 for the determination of insulin in plasma. It continued to remain in extensive use for the estimation of thyroid and fertility hormones like T3 (Triiodothyronine), T4 (thyroxine), Thyroid Stimulating Hormone (TSH), Luteinizing Hormone(LH), Follicle Stimulating Hormone (FSH) and Prolactin for several years thereafter as it provided acceptable sensitivity and specificity. This technique, however, suffered from several disadvantages such as the need for radio-labelled reagents that could pose a radiation hazard. It required specially trained personnel to conduct the test. The labs in addition, required a special license to store, handle and dispose-off the radioactive material.

The adoption of RIA technique in labs started declining once the Enzyme Linked Immuno Sorbent Assay (ELISA) was introduced. ELISAs did not require the use of radioactive reagents and thus was considered to be safe.

This method used enzymes and substrates to measure the intensity of antigen and antibody reactions, using a photometer for microwells - the ELISA reader. Though the first ELISA test was introduced in the 1970s, the method gained momentum after the first ELISA assay for HIV-1 became available for the screening of blood and blood products in 1985.

The ELISA method proved to be very sensitive and specific specially for qualitative determinations and has been one of the most extensively deployed immunoassays. In fact ELISA is still the only approved method by the Govt. of India, for the screening of Human Immunodeficiency Virus (HIV 1&2), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in the blood banks. These tests provide a sensitivity of > 99.5% and a specificity of > 98%.

The ELISA method has evolved through several generations which could be traced by the classic example of ELISA tests for the screening of HIV : Table -1.

The ELISA method advanced also through the introduction of its several formats over the years. Each one of these formats was specifically suited for the detection of IgG antibodies, antigens, IgM antibodies and small proteins e.g. the Indirect

Table 1: ELISA tests for the screening of HIV

1st Generation	Used the viral lysate on the microwells for the detection of IgG antibodies to HIV.
2nd Generation	Used recombinant antigens on the microwells for the detection of IgG antibodies to HIV 1/2.
3rd Generation	Used synthetic peptides on the microwells for the detection antibodies to HIV 1/2.
4th Generation	Used a combination of antigens and antibodies for the detection antibodies to HIV 1/2 and the p24 antigen.
With each successive generation, the 'window period' for the detection of HIV was shortened by several weeks ensuring early detection.	

ELISA, Sandwich ELISA, Immunocapture ELISA and Competitive ELISA methods. The ELISA methodology, however, lacked the desired sensitivity and reliability for the quantitative determinations for hormones, vitamins, drugs etc.

This necessitated the development of Enzyme Linked Fluorescent Assays (ELFA) and more notably Chemiluminescence Immuno Assays (CLIA), in the early 2000s. Chemiluminescence (CL) is the luminescence produced by a chemical reaction that induces the transition of an electron from its ground state to an excited electronic state. When the excited molecule decays to its ground state, CL emission at different wavelengths occurs, which can be measured by a luminometer. These assays use a luxogenic (light generating) substrate in place of a chromogenic (colour generating) substrate. Compared to absorbance based assays like ELISA, chemiluminescence assays have a lower background signal resulting in higher sensitivity.

The sensitivity of a CLIA assay is estimated to be ten times higher than that of an ELISA assay. CLIA is currently the most acceptable immunoassay method for quantitative determination of hormones, vitamins, proteins etc. for its high sensitivity, dynamic range and complete automation (Figure 2):

Hematology

The most common test conducted in any lab is the Complete Blood Count (CBC). A CBC test determines the number of Red blood cells, White blood cells, Hemoglobin, Platelets, Hematocrit along with other parameters such as Red Blood Cell Volume Distribution Width (RDW), Mean Platelet Volume (MPV), Platelet Volume Distribution Width (PDW) etc. in a person's blood sample.

During the 1950s, laboratory technicians counted individual blood cells underneath a microscope. This tedious and inconsistent method was replaced with the first, very basic hematology analyser, designed by Wallace Coulter. Since then hematology analysers have been evolving regularly offering counts for an increasing number of parameters with complete automation. These analyzers are engineered on the principles of impedance and flowcytometry.

The CBC analyzers have evolved as under based on the types of subpopulations of the WBCs that they are able to report (Table 2):

Recent innovations in hematology include the use

Table 2: CBC analyzers based on the types of subpopulations of the WBCs

2 Part differential Hematology Analysers	Report only 2 types of WBCs. (Granulocytes, Lymphocytes/Monocytes)
3 Part differential Hematology Analysers	Report only 3 types of WBCs. (Lymphocytes, Monocytes & Granulocytes)
5 Part differential Hematology Analysers	Report all the 5 types of WBCs separately. (Neutrophils, Lymphocytes, Basophils, Eosinophils, and Monocytes)
6 Part differential Hematology Analysers	Report all the 5 types of WBCs separately and classify immature Granulocytes (IG) as a sixth subpopulation of the WBCs.

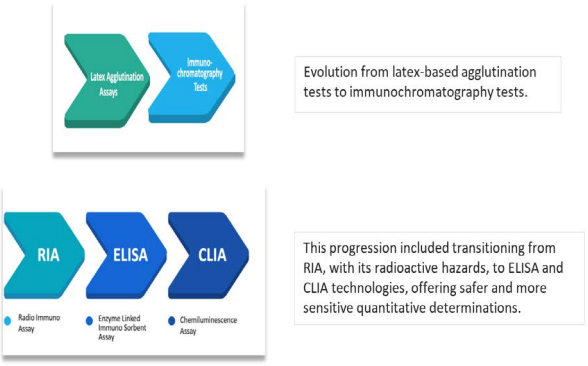

Figure 2: Evolution of Testing Methods in Immunology

of artificial intelligence (AI) to identify and classify cell morphologies, enabling the detection of rare cell types and subtle changes indicative of disease.

Microbiology

The field of microbiology has similarly experienced transformative changes, moving from labour-intensive 'blood smear' examinations under the microscope to rapid, molecular-based methods such as real-time Polymerase Chain Reaction (RT-PCR).

RT PCR has revolutionized the way laboratories diagnose human microbial infections. This testing method combines PCR chemistry with fluorescent probe detection of the amplified product in the same reaction tube.

RT PCR is fast emerging as the preferred test for the detection of a large number of infectious agents. It offers the benefits of high sensitivity, accuracy and fast diagnosis in addition to its high throughout and quantification.

The RT PCR was notified as the Gold Standard test for the detection of Covid -19 in April, 2020 by the Indian Council of Medical Research.

Cultures of blood, cerebrospinal fluid, stool, tissue and other human samples for aerobic, anaerobic, mycobacterial and fastidious bacterial species as well as fungus are widely conducted in microbiology labs.

Cultures also determine the susceptibility and resistance of specific pathogens to a wide range of antimicrobial agents. This information is pertinent for facilitating immediate institution of proper treatment regimens to the patients.

Culture is still considered as a Gold Standard in diagnostic microbiology.

Automated blood culture systems are also available now to conduct these tests more efficiently.

The application of next-generation sequencing (NGS) technologies in microbiology represents the next frontier, offering unparalleled depth and breadth in pathogen detection, antimicrobial resistance profiling and microbial community analysis.

CONCLUSION

Technology based evolution will continue to take place in the diagnostics space to provide more reliable and faster diagnosis. Moreover, artificial intelligence (AI) and machine learning (ML) algorithms are now being employed to interpret complex biochemical data to predict outcomes and tailor patient management plans, signifying a new era in diagnostics.

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