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En vue de l'obtention du diplôme

MASTER

Thème

Les techniques de biologie moléculaire appliquées au diagnostic microbiologique

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DEDICATION

«Praise be to Almighty God, who allowed me to see this long-awaited day». From the depths of my heart, I dedicate this work:

To the light of my life, the strength behind every step, the quiet flame that warms my heart my dearest mother, Malia. No words, however heartfelt, can truly capture the love and gratitude I hold for you. Through every challenge and every triumph, you stood beside me with unwavering support and tender comfort. You believed in me when I doubted myself and carried me with your strength when I was weak. On this day that marks the fruit of my journey, I offer this work to you, with all my love, as a tribute to your endless devotion.

To my father, Elyamine the man whose strength, values, and quiet guidance shaped the person I have become. Your discipline, your patience, and the example you set taught me far more than words ever could. You stood firm through every season of my life, often in silence, always with purpose. I owe you not only my progress, but also the foundation of my character. On this meaningful day, as I reach a milestone that you helped make possible, I dedicate this work to you with deep respect and lasting gratitude.

To my sister Zineb, the future pharmacist the sister, friend, and companion whose presence has always brought comfort and strength. I wish for you a path filled with success beyond anything I have known, and a life full of the happiness you truly deserve. All the love in the world would not be enough to express what I feel for you. And to my little angel Maryam your innocence lights up our home. Every time I see you, I remember the child I once was. I wish you both endless joy and success. With love, your big brother.

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DEDICATION

To the friends untouched by time, the constant stars in my ever-changing sky Atef, Arif, Chamesdinne, and Abdelallah. When many drifted away, you remained steadfast, sincere, and true. Our friendship has been a refuge in moments of doubt, a source of laughter in times of gloom, and a reminder that loyalty still exists in its purest form. This journey would have been lonelier without your presence, and today, I honor the bond we share one built on trust, warmth, and memories that no distance or time could ever erase.

I never had a brother neither younger nor older but if ever there were someone I'd call a brother, it would be you. It was only a journey of two years, yet the experiences we shared, the advice you offered, and the kindness you showed me were, without doubt, the heartfelt care of an elder brother toward his younger one. Words of thanks will never be enough, and it is truly an honor to call you my brother, Fateh.

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SUMMARY

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LIST OF ABBREVIATIONS

A-DNA: A-form of DNA

Amp: Amplicon

API: Analytical Profile Index

BAL: Bronchoalveolar Lavage

B-DNA: B-form of DNA

blaKPC: Klebsiella pneumoniae carbapenemase gene

blaNDM-1: New Delhi metallo-beta-lactamase-1 gene

blaTEM: Beta-lactamase TEM-type gene

cDNA: Complementary DNA

CMV: Cytomegalovirus

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

DBS: Dried Blood Spot

ddNTPs: Dideoxynucleoside Triphosphates

DETECTR: DNA Endonuclease Targeted CRISPR Trans Reporter

dNTPs: Deoxynucleoside Triphosphates

EBERs: Epstein–Barr virus-encoded RNAs

eae: Intimin gene (E. coli)

ELISA: Enzyme-Linked Immunosorbent Assay

env: Envelope gene (HIV)

ER: Endoplasmic Reticulum

FAME: Fatty Acid Methyl Esters

FISH: Fluorescence In Situ Hybridization

gag: Group-Specific Antigen (HIV gene)

GC-MS: Gas Chromatography-Mass Spectrometry

gRNA: Guide RNA

GWAS: Genome-Wide Association Study

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

HIV: Human Immunodeficiency Virus

hlyA: Hemolysin A gene

IFNGR1: Interferon Gamma Receptor 1

Ig: Immunoglobulin

inhA: Isoniazid resistance gene

IS6110: Insertion Sequence 6110

ITS: Internal Transcribed Spacer

katG: Catalase-peroxidase gene

L1: Late gene 1 of HPV

LAMP: Loop-mediated Isothermal Amplification

LC-MS: Liquid Chromatography–Mass Spectrometry

mecA: Methicillin resistance gene

MDR: Multidrug Resistance

metA: Methionine biosynthesis gene

MOMP: Major Outer Membrane Protein

mRNA: Messenger RNA

mtDNA: Mitochondrial DNA

N: Nucleocapsid gene

NGS: Next Generation Sequencing

NS1: Non-structural protein 1

NS3: Non-structural protein 3

NS5A: Non-structural protein 5A

NS5B: Non-structural protein 5B

ompA: Outer Membrane Protein A

ORF1ab: Open Reading Frame 1ab

ospC: Outer Surface Protein C

p24: HIV capsid protein (gag gene product)

PCR: Polymerase Chain Reaction

pol: Polymerase gene (HIV)

PRNP: Prion Protein Gene

qPCR: Quantitative Polymerase Chain Reaction

RBPs: RNA-binding Proteins

rDNA: Ribosomal DNA

RdRp: RNA-dependent RNA Polymerase

rpoB: RNA Polymerase Beta Subunit

rRNA: Ribosomal RNA

RT-PCR: Reverse Transcription PCR

S: Spike gene

SHERLOCK: Specific High Sensitivity Enzymatic Reporter Unlocking

Sm: Smith antigen

stx1: Shiga toxin 1

stx2: Shiga toxin 2

SYBR: SYBR Green dye

tRNA: Transfer RNA

UL83: Unique Late gene (CMV)

vanA: Vancomycin resistance gene

VOC: Volatile Organic Compounds

WGS: Whole Genome Sequencing

Z-DNA: Z-form of DNA

Introduction

Molecular biology elucidates molecular-level cellular interactions and transformations, centering on the structure, function, processing, regulation, and evolution of macromolecules, particularly genes and proteins **(Schleif, 2023).** This understanding is critical for discerning fundamental life mechanisms and disease pathogenesis. Key advancements, exemplified by Mendel's laws of inheritance and the elucidation of DNA's double-helix structure, have propelled molecular comprehension, fostering progress in diagnostics and biotechnology.

The late 20th century saw a paradigm shift in microbiological diagnosis from restrictive traditional (culture/microscopy) to sophisticated molecular approaches. Conventional methods were limited by protracted turnaround times, inherent insensitivity, and challenges with fastidious/unculturable organisms. This necessitated swifter, more accurate, and highly sensitive diagnostic tools, particularly for emerging infections **(Houpikian & Raoult, 2002).** Molecular diagnostics, leveraging understanding of microbial genetic material and proteins, provided unprecedented disease detection capabilities, driving their widespread adoption **(Cardenas, 2021).**

The molecular diagnostics revolution is founded on techniques like Polymerase Chain Reaction (PCR) and its variants (RT-PCR, qPCR, LAMP), enabling rapid and highly sensitive amplification and detection of specific nucleic acid segments for diverse pathogens. Further advancements include sophisticated sequencing (Sanger, NGS) and hybridization (FISH) technologies, which allow comprehensive genome characterization, pathogen variant identification, and high-resolution visualization of DNA sequences, extending to holistic microbiome profiling via metagenomics. The ongoing pursuit of efficient diagnostics drives the development of cutting-edge tools such as CRISPR-Cas systems for rapid and specific pathogen detection, nanotechnology for sensitive sensing, and microarray technology for multiplexed genetic analysis. These genetic breakthroughs are complemented by proteomics and antigen detection methods (e.g., chromatography, immunofluorescence, Western blot), which provide specific insights into pathogenic proteins, collectively transforming precision microbiology **(Chiu et al., 2015).**

The profound impact of advanced molecular and proteomic techniques on precision microbiology highlights their central significance within this specialized academic domain. This continuously and rapidly evolving field represents a highly dynamic and contemporary research area. This thesis, driven by the critical importance of rapid, accurate, and

comprehensive microbiological diagnosis for public health, clinical management, and biomedical knowledge, will thoroughly explore these pivotal diagnostic advancements. The research is structured: Chapter 1 establishes molecular biology fundamentals and historical evolution of diagnostics; Chapter 2 details principles, applications, advantages, and limitations of key molecular and antigen detection techniques. Subsequent chapters will cover specific clinical applications, challenges, and future directions.

However, despite these transformative advances, significant challenges remain in optimizing molecular diagnostic strategies for routine clinical use. Variability in sensitivity, cost-effectiveness, infrastructure requirements, and applicability across diverse pathogens raise critical questions about the universal accessibility and integration of these technologies. This thesis aims to address these concerns by systematically examining the principles, applications, and limitations of current molecular and proteomic diagnostic methods within the evolving landscape of precision microbiology.

I. Introduction to molecular biology techniques

I.1. Fundamental concepts in molecular biology

I.1.1 Introduction to molecular biology

Molecular biology is a branch of biology that studies the interaction and transformation in the cells at a molecular level, living things are made of chemicals just as non-living things are, so a molecular biologist studies how molecules interact with each other in living organisms to perform the functions of life **(Alberts et al., 2014).** In order to gain a micro-level understanding of how life functions, molecular biologists do experiments to examine the (structure, function, processing, control, evolution) and interactions of biological molecules, even though all living things contain a wide variety of molecules, the majority of biologists concentrate on genes and proteins because they carry out a vast array of tasks in live cells and hold the instructions needed to produce new proteins **(Gannon, 2002).** Genes are segments of information stored in large nucleic acid molecules, while proteins are distinct functional molecules, making both of these substances and the interaction between them, extraordinarily important to study **(Hutchins, 2014).** The long chain of these interactions is what many molecular biologists seek to document, each step in a functional pathway is something that a disease can disrupt, understanding the role of each such molecule is essential to grasp more complex aspects of how organisms live and work to fully understand the structure of a molecule **(Yanofsky, 1967).** Genetics and biochemistry are two related sciences that share many similarities with molecular biology. All three disciplines focus on how molecules function in living organisms. However, each has distinct applications.

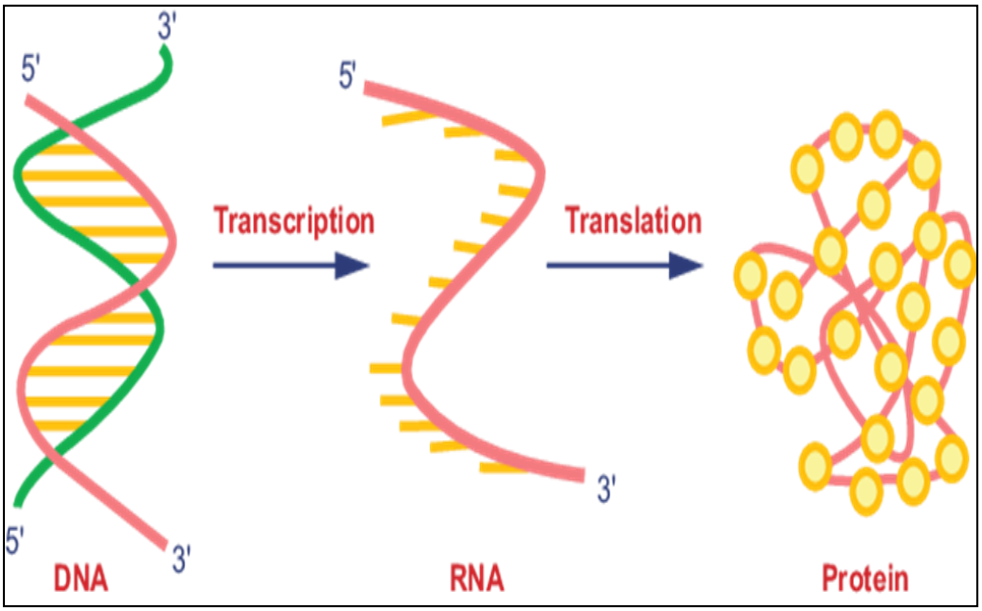

Figure 1. The relationship between genes and proteins **(Kaushik & Singh, 2023).**

I.1.2 The Central dogma of molecular biology

Molecular biology's core tenet is the idea that genetic information can only move from DNA to RNA to protein or from RNA to protein directly **(Leavitt, 2004).** Francis Crick created the basic hypothesis of the central dogma in 1958, the idea that information does not go from proteins to nucleic acids was part of his slightly more universal version **(Cobb, 2017).** Researchers have found a number of exceptions to the idea, the example of prions is one that stands out in particular, infectious proteins called prions reproduce without the use of DNA or RNA, the neurologic condition known as Creutzfeldt-Jakob disease is caused by these prions **(Prusiner, 1984).** DNA replication is the process by which cells duplicate the DNA in their genome, A cell must first replicate its entire genome before division, to ensure that each daughter cell recevies a set of genetic information **(Kornberg & Baker, 2005).**

Transcription is the process of creating an RNA copy of the DNA sequence of a gene. This copy, known as messenger RNA (mRNA) contains the DNA-encoded protein information for synthesizing a protein. In humans and other complex organisms, the mRNA must travel from the cell nucleus to the cytoplasm, where protein synthesis occurs. Translation is the mechanism by which mRNA-encoded information directs the addition of amino acids during protein synthesis. This process takes place in the cytoplasm, where ribosomes read the mRNA and assemble the corresponding chain of amino acids, ultimately forming the resulting protein **(Krause, 1995).**

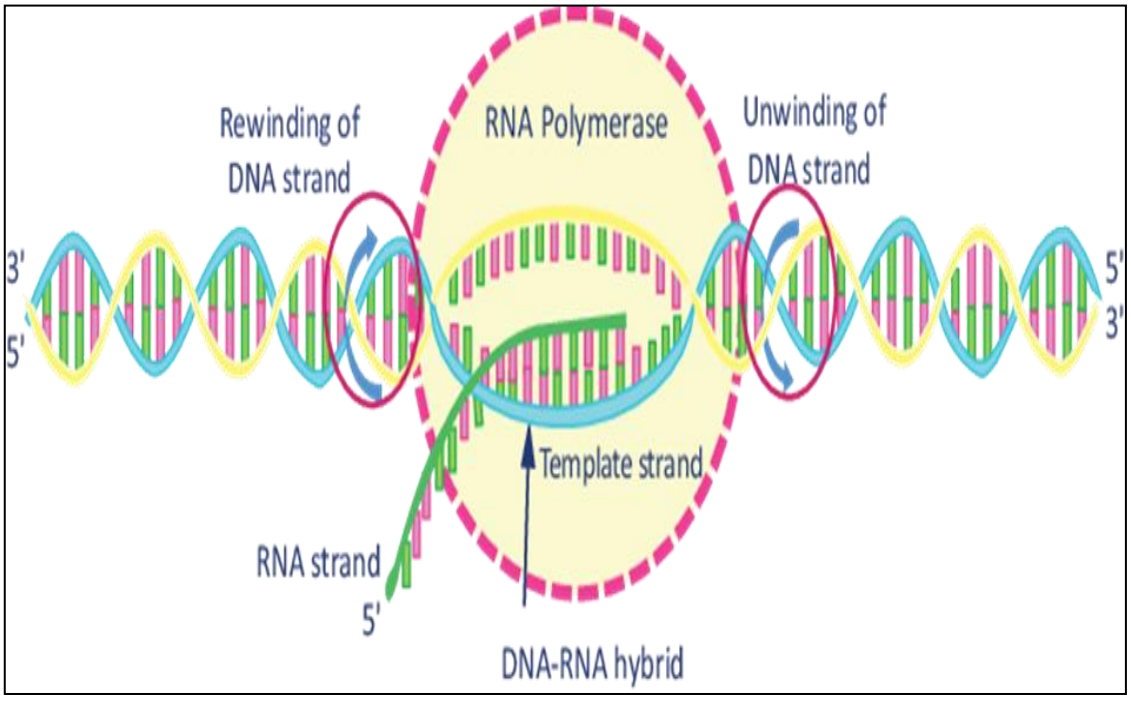

Figure 2. Trancription **(Kaushik & Singh, 2023).**

RNA replication is the process by which an RNA molecule is copied into a new RNA molecule. Many RNA viruses use this mechanism. RNA-dependent RNA polymerases, which are enzymes that copy RNA to new RNA, are also present in many eukaryotes and participate in RNA silencing **(Ahlquist, 2002).** Transferring information from RNA to DNA is known as reverse transcription, which is the reverse of regular transcription. This process occurs in eukaryotes during telomere synthesis and in retrotransposons, as well as in retroviruses such as HIV. It involves a family of enzymes called reverse transcriptases **(Varmus, 1987).**

I.1.3 Structure and role of DNA

DNA, or deoxyribonucleic acid, is the molecule that contains the genetic information necessary for an organism's growth and function, it is composed of two interconnected strands that loop around one another to form a double helix, resembling a twisted ladder **(Plomin & Crabbe, 2000).** The backbone of each strand is composed of alternating deoxyribose sugar units. Attached to each sugar is one of four nitrogenous bases; adenine (A), cytosine (C), guanine (G), or thymine (T). Hydrogen bonds between cytosine and guanine, and between adenine and thymine, hold the two strands together. The biological information such as instructions for synthesizing proteins and RNA molecules is encoded in the sequence of these bases along the DNA backbone **(Travers & Muskhelishvili, 2015).**

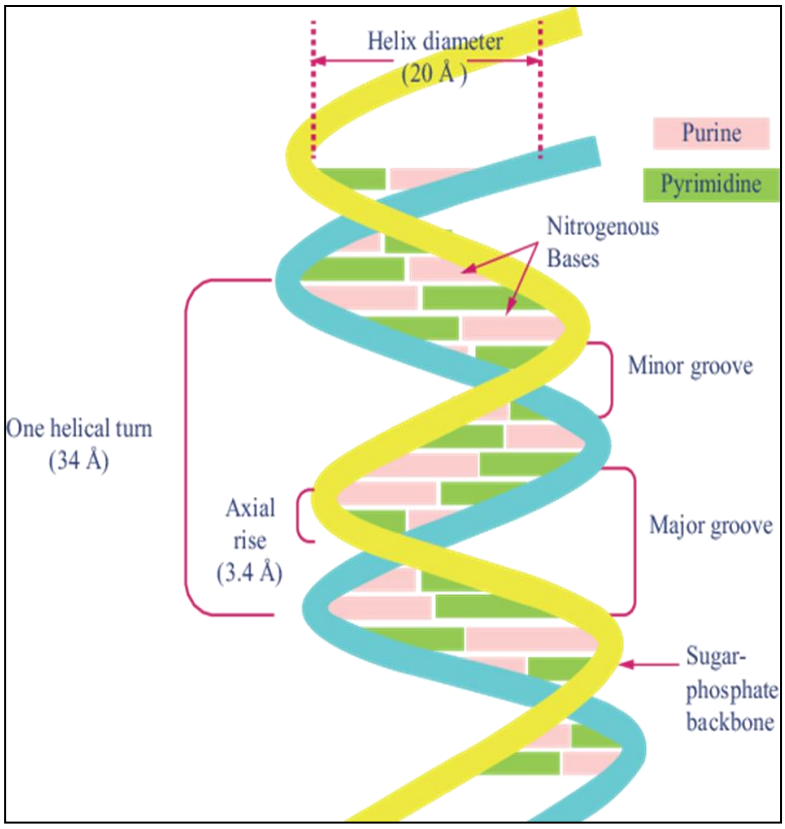

Figure 3. DNA structure **(Kaushik & Singh, 2023).**

Table 1. Major differences between the three main types of DNA **(Rich, 1993).**

Feature	**A-DNA**	**B-DNA**	**Z-DNA**	**mtDNA**
Helix direction	Right-handed	Right-handed	Left-handed	Circular (not helical in the same way)
Shape	Short & wide	Long & thin	Elongated & zigzag	Circular, closed-loop
Base pairs per turn	~11	~10.5	12	Varies (species-specific)
Helix diameter	~23 Å	~20 Å	~18 Å	Varies
Major groove	Deep, narrow	Wide, deep	Flat, hardly visible	Not defined the same way
Minor groove	Wide, shallow	Narrow, deep	Narrow, deep	Not defined the same way
Occurrence	Dehydrated DNA, lab conditions	Most DNA under physiological conditions	Certain sequences (e.g., CG repeats) under supercoiling	In mitochondria of eukaryotic cells
Stability	Less stable than B-DNA	Most stable form	Least stable	Stable in mitochondria
Biological function	Rare, not common in vivo	Standard form for genetic storage	May regulate transcription	Involved in energy production (OXPHOS)
Discovered by	Rosalind Franklin (1953)	Watson & Crick (1953)	Rich & Zhang (1979)	Discovered through mitochondrial studies
Location	Artificial conditions	Found in nucleus of all cells	Sometimes in nucleus, regulatory roles	Found in mitochondria

I.1.4 RNA and its functions

The nucleic acid known as ribonucleic acid or RNA, is found in all living cells and shares structural similarities with DNA. However, unlike DNA, RNA is typically single-stranded **(Copley et al., 2007).** Instead of DNA's deoxyribose, the backbone of an RNA molecule is composed of alternating phosphate groups and the sugar ribose. Each ribose sugar is bonded to one of four nitrogenous bases: adenine (A), guanine (G), cytosine (C), or uracil (U) **(Conn & Draper, 1998).**

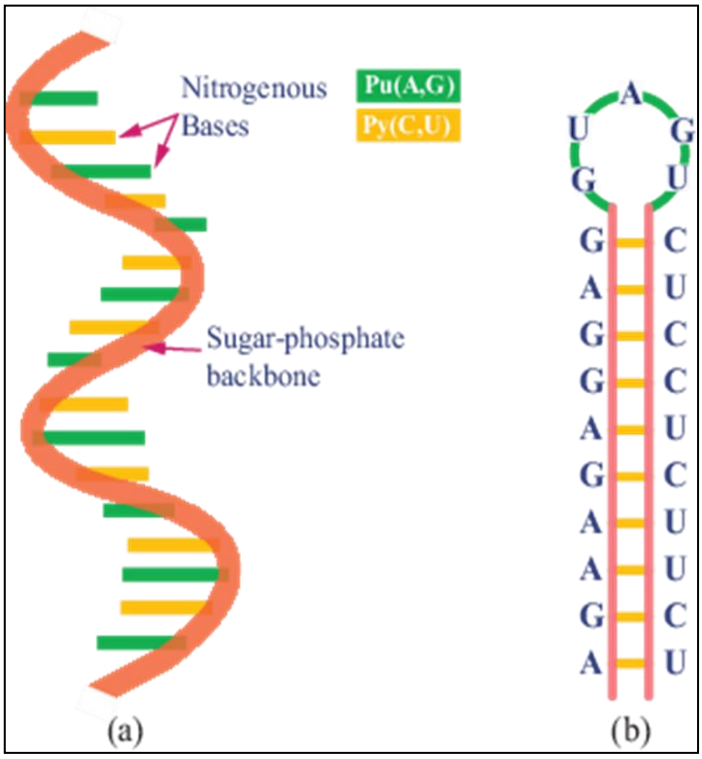

Figure 4. RNA structure **(Kaushik & Singh, 2023).**

Messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA) are the three different forms of RNA found in cells. Certain RNAs also play a role in controlling the expression of genes, the genetic material of certain viruses is RNA **(Liu et al., 2020).**

Table 2. Comparing different RNA types **(Brosius & Raabe, 2016).**

Feature	**mRNA (Messenger RNA)**	**tRNA (Transfer RNA)**	**rRNA (Ribosomal RNA)**
Function	Carries genetic code from DNA to ribosomes	Brings amino acids to ribosomes	Forms core of ribosome structure and catalyzes protein synthesis

Structure	Single-stranded, linear	Cloverleaf-shaped with loops	Complex structure with multiple loops and stems
Location	Synthesized in nucleus, functions in cytoplasm	Cytoplasm	Part of ribosomes in cytoplasm or rough ER
Size	Variable; relatively long	Short (~70-90 nucleotides)	Varies (depends on organism and ribosome type)
Stability	Unstable, short-lived	More stable than mRNA	Very stable
Codon/Anticodon	Has codons (3-base sequences)	Has anticodons that match codons	No codons or anticodons
Role in Protein Synthesis	Template for translation	Delivers amino acids during translation	Forms ribosomes, catalyzes peptide bond formation

I.1.5 Proteins and their biological significance

Proteins are vital macromolecules composed of amino acids which perform diverse functions necessary for the organisms.

A protein's structure is determined by the arrangement of its amino acid chains. The twisting and folding of these chains determine whether it transforms a more complex structure, Proteins structures can be classified as primary, secondary, tertiary, or quaternary **(Pour-El, 1981).**

These molecules perform complex roles. For example, enzymes catalyze biochemical reactions, such as insulin which regulates blood sugar levels, transport proteins such as hemoglobin facilitate the movement of molecules by transporting oxygen and carbon dioxide, structural proteins like collagen provide mechanical support especially in the process of stopping bleeding **(Miege, 1982).**

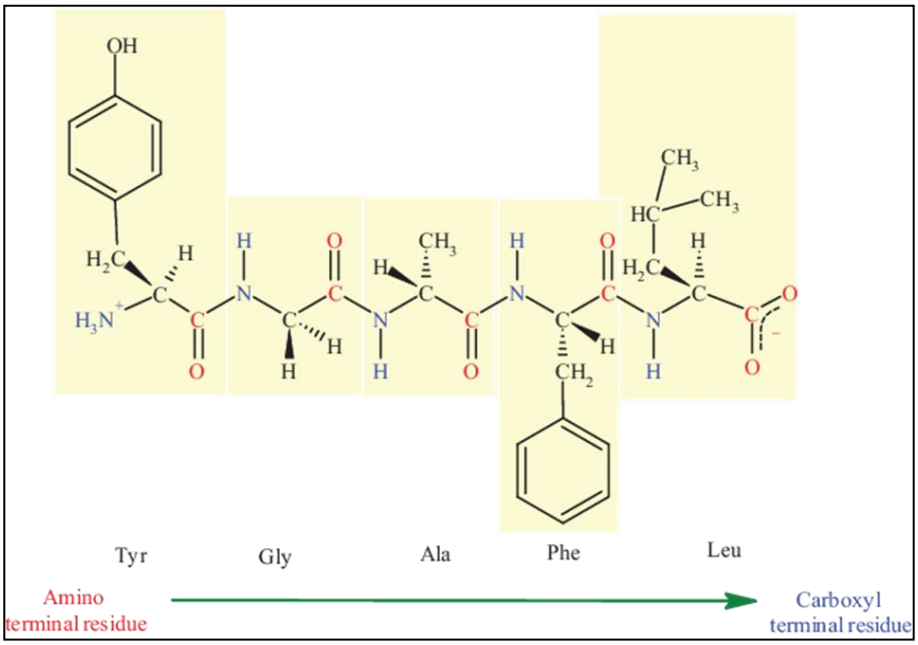

Figure 5. Primary structure of a protein **(Kaushik & Singh, 2023).**

Table 3. Functions of the most important proteins **(Whitford, 2013).**

Protein Type	**Primary Function**	**Examples**	**Relevance in Molecular Biology**
Enzymes	Catalyze biochemical reactions	Polymerases, helicases, ligases	Essential for DNA/RNA replication, repair ans transcription
Gene Regulatory Proteins	Control gene expression (activation/repression)	Transcription factors	Determine when and where genes are transcribed
Structural Proteins	Support cell or complex structures	Histones	Package DNA into chromatin and regulate access
Transport Proteins	Transport biomolecules like RNA or amino acids	tRNA, nuclear transport proteins	Key in translation and genetic code delivery
Signal Proteins	Receive and transmit	Surface receptors,	Transmit regulatory

	signals within or between cells	kinases	signals that affect gene expression
Modifier Proteins	Modify proteins or nucleic acids (e.g., phosphorylation, methylation)	Methyltransferase, kinase	Alter gene activity or protein function
DNA Repair Proteins	Detect and fix DNA damage	Nucleases, repair polymerases	Prevent mutations and maintain genome stability
RNA-binding Proteins	Bind RNA to regulate processing, transport, translation, or decay	RNA-binding proteins (RBPs)	Control the post-transcriptional fate of RNA

Advances in proteomics and structural biology continue to expand our understanding of protein function. These developments contribute to the creation and refinement of molecular diagnostic techniques by helping to overcome their limitations **(Aslam et al., 2016).**

I.2 History and evolution of techniques

I.2.1 Early foundations of molecular biology

Decades before scientists were able to understand genes at the molecular level, Gregor Mendel had already laid the foundations of genetics. In the 1860s, he conducted experiments on pea plants that led to the discovery of the fundamental laws of inheritance, although the molecular explanation of his findings would not emerge for many decades **(Ellis et al., 2011).** At the turn of the 20th century, biologists began to integrate chemistry, genetics, and microbiology in order to study living organisms at the molecular level. This new approach combined knowledge from chemistry, genetics, and microbiology that helped understanding how genes and cells work more precisely, something previous sciences could not accurately explain **(Morange, 2000).** At the beginning of the 20th century, a series of experiments began to change scientists' view of heredity. Frederick Griffith and Oswald Avery experiments helped prove that DNA was the true genetic material not proteins as previously believed, these discoveries in the 1920s and 1940s contributed to building the foundation upon which

we today understand how genetic information is stored, transmitted, and expressed within cells **(Morange, 2021).**

Table 4. Key experiments in molecular biology **(Moss, 2019).**

Year	**Scientist(s) or Experiment**	**Purpose or Hypothesis**	**Outcome or Conclusion**
1866	Gregor Mendel	To understand how traits are inherited	Established the laws of inheritance and foundation of classical genetics
1869	Friedrich Miescher	Study the contents of cell nuclei	Discovered 'nuclein' (now known as DNA) in pus cells
1910s–1920s	Phoebus Levene	Study the chemical structure of nucleic acids	Proposed DNA is made of nucleotide units (sugar, phosphate, base)
1928	Frederick Griffith	Investigate transformation in bacteria	Discovered bacterial transformation: harmless bacteria became virulent
1944	Avery, MacLeod, McCarty	Identify Griffith's 'transforming principle'	Proved that DNA, not protein, is the genetic material
1950	Erwin Chargaff	Analyze base composition of DNA across species	Found A=T and G=C → Chargaff's rules
1952	Hershey & Chase	Confirm the genetic material in viruses	Demonstrated that DNA (not protein) enters host cells in bacteriophages
1953	Watson & Crick (with Franklin & Wilkins)	Discover DNA's 3D structure	Proposed the double helix model using X-ray data
1961	Crick, Brenner, et al.	Decipher the genetic code	Discovered the triplet code (codons) and

			that it is non-overlapping
1970	Smith & Nathans	Study DNA-cutting enzymes	Discovered restriction enzymes and tools for DNA manipulation
1977	Frederick Sanger	Develop DNA sequencing method	Introduced Sanger sequencing and key to genomics
1983	Kary Mullis	Develop a method to amplify DNA	Invented PCR – revolutionized diagnostics and forensics
2003	Human Genome Project	Map all human genes	Completed the first full human genome sequence

I.2.2 Discovery of the DNA structure and its impact

James Watson and Francis Crick's 1953 discovery of the DNA structure is regarded as a major turning point in the history of molecular biology. Their research demonstrated that DNA has a double-helix structure, based on X-ray crystallography data provided by Rosalind Franklin and Maurice Wilkins **(Crouse, 2007).** This discovery not only established DNA as the molecule responsible for genetic inheritance, but also revealed how genetic information could be replicated. The elucidation of DNA's structure gave scientists the tools to study gene expression, replication, and function. This foundational knowledge ultimately led to the development of recombinant DNA technology in the 1970s, along with new methods for manipulating and analyzing DNA in laboratory settings **(Moss, 2019).**

I.2.3 Advancements in genetic sequencing techniques

Sequencing DNA became a major objective in molecular biology after the discovery of DNA structure, Frederick Sanger invented the chain-termination technique commonly referred to as Sanger Sequencing in the late 1970s, this technique made it possible to precisely determine the nucleotide sequence **(Valencia et al., 2013).** The early 2000s saw the emergence of next-generation sequencing (NGS) as a response to the growing need in the word for quicker and more precise techniques. NGS was completed in 2003 which mapped

the whole gene structure of the human genome; this advancement is one of the most transformative changes in the history of molecular biology **(Behjati & Tarpey, 2013).**

Table 5. DNA sequencing over the years **(Heather & Chain, 2016).**

Technology	**Year Introduced**	**Key Features**	**Limitations**	**Advancements Over Previous Methods**
Sanger Sequencing	1977	First widely used method; high accuracy	Slow, labor-intensive, expensive for large genomes	Pioneered DNA sequencing; laid foundation
Automated Sanger Sequencing	1986	Fluorescent dyes, capillary electrophoresis	Still slow for large-scale projects	Higher throughput and automation
Next Generation Sequencing (NGS)	2005	Massive parallel sequencing; high throughput	Short read lengths, complex data analysis	Enabled whole-genome and transcriptome studies
Third Generation Sequencing (e.g. PacBio)	2011	Long reads, real-time sequencing	Higher error rates, expensive	Solved issues of short reads in NGS
Nanopore Sequencing	2014	Portable, real-time long-read sequencing	Moderate accuracy, requires optimization	Field applications; direct RNA sequencing possible

I.2.4 Development of the polymerase chain reaction (PCR)

Kary Mullis introduced a new diagnostic method that revolutionized molecular diagnostic. By using polymerase chain reaction, it has become possible to multiply a small sample of DNA into a quantity large enough for diagnostic purposes. This was previously unfeasible due to the limited sample availability **(Karnath, 2019).** PCR technology has undergone numerous developments over the decades since its first invention in the early 1980s. Special thermal scalers have been used to assist automated reactions. The heat-

resistant Taq polymerase enzyme was first introduced in 1988 **(Newton et al., 1997).** RT-PCR emerged in the mid-1990s as a method to analyze RNA, and in 1996, it evolved into quantitative PCR (qPCR), which allowed real-time monitoring of amplification reactions. In the early 2000s, digital PCR was developed, offering even greater accuracy for quantitative detection and further expanding the applications of PCR in both clinical and research settings **(Clementi et al., 1993).**

I.3 Evolution and foundations of microbiological diagnosis

I.3.1 Historical evolution of microbiological diagnostic Methods

In 1670, Leeuwenhoek observed microorganisms, initiating the study of their link to disease. In 1860, Pasteur confirmed their role in fermentation and some illnesses **(Guardino, 2005).** In the 1880s, Robert Koch developed culture techniques and his four postulates. In 1884, the Gram stain was introduced, enhancing bacterial classification and establishing microscopy as a key diagnostic tool **(Wang et al., 2022).**

In the 20th century, culture techniques advanced with the use of agar, biochemical tests like catalase, oxidase and API strips. Enhancing bacterial identification accuracy in laboratories **(Collard & Collard, 1976).**

Immunodiagnostic techniques like ELISA and agglutination emerged in the 1970s for rapid pathogen detection, followed by the introduction of PCR in 1990, which revolutionized diagnostic accuracy and speed, complementing traditional methods **(Levieux, 2007).**

Table 6. Phases of microbiological diagnostic technique development **(Ivashko et al., 2023).**

Era	Technique	Key Contributions	Limitations	Reason for Evolution
1670s	Simple Microscopy (Leeuwenhoek)	First visual observation of microbes	No link to disease, no identification or culture capability	Need to establish microbial role in disease
1860s	Pasteur's Germ Theory	Linked microbes to fermentation and some diseases	Lacked precise methods to isolate or identify organisms	Prove causation and isolate pathogens
1880s	Koch's Culture Techniques	Isolated specific bacteria and formulated Koch's	Limited to cultivable organisms only	Need to grow microbes for study and

		postulates		verification
1884	Gram Staining	Enabled classification of bacteria into Gram + and -	Cannot identify species or detect non-stainable pathogens	Need for rapid classification before culturing
Early 1900s	Solid Media & Biochemical Tests	Use of agar, catalase/oxidase, API strips for accurate ID	Slow process, variable sensitivity/specificity	Need for more specific, faster identification
1970s	Immunodiagnostic (ELISA, Agglut.)	Rapid detection of microbial antigens/antibodies	Cross-reactivity, sometimes less sensitive	Faster, antigen-based identification
1990s–present	Molecular Techniques (PCR, etc.)	Direct detection of microbial DNA/RNA with high sensitivity and speed	Cost, requires skilled personnel and equipment	Precision, speed, and detection of unculturable or rare pathogens

I.3.2 Transition to molecular-based approaches: context and necessity

Significant challenges emerged with traditional diagnostic methods by the end of the 20th century. These included slow turnaround times, low sensitivity, and difficulties in detecting certain organisms, such as slow-growing bacteria or viruses **(McPartlin & O'Kennedy, 2014).**

This created a demand for faster and more accurate diagnostic tools, especially in cases of acute infections or epidemics.

The ELISA technique improved the speed of diagnosis but it lacks sufficient precision and specificity. Meanwhile, the emergence of molecular biology techniques, supported by major advances in understanding microbial genetic material, provided a solution by enabling rapid and highly accurate diagnoses.

Over time, reliance on PCR technology grew significantly and particularly with the spread of epidemics such as HIV, establishing molecular diagnostics as a foundational pillar of modern precision microbiology **(Olby, 2006).**

II. Molecular biology techniques applied to microbiological diagnosis

II.1 PCR and its variants

II.1.1 Classical PCR

The first invention of the polymerase chain reaction (PCR) was in 1983 by the American scientist Kary Mullis, who developed the technique to amplify specific regions of DNA for laboratories researches, allowing quick and precise detailed studies of genetic molecules **(Bartlett & Stirling, 2003).** PCR is a technique that amplifies multiple copies of a specific DNA segment through repeated enzymatic reaction under differnts thermal cycles, the three-step cycles that implifie DNA are: denaturation of DNA strands, annealing of primers and finaly the extension of the new strand by the enzyme Taq polymerase which have highly temperatures resistant **(Schochetman, Ou, & Jones, 1988).**

In laboratory implementation, the reaction mixture is first prepared, containing the sample DNA, specific primers, Taq polymerase enzyme, free nucleotides (dNTPs), and a reaction buffer. This mixture is then placed in a thermocycler. Multiple thermal cycles are carried out, each consisting of three phases: a denaturation phase at around 94–95°C to separate the DNA strands, an annealing phase at 50–65°C for the primers to bind to specific sites, and an extension phase at 72°C where the enzyme extends the new DNA strand. This cycle is repeated 25 to 40 times to generate an adequate quantity of DNA **(Saiki et al., 1988).**

The PCR diagnostic process begins with taking an appropriate sample, such as blood, biofluid, or swabs. DNA is then extracted from the sample using chemical or physical extraction methods. After extraction, the reaction mixture is prepared with the necessary components. This mixture is placed into a thermocycler to carry out the PCR reaction. Once the reaction is complete, the amplification results are analyzed, often using agarose gel electrophoresis to detect the presence of the target DNA strand. The final results are interpreted based on the presence or absence of the specific DNA strand in the gel electrophoresis **(Valones et al., 2009).**

Classical PCR has played a transformative role in molecular diagnostics by enabling early and precise detection of various pathogens through the amplification of specific genetic regions. In HIV diagnosis, amplification of the gag gene allowed for the early identification of infection (Ou et al., 1988). Amplification of the L1 gene in human papillomavirus (HPV) allowed for the detection of high-risk oncogenic types associated with cervical cancer (Manos et al., 1989)

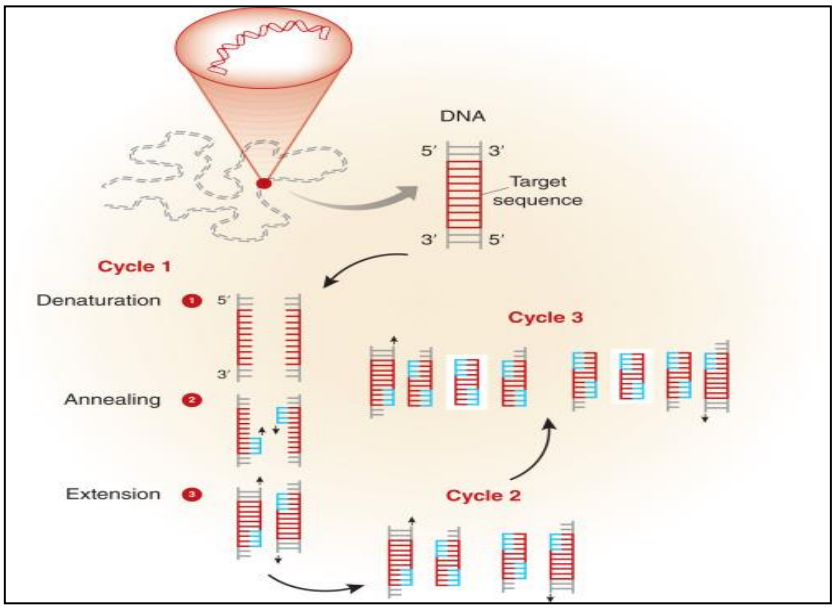

Figure 6. PCR principle and steps **(Garibyan & Avashia, 2013).**

II.1.2 RT-PCR (Reverse transcription PCR)

The first appearance of reverse transcription PCR was following the integration of two key technologies in the late 1980s: reverse transcription and polymerase chain reaction, and quickly was used to diagnose retroviruses like HIV and hepatitis **(Coffin & Fan, 2016).** RT-PCR is used to convert RNA molecules into complementary DNA (cDNA) using the enzyme reverse transcriptase, this cDNA is amplified using PCR thecnique allowing the detection of viruses or microorganisms whose genetic material contains RNA, the technique relies on two main steps: reverse transcription followed by amplification **(Bachman, 2013).**

The technique begins by isolating RNA from the sample using chemicals such as Trizol or specialized extraction kits. Reverse transcription is then performed to convert the RNA into complementary DNA (cDNA). A PCR mixture is prepared containing the cDNA, primers, Taq polymerase, dNTPs, and a buffer. This mixture is then inserted into a thermocycler. Repeated PCR cycles are carried out as in the classical technique as a result, multiple copies of the target cDNA are obtained **(Bustin, 2000).**

The diagnostic process using RT-PCR starts with taking a sample, usually a nasal or throat swab in the case of a respiratory virus. RNA is then extracted from the sample. A reverse transcription step is performed to convert the RNA into complementary DNA (cDNA). The reaction mixture is prepared and placed into a thermocycler to carry out the amplification process. After the reaction, the results are analyzed using a technique such as gel electrophoresis or fluorescence reading, especially if Real-Time RT-PCR is used. The

diagnosis is confirmed based on the presence of amplification of the target gene **(Corman et al., 2020).**

Reverse Transcription PCR (RT-PCR) has enabled significant advancements in the diagnosis of RNA viruses by converting viral RNA into complementary DNA (cDNA) for subsequent amplification. In the case of SARS-CoV, the **N** (nucleocapsid) gene served as a reliable molecular marker for early detection **(Drosten et al., 2003).** During the COVID-19 pandemic, targeting the **E** gene of SARS-CoV-2 enabled rapid and specific diagnosis, with the **RdRp** gene offering an additional highly specific target **(Corman et al., 2020).** Thus, RT-PCR provided a highly sensitive and specific approach to RNA virus detection by focusing on conserved and functionally relevant genomic regions **(Bustin & Nolan, 2004).**

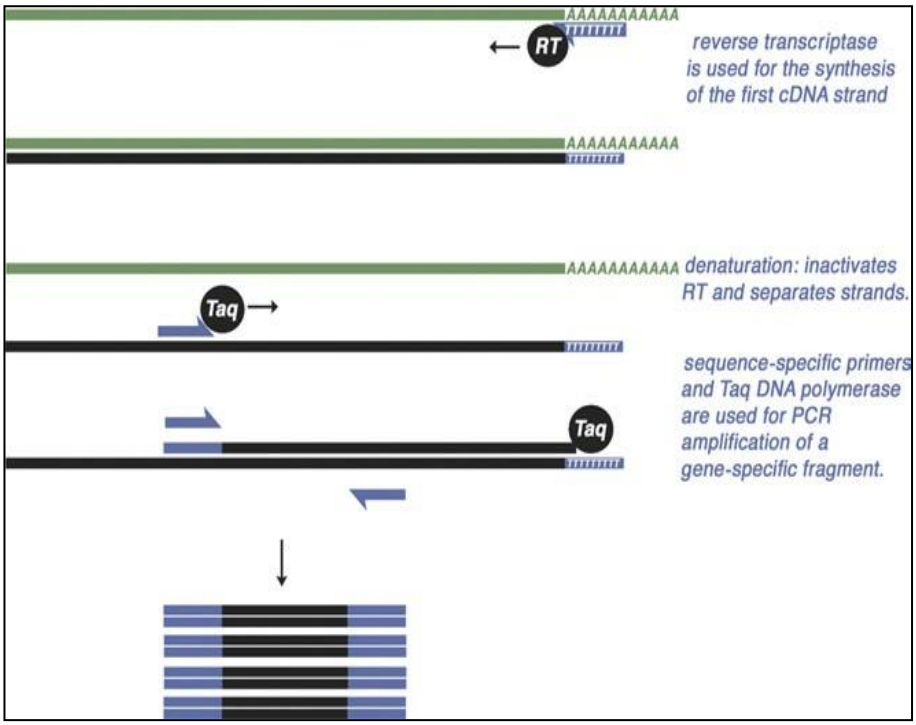

Figure 7. Reverse transcriptase PCR steps **(Fraga, Meulia, & Fenster, 2008).**

II.1.3 Quantitative PCR (qPCR)

Real-time PCR emerged in the late 1990s after the development of PCR techniques, allowing the amount of DNA replicated to be measured in real time during the amplification process **(Jyothy, Sabu, & Dharan, 2020).** The technique precisely amplifies and quantifies the target DNA during the amplification cycle, using fluorescent markers that increases with the quantity of cloned DNA, qPCR relies on the detection of the fluorescence generated during DNA amplification, allowing to determines the amount of genetic material in a sample without the need for subsequent analysis **(Jung, Soondrum, & Neumaier, 2000).**

The technique begins by preparing the reaction mixture, which contains the sample DNA, specific primers, Taq polymerase enzyme, nucleotides (dNTPs), and fluorescent materials such as SYBR Green dye or a special fluorescent probe. This mixture is then loaded into a quantitative PCR machine (real-time PCR machine). The machine is started to perform the amplification cycles, which include denaturation, annealing, and extension. During each cycle, the fluorescence signal is measured, and this signal is directly proportional to the amount of replicating DNA **(Bustin et al., 2009).**

The diagnostic process using quantitative PCR begins with collecting the appropriate sample, such as blood, fluid, or swabs. DNA is then extracted from the sample. A reaction mixture is prepared with fluorescent additives and placed into a specialized device to perform quantitative PCR amplification. Fluorescence measurements are monitored and recorded in real time during the amplification process. The results are then analyzed to accurately quantify the target DNA, which can confirm the presence of a microbial pathogen or determine the microbial load in the sample **(Yang & Rothman, 2004).**

Quantitative PCR (qPCR) brought a major breakthrough in molecular diagnostics by enabling not only detection but also quantification of pathogen load through real-time monitoring of amplification. In HIV diagnostics, quantification of the **gag** or **pol** genes allowed clinicians to accurately assess viral load and monitor treatment efficacy over time **(Lin et al., 2024).** For Mycobacterium tuberculosis, qPCR targeting **IS6110** provided rapid estimation of bacterial burden in pulmonary and extrapulmonary samples **(Sánchez-Carvajal et al., 2021).** In cytomegalovirus (CMV) infections, amplification of the **UL83** gene offered a sensitive method for monitoring viral reactivation in immunocompromised patient **(Razonable et al., 2020).** In the case of Epstein-Barr virus (EBV), the **BamHI-W** region was quantified to evaluate the risk of post-transplant lymphoproliferative disorders **(Buonavoglia et al., 2021).** In hepatitis B, quantification of the S gene allowed precise tracking of viral replication **(Erken et al., 2022). S**imilarly, in hepatitis C virus, quantification of the 5′UTR region facilitated monitoring of therapeutic response **(Ampoot et al., 2025).**

Moreover, in SARS-CoV-2, real-time PCR targeting the **N** and **ORF1ab** genes became the gold standard for both diagnosis and viral load estimation, especially during patient follow-up **(Corman et al., 2020).** Through precise quantification of specific genetic targets, qPCR has become essential for diagnosis and treatment monitoring in infectious diseases.

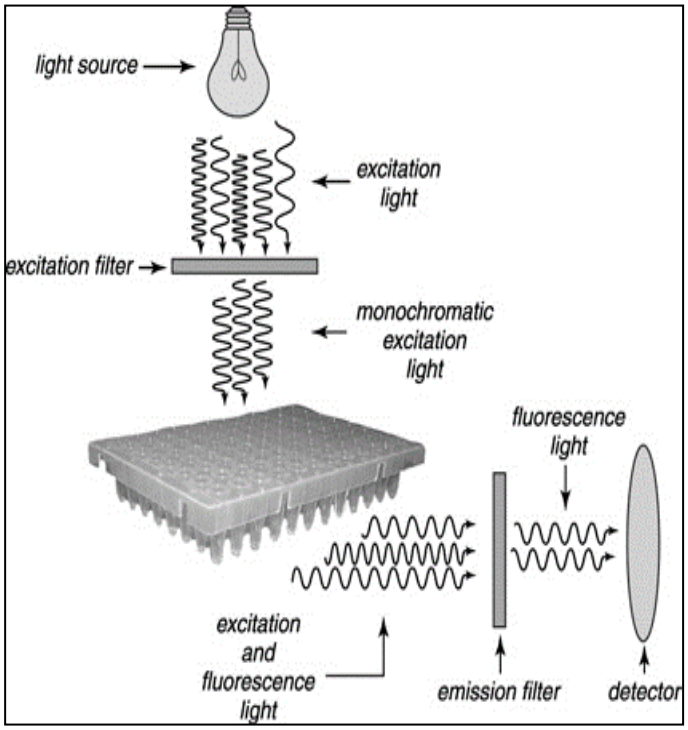

Figure 8. Detection of fluorescence in RT-PCR thermal cycler **(Fraga, Meulia, & Fenster, 2008).**

II.1.4 LAMP (Loop-mediated isothermal amplification)

A research group led by Notomi et al devoloped the LAMP technique in in the year 2000, the goal was to create a fast and simple alternative to PCR that could be applied without a thermocycler, it was used to diagnose parasitic diseases in the beginign but eventualy expanded to include a wide range of microbes, especially in areas lacking advanced laboratory facilities **(Gellrich & Schmidt, 2013).** LAMP is a method for isothermal amplification of DNA, it means that at a constant temperature (usually between 60-65°C) without the need for temperature changes like in PCR, the technique relies on the use of 4 to 6 specific primers that recognize 6 to 8 different sites on the target gene, along with a DNA polymerase enzyme with strand displacement activity, the principle is based on generating a large number of DNA copies at high speed and repeatedly, resulting in rapid and visible accumulation of DNA **(Wong, Othman, Lau, Radu, & Chee, 2018).**

The technique begins by preparing the reaction mixture, which contains the template DNA (either DNA or cDNA in the case of RNA viruses), a set of 4 to 6 specific primers, DNA polymerase enzyme, nucleotides (dNTPs), and a visual indicator such as SYBR Green or a visual turbidity indicator. The mixture is then incubated at a constant temperature, typically around 63°C, for 30 to 60 minutes. The appearance of the amplification product is

determined by observing a color change, measuring turbidity, or detecting fluorescence **(Notomi et al., 2000).**

The diagnostic process using LAMP begins with collecting samples such as urine, throat swab, or blood. DNA or RNA is then extracted from the sample. In the case of RNA pathogens, reverse transcriptase is used to convert the RNA into cDNA. A reaction mixture is prepared and added into a tube. The reaction tube is then incubated at a constant temperature of approximately 63°C. After the reaction is complete, the result is checked directly by observing a color change or using a simple fluorescence reading device, providing results in less than an hour **(Tanner & Evans, 2014).**

LAMP technology has provided a rapid and highly specific alternative for nucleic acid detection, especially in low-resource settings due to its isothermal nature. In malaria, amplification of the **18S rRNA** gene allowed species-specific identification of Plasmodium parasites, crucial in endemic regions **(Chen et al., 2021).** In the context of Zika virus, targeting the **NS5** gene through LAMP facilitated field-level detection during outbreaks **(Kaarj & Yoon, 2023).** In SARS-CoV-2 diagnostics, amplification of the **N** and **ORF1ab** genes offered a fast and sensitive alternative to qPCR, especially useful for decentralized testing **(Lu et al., 2020).** For Listeria monocytogenes, detection of the **hlyA** gene allowed for foodborne pathogen identification with minimal equipment **(Fiore et al., 2023).** Thus, LAMP has extended molecular diagnostics beyond central laboratories, enabling effective detection of pathogens by targeting conserved and functionally significant genomic sequences.

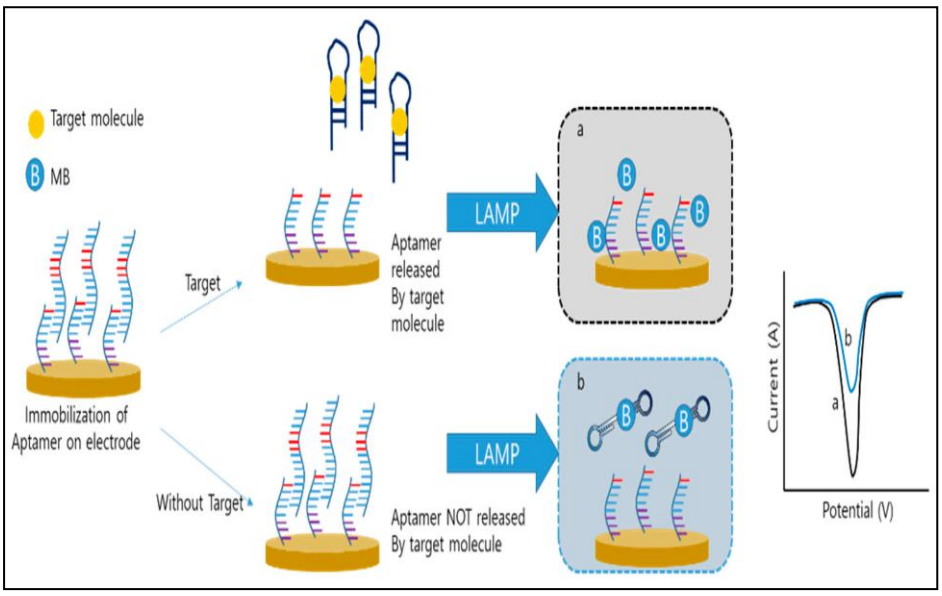

Figure 9. Electrochemical detection of small molecule with LAMP **(Park, 2022).**

II.1.5 Advantages and limitations of PCR and its variants

PCR-based techniques (classical PCR, RT-PCR, qPCR, and LAMP) offer high sensitivity and specificity for nucleic acid detection, with qPCR and LAMP enabling rapid and accurate diagnostics. However, they share limitations such as contamination risk, complex primer design, and high costs for equipment or reagents especially in qPCR and LAMP **(Zhu et al., 2020).**

II.2 DNA and whole genome sequencing (WGS)

II.2.1 Sanger sequencing

The chain termination method or sanger sequencing was developed in 1977 by the british scientist Frederick Sanger and his team, he won a second Nobel Prize in Chemistry for devolping this technique, which was the base of the Human Genome Project that began in the 1990s and considered the first accurate large-scale DNA sequencing technology **(Valencia et al., 2013).** The technique amplifies DNA using a polymerase enzyme, with the incorporation of modified nucleotides known as dideoxynucleotides, they are nucleotides that lack a 3' hydroxyl group causing the expansion to stop when it is inserted. each ddNTP is labeled with a different fluorescent dye (in modern versions), allowing to identifie the last nucleotide base in each of the replicated DNA fragments **(Men, Wilson, Siemering, & Forrest, 2008).**

The technique begins by preparing the reaction mixture, which contains the DNA template to be sequenced, one primer, DNA polymerase enzyme, four dNTPs (A, T, C, G), and four ddNTPs, each labeled with a different color. The replication reaction takes place in a thermocycler, where ddNTPs are randomly incorporated, producing DNA fragments of varying lengths, each ending with a ddNTP. These reaction products are then passed through a capillary electrophoresis device, where the fragments are separated by size, and the fluorescent signal of each fragment is read. The signals are then converted into a letter sequence representing the original DNA sequence **(Prober et al., 1987).**

The diagnostic process using Sanger sequencing begins by taking a biological sample, such as blood or a swab from the patient. DNA is then extracted from the sample. A specific gene or gene segment is selected for testing, and a suitable primer is prepared. The Sanger sequencing reaction is performed using the prepared reaction mixture. After the reaction is complete, the resulting products are separated, and the fluorescent signals are analyzed using a sequencing device. The obtained sequence is then compared with a reference database to detect mutations or accurately identify the pathogen **(Furutani et al., 2022).**

Sanger sequencing has played a foundational role in molecular diagnostics by enabling precise identification of nucleotide-level variations in pathogenic genomes. In HIV, sequencing of the **pol** gene allowed for the detection of resistance mutations to antiretroviral drugs, shaping personalized treatment approaches **(Shafer et al., 2007).** For Mycobacterium tuberculosis, sequencing of the **rpoB** gene was pivotal in identifying rifampicin resistance, which is a marker of multidrug-resistant TB **(Telenti et al., 1993).** In hepatitis C virus (HCV), sequencing the **NS5B** region enabled accurate genotyping, critical for guiding antiviral therapy **(Simmonds et al., 2005).** For HPV, full sequencing of the **L1** gene provided precise classification of viral genotypes and differentiation between high-risk and low-risk strains **(Bernard et al., 1994).** In SARS-CoV-2, early sequencing of the **S** gene was essential in identifying variants with altered infectivity and immune escape profiles **(Korber et al., 2020).** Additionally, in hereditary diseases caused by infectious triggers, Sanger sequencing of host susceptibility genes such as **IFNGR1** has been used to understand predispositions to severe infections **(Jouanguy et al., 1997).** Through high-fidelity reading of genetic code, Sanger sequencing has enabled definitive molecular diagnosis and mutation tracking in both pathogens and host genomes.

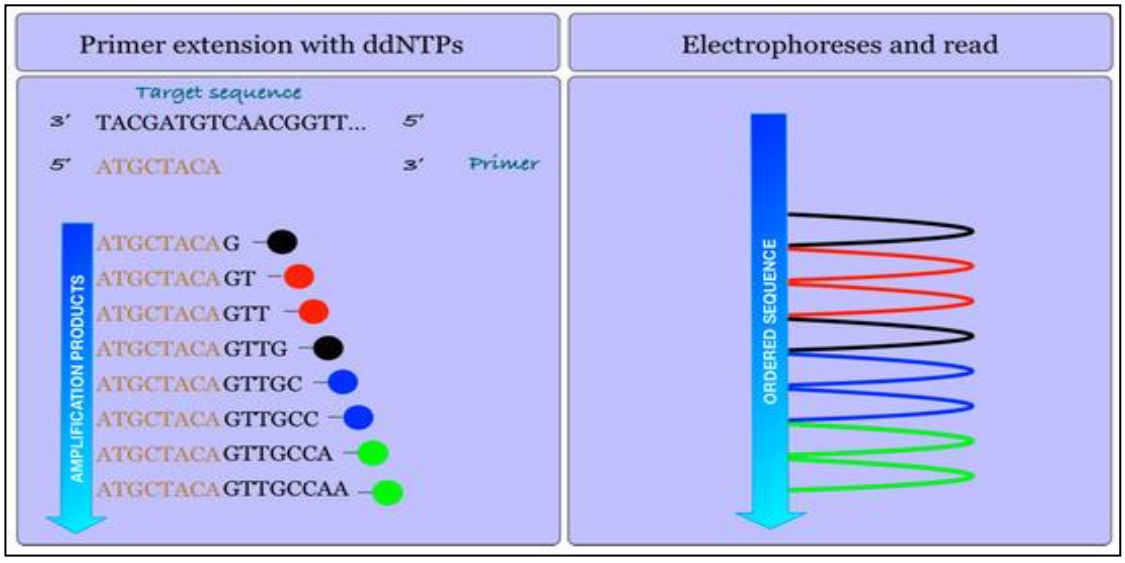

Figure 10. The sanger dideoxy sequencing method **(Garrido-Cardenas et al., 2017).**

II.2.2 Next generation sequencing

High-throughput sequencing technologies emerged in 2005, responding to the need to sequence large quantities of DNA quickly and at a lower cost compared to Sanger sequencing, the 454 Life Sciences platform was the first commercial NGS technology, after that more accurate and efficient technologies emerged such as Illumina, SOLiD and Ion Torrent that played a key role in accelerating projects like the sequencing of the human genome,

metagenomic studies and the diagnosis of infectious diseases **(Kulski, 2016).** NGS is a set of platforms and technologies that sequence millions to billions of DNA molecules in parallel, rather than one piece at a time as in Sanger sequencing, each technology has its own mechanism but the general principle is: Dividing DNA into small fragments, Attaching the fragments to a surface or small beads, replicating each fragment at its site to form clusters and sequencing each fragment in parallel using techniques such as sequencing by synthesis or hybridization and sensing **(Hu, Chitnis, Monos, & Dinh, 2021).**

The technique begins by cutting DNA into small fragments using enzymes or ultrasonic waves. Adapters are then added to the ends of these fragments to prepare them for binding to a surface or beads. The fragments are immobilized onto a chip surface, such as a flow cell on the Illumina platform, or onto beads, as in the 454 system. Local amplification is performed to form colonies of identical fragments. The reaction is then initiated by adding nucleotide bases labeled with detectable dyes. After each cycle, an image is taken to identify the added base for each fragment based on the color of the signal. This process continues for several cycles until the full sequence of each fragment is read **(Metzker, 2010).**

The diagnostic process using Next-Generation Sequencing (NGS) starts with collecting a sample from the patient, such as blood, tissue, or a swab. DNA or RNA is then extracted from the sample, with RNA being converted to cDNA if necessary. A sequencing library is prepared by cleaving the molecules and adding linkers. This library is loaded onto the sequencing platform, where the sequencing reaction is run. After completion, the biological data is analyzed using specialized software to extract sequences and compare them to a reference database. The results are used to detect mutations, accurately identify pathogens, or analyze the microbiome **(Goodwin et al., 2016).**

Next-Generation Sequencing (NGS) revolutionized molecular diagnostics by enabling comprehensive, high-throughput sequencing of entire genomes or targeted regions simultaneously.

This allowed for rapid identification of pathogen variants and mixed infections with unprecedented resolution. In infectious diseases, NGS facilitated detection of minority drug-resistant mutations in HIV by sequencing the **pol** gene, significantly improving personalized therapy **(Ji et al., 2020).** For tuberculosis, whole-genome sequencing uncovered complex resistance patterns in Mycobacterium tuberculosis, targeting multiple loci including **rpoB** and **katG (Walker et al., 2015).**

NGS also enabled full genomic characterization of hepatitis viruses, allowing distinction of genotypes and subtypes via regions like **S** and **NS5B (Garcia-Garcia et al., 2021).** During the COVID-19 pandemic, sequencing of the full SARS-CoV-2 genome, especially the **S** gene, tracked the emergence and spread of variants of concern **(Chiara et al., 2021).** Moreover, NGS supports metagenomic approaches that identify novel or unexpected pathogens by sequencing all genetic material in clinical samples **(Wilson et al., 2019).** Thus, NGS transformed diagnostics by providing detailed genetic insights critical for disease management and outbreak control.

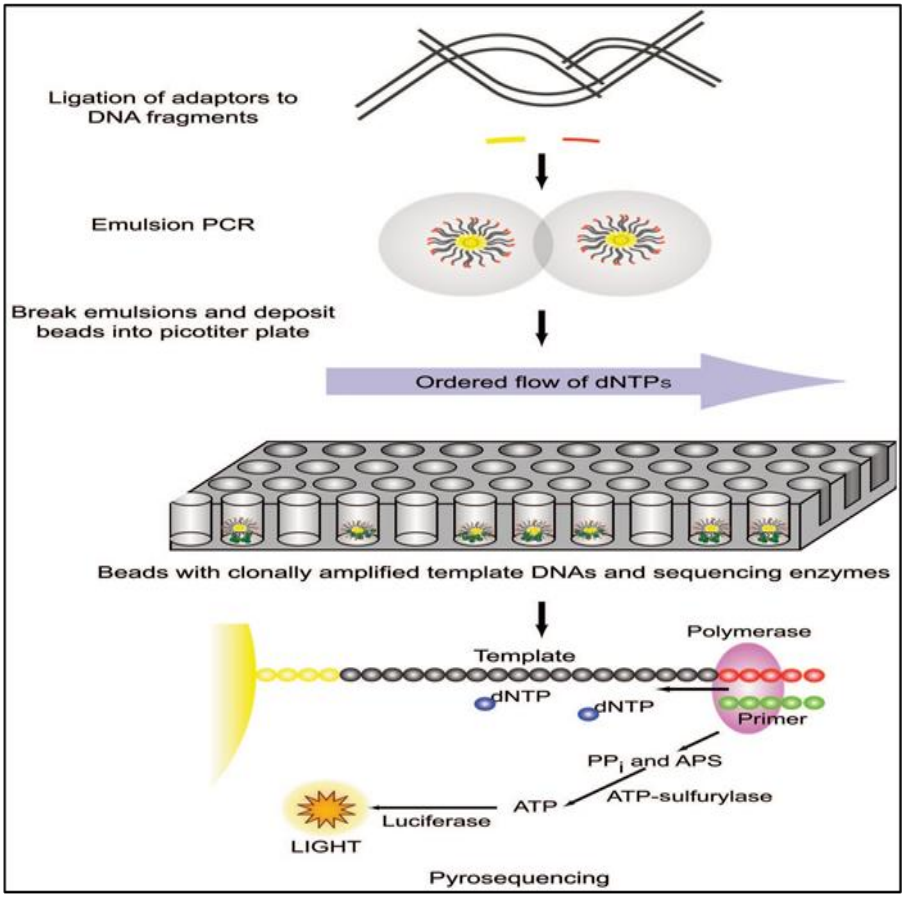

Figure 11. NGS sequencing **(Voelkerding et al., 2009).**

II.2.3 Advantages and limitations of Sanger and NGS sequencing

Sanger sequencing and NGS are essential tools for DNA analysis. Sanger offers high accuracy for short sequences, while NGS enables massive parallel sequencing, allowing whole genomes to be analyzed rapidly and cost-effectively.

However, Sanger is time-consuming and low-throughput, whereas NGS requires complex data analysis, expensive equipment, and can suffer from higher error rates in repetitive regions **(Dey, 2023).**

II.3 FISH (Fluorescence in Situ Hybridization)

FISH was developed in the early 1970s, but it was not widely used as a reliable source of diagnostics and genetics until the 1980s, its previous use was limited to identifying genes on chromosomes, over time it has become an important tool in diagnosing genetic diseases and cancers and directly detecting microorganisms in biological samples analyzed in laboratories **(Huber, Von Voithenberg, & Kaigala, 2018).** This technique uses short probes of DNA or RNA labeled with fluorescent dyes, which bind to a specific gene sequence on chromosomes or in cells, the probe then hybridizes with the target sequence inside the cell, where the presence of the fluorescent signal is detected using a fluorescence microscope, allowing the precise location of the gene or pathogen to be visualized **(Volpi & Bridger, 2008).** The principle of the technology is based on the property of molecular hybridization, meaning that the probes specifically integrate with their complementary sequence only, giving the technology high accuracy in identifying targets **(Liehr, 2017).**

The laboratory implementation of the technique begins with sample preparation by mounting cells, such as blood cells or biopsies, on a glass slide. The genetic material is then

fixed using a chemical fixative like formaldehyde to preserve the cellular structure. DNA denaturation follows, where the DNA strands within the cells are opened through heat or chemical treatment to allow the probes to bind. Fluorescent probes, which contain sequences complementary to the target and are labeled with fluorescent dyes, are then added. Hybridization is carried out by incubating the membrane under specific conditions to allow the probes to bind to the complementary sequence. Unbound probes are washed away to prevent non-specific background signals. Finally, fluorescence microscopy is used to detect and localize the fluorescent signal using special filters, indicating the presence of the target sequence **(Pinkel et al., 1986).**

The diagnostic process using FISH begins with collecting a sample, such as infected tissue, blood cells, or bone marrow. The sample is then mounted and prepared on a slide. Appropriate fluorescent probes are applied depending on the diagnostic purpose—whether to detect a specific gene or microorganism. After hybridization, the slide is washed to remove unbound probes.

The results are then examined under a fluorescence microscope: the presence of a fluorescent signal indicates the presence of the target gene or microorganism, while the absence of a signal indicates that the target sequence is not present. These results are used to

identify gene mutations, chromosomal rearrangements, or to detect specific pathogens **(Speicher & Carter, 2005).**

Fluorescence In Situ Hybridization (FISH) has enabled precise, spatially-resolved detection of nucleic acid sequences directly within cells or tissues, making it invaluable for identifying chromosomal and pathogen-specific DNA or RNA. In bacterial diagnostics, FISH targeting the **16S rRNA** gene allowed rapid species-level identification of pathogens in blood cultures, bypassing lengthy cultivation steps **(Amann, Ludwig, & Schleifer, 1995).** In Mycobacterium tuberculosis, detection of **rRNA** sequences directly in sputum provided a fast and specific alternative for TB diagnosis **(Kaattari et al., 2006).** For Chlamydia trachomatis and Neisseria gonorrhoeae, FISH probes targeting unique genomic regions enabled simultaneous visualization and differentiation in clinical samples **(Poppert et al., 2002).** In viral diagnostics, FISH has been used to detect EBV-encoded RNAs **(EBERs)** in tissue biopsies, serving as a key diagnostic marker for EBV-associated cancers **(Weiss et al., 1991).** Additionally, in cytogenetic applications, pathogen-induced chromosomal abnormalities can be identified through FISH targeting specific loci, aiding in the diagnosis of virus-induced malignancies **(Moter & Göbel, 2000).** Thus, FISH contributes to both pathogen detection and understanding host-pathogen genomic interactions at the cellular level.

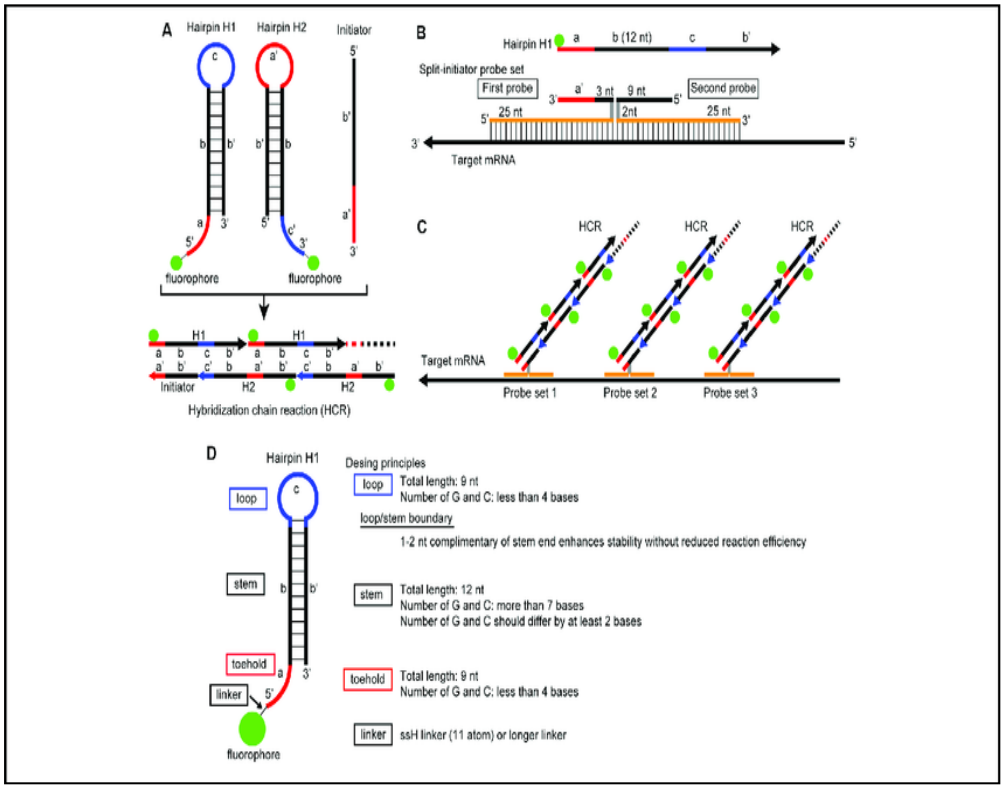

Figure 12. The principles of FISH **(Veselinyová et al., 2021).**

II.3.1 Advantages and Limitations of Fluorescence In Situ Hybridization

Fluorescence In Situ Hybridization (FISH) is a robust cytogenetic method that enables the visualization of specific DNA sequences within chromosomes or cells with high spatial resolution. It offers rapid and precise detection of genetic abnormalities, chromosomal rearrangements, and microbial identification without the need for DNA extraction. However, FISH has limitations including relatively low sensitivity compared to PCR-based methods, dependence on high-quality probes, and limited multiplexing capacity. Additionally, it requires specialized fluorescence microscopy equipment and skilled personnel, which can restrict its accessibility in some laboratories **(Leung et al., 2022).**

II.4 Metagenomics and microbiome analysis

The term metagenomics emerged in the early 1990s, but its use was not widespread until the early 2000s due to the development of high-throughput sequencing (NGS) technologies, this technology was first widely applied to analyze bacterial communities in soil and water, and later to analyze the human gut microbiome revolutionizing our understanding of the relationship between microbes and human health **(Olm et al., 2017).** Metagenomics is the study and analysis of genetic material collected from entire microbial communities taken from an environmental sample without the need to isolate or culture the microorganisms, it is used in microbiological diagnostics to identify the identity and diversity of microbes in a given sample, especially when the organisms are not culturable in the laboratory, the principle is based on sequencing specific DNA fragments (usually the 16S rRNA gene for bacteria or ITS for fungi) or sequencing the entire genome of the sample **(Sleator, Shortall, & Hill, 2008).**

In metagenomics, the first technique involves the amplification of genes such as the 16S rRNA gene. This starts with extracting DNA from an environmental sample like feces, saliva, or body fluids. Primers targeting the bacterial 16S rRNA gene are then used to amplify the sequence through PCR. The PCR product is sequenced using a next-generation sequencing (NGS) platform, followed by bioinformatic data analysis to identify the microbial taxa present. The second approach is targeted gene sequencing, which begins with DNA extraction. Primers are designed specifically for the target gene, such as those related to resistance or virulence. PCR is performed for the gene of interest, and the resulting fragment is sequenced. The sequence is then analyzed and compared to a database to determine its type or function **(Quince et al., 2017).**

The diagnostic steps using metagenomics begin with collecting a biological sample, such as body fluid or clinical specimens. Total DNA is then extracted from the sample. Marker genes like 16S rRNA are amplified, unless comprehensive sequencing is used, in which case amplification may be skipped. Sequencing is performed using next-generation sequencing (NGS) technology. The resulting data is analyzed bioinformatically to identify microbial species and estimate their relative proportions. Finally, the results are interpreted in relation to clinical indicators to identify the pathogen or to understand the microbiome balance **(Chiu & Miller, 2019).**

Table 7. Clinical applications of 16S rRNA and ITS-based metagenomics in infectious disease diagnostics **(Jobin et al., 2019).**

Clinical Area / Condition	Type of Analysis	Benefit / Diagnostic Insight
Polymicrobial sepsis or meningitis	16S rRNA sequencing	Detection of unexpected or fastidious bacterial pathogens, including anaerobes and unculturable species.
Pulmonary infections in immunocompromised patients	16S profiling of BAL samples	Identification of mixed bacterial populations to guide targeted antibiotic therapy.
Invasive fungal infections	ITS-based fungal profiling	Accurate diagnosis of infections by species such as *Aspergillus* or *Candida*, even when culture is negative.
Rare or emerging infections	Metagenomic analysis	Discovery of novel pathogens by aligning unknown sequences to reference databases.
General infectious disease diagnostics	16S and ITS molecular signatures	Development of microbiome-based, culture-independent diagnostic tools.

II.5 Advanced molecular techniques

II.5.1 CRISPR-Cas

CRISPR sequences were first discovered in 1987 in E. coli bacteria, without further investigation due to the limitations of technology at the time, However their role in bacterial immune defense was discovered in 2005, and seven years later Jennifer Doudna and Emmanuelle Charpentier published the first practical application of gene editing using the CRISPR-Cas9 system revolutionizing molecular biology, CRISPR has subsequently been used in microbiological diagnostics since 2016 with tools such as SHERLOCK and DETECTR **(Ishino, Krupovic, & Forterre, 2018).** CRISPR-Cas relies on a natural mechanism in bacteria that cuts foreign DNA, such as viral DNA, for diagnosis a Cas enzyme (such as Cas9, Cas12, or Cas13) is programmed to identify a specific DNA or RNA sequence in a sample, upon recognition a signal (fluorescent or otherwise) is released indicating the presence of a pathogen **(Hille & Charpentier, 2016).**

The technique steps begin by designing a guide RNA (gRNA) that targets a specific DNA or RNA sequence. This gRNA is then mixed with the appropriate Cas enzyme. The resulting mixture is added to a biological sample. When the target sequence is present and matches the gRNA, the Cas enzyme cuts the genetic material, releasing a detectable signal **(Gootenberg et al., 2017).**

For the diagnostic steps using CRISPR, the process starts with sample collection, such as blood, sputum, or urine. DNA or RNA is then extracted from the sample. Depending on the protocol, the target sequence may be amplified. A CRISPR-Cas system is then prepared using a suitable gRNA and applied to the sample. The presence of the pathogen is determined by detecting the resulting signal **(Broughton et al., 2020).**

CRISPR-Cas systems have emerged as highly specific molecular diagnostics by exploiting guide RNA-directed recognition of nucleic acid sequences, enabling rapid, low-cost detection of infectious agents. In SARS-CoV-2 diagnosis, **CRISPR-Cas12** and **Cas13-based** assays targeted conserved regions such as the ***N*** and ***ORF1ab*** genes, providing sensitive detection within an hour, even in low-resource settings **(Xiong et al., 2020).** In tuberculosis, CRISPR-based diagnostics have been developed to identify *Mycobacterium tuberculosis* by detecting the ***IS6110*** insertion element with high specificity **(Ai et al., 2019).** For human papillomavirus (HPV), detection of ***E6*** and ***E7*** oncogenes using CRISPR tools allowed accurate typing of high-risk strains associated with cervical cancer **(Yoshiba et al.,**

2019). In antimicrobial resistance diagnostics, CRISPR-Cas13 was used to identify resistance-associated mutations in the ***blaNDM-1*** and ***mecA*** genes, associated with carbapenem and methicillin resistance, respectively **(Cao et al., 2024).** Additionally, in Zika and Dengue virus infections, Cas13 assays enabled discrimination between closely related flaviviruses by targeting the ***NS1*** gene **(Myhrvold et al., 2018).** These pathogen and resistance specific genetic signatures made CRISPR-Cas technologies a transformative tool in rapid, point-of-care molecular diagnostics **(Gootenberg et al., 2017).**

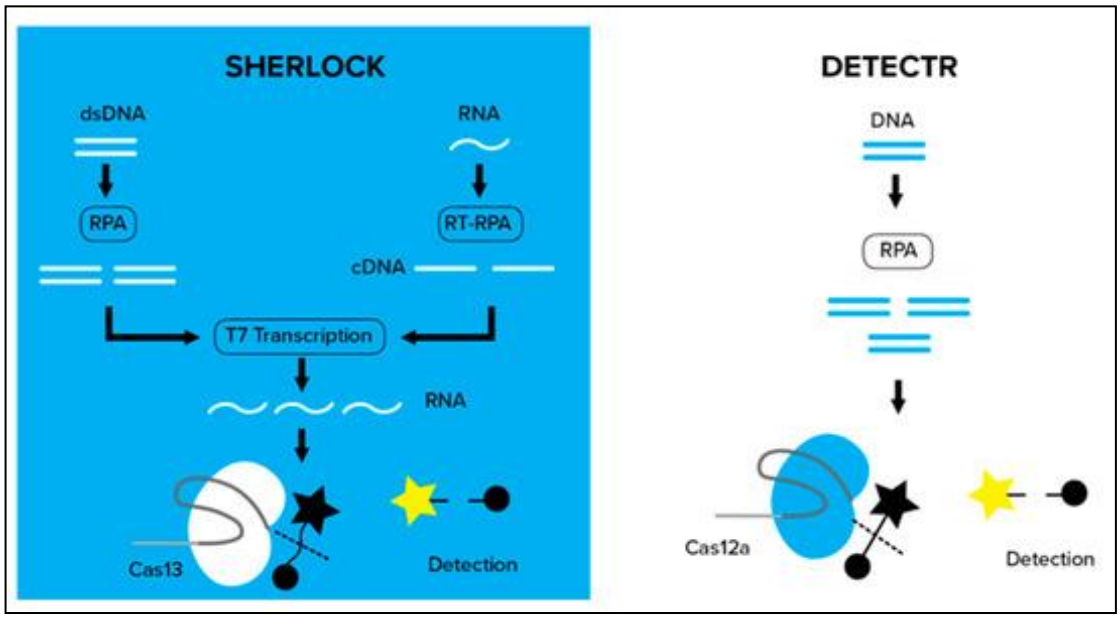

Figure 13. SHERLOCK and DETECTR **(Sauvagère & Siatka, 2023).**

II.5.2 Nanotechnology

Research into nanotechnology began in the 1960s, but its first practical applications in medical science were at the beginning of the 2000s, when nanoparticles (such as gold and silver) were used for biological diagnosis through the development of nanoprobes that detect pathogens **(Tolochko, 2009).** This technique relies on the use of nanoparticles (less than 100 nanometers) that are able to interact with microbial genetic sequences or proteins, and exhibit a change in properties (such as color or radiation) when bound to the pathogen **(McNeil, 2005).**

The technique steps start with preparing metallic or polymeric nanoparticles, then modifying their surfaces to make them specific for a target antigen or gene sequence. These functionalized nanoparticles are then integrated with the sample, and any resulting optical change or signal is monitored **(Giljohann et al., 2020).**

For the diagnostic steps using nanotechnology, a biological sample is first collected and incorporated into the functional nanoparticles. After waiting for a period of time to allow the target to react, detection is performed visually or through an indicative method, such as a clear color change. The resulting signal is then interpreted to determine the presence of the pathogen **(Dreaden et al., 2012).**

Nanotechnology has advanced molecular diagnostics by enabling ultra-sensitive detection of nucleic acids through engineered nanoparticles and nanostructures. In viral infections such as SARS-CoV-2, gold nanoparticles functionalized with probes targeting the **N** gene allowed colorimetric detection of viral RNA without amplification, achieving high specificity at low viral loads **(Moitra et al., 2020).** In tuberculosis, magnetic nanoparticle-based assays were developed to capture and detect **IS6110** DNA fragments directly from sputum, significantly reducing diagnostic time **(Kaittanis et al., 2007).** For HIV, quantum dot-labeled probes targeting the **gag** gene enabled single-molecule level detection and viral load estimation in complex samples **(Zhang et al., 2005).** Additionally, in antimicrobial resistance surveillance, nanobiosensors detecting resistance genes like **blaKPC**, **mecA**, and **vanA** provided quick and multiplexed identification of resistant pathogens **(Baranwal et al., 2022).** These nanoscale platforms offered precise, portable, and amplification-free alternatives to conventional molecular tools, enhancing early detection and point-of-care diagnostics in clinical microbiology.

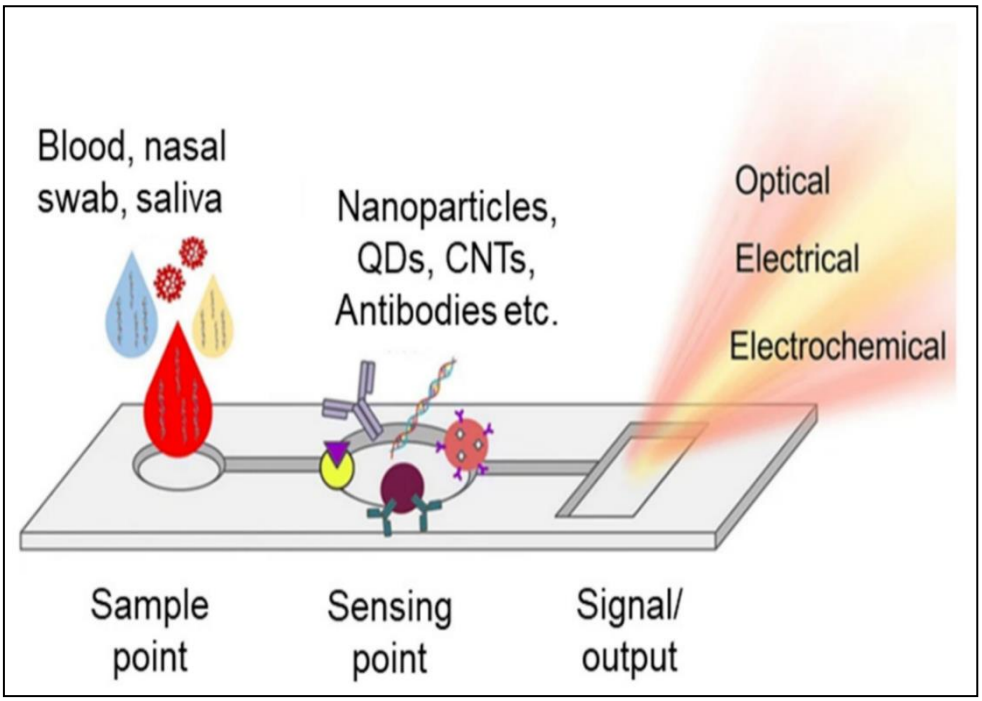

Figure 14. Nanotechnology diagnostic steps **(Thwala et al., 2023).**

II.5.3 Microarray

Microarray technology was developed in the 1990s, and its first application was in the detection of gene expression polymorphisms, and it was later used to identify mutations and gene sequences specific to pathogens **(Ewis et al., 2005).** This technique relies on small plates containing thousands of tiny probes attached to a solid surface, each carrying a known DNA sequence, when a sample containing the target DNA or RNA is added hybridization occurs, and a fluorescent signal is detected to identify the pathogen **(Choudhuri, 2004).**

The technique steps begin with designing a microarray that contains hundreds or thousands of probes. DNA is then extracted from the sample and labeled with a fluorescent dye. This labeled DNA is applied to the array, where it hybridizes with the complementary probes. After hybridization, the slide is scanned, and the signals are analyzed **(Schena et al., 1995).**

For the diagnostic steps using microarray, a biological sample is collected, and DNA or RNA is extracted. The extracted genetic material is labeled with a fluorescent dye and applied to a microarray chip. The fluorescent signal is then read using a special scanner, and the data is analyzed to identify the pathogen **(Wilson et al., 2002).**

Microarray technology enabled the parallel detection of thousands of nucleic acid sequences, making it a powerful tool for identifying pathogens and resistance genes in a single assay. In respiratory infections, microarrays targeting conserved regions of the ***hemagglutinin*** and ***neuraminidase*** genes facilitated subtype identification of influenza viruses, including emergent pandemic strains **(Kessler et al., 2004).**

In bacterial diagnostics, arrays detecting the ***16S rRNA*** gene alongside virulence factors such as ***stx1***, ***stx2***, and ***eae*** enabled rapid differentiation of Shiga-toxin producing *Escherichia coli* strains **(Call, Borucki, & Loge, 2003).** For *Mycobacterium tuberculosis*, hybridization to probes covering mutations in the ***rpoB***, ***katG***, and ***inhA*** genes allowed simultaneous detection of rifampicin and isoniazid resistance, revolutionizing the diagnosis of multidrug-resistant TB **(Mikhailovich et al., 2001).**

In HIV and HCV genotyping, microarrays targeting sequence variations in the ***env***, ***gag***, or ***NS5B*** genes provided high-throughput subtyping essential for guiding antiviral therapy **(Wang et al., 2002).** Additionally, in nosocomial infection surveillance, microarrays

detecting a panel of resistance genes including ***blaTEM***, ***mecA***, and ***vanA*** facilitated early identification of multi-resistant pathogens **(Perreten et al., 2005).**

This multiplexed, sequence-specific hybridization platform brought significant advancements to clinical diagnostics through its capacity to detect multiple genetic targets simultaneously **(Schena et al., 1995).**

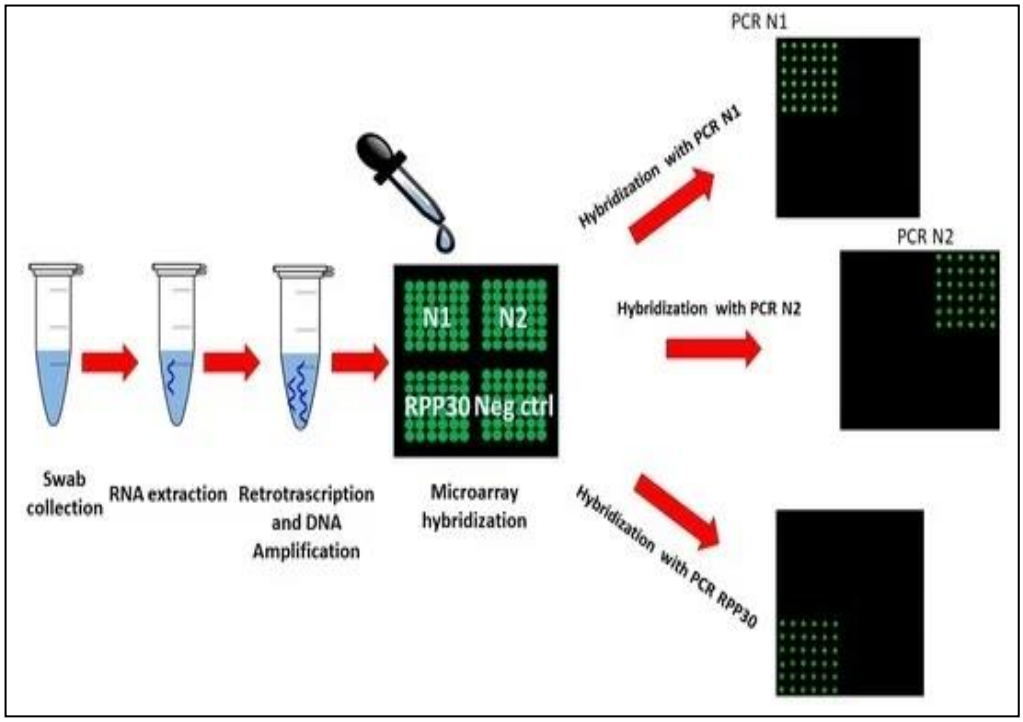

Figure 15. Microarrays diagnostic steps **(Damin et al., 2021).**

II.5.4 Advanced Molecular Techniques: advantages and limitations

CRISPR-Cas, nanotechnology, and microarray platforms represent cutting edge tools in molecular diagnostics, offering high specificity, multiplexing capability, and potential for miniaturization and point-of-care use. These technologies allow rapid and comprehensive genetic analysis. However, they face challenges such as off-target effects (in CRISPR), complex data interpretation (in microarrays), and scalability or biocompatibility issues (in nanotechnology), which limit routine clinical adoption **(Wang et al., 2021).**

II.6 Proteomics and antigen detection

II.6.1 Chromatography-based techniques

Mikhail Tsvet developed the technique in 1906, but its use in molecular biology and protein analysis did not occur until the middle of the twentieth century using techniques like HPLC **(Weil & Williams, 1951).** Chromatography technique principale is separating

molecules like proteins or antigens according to their physical and chemical properties, special columns containing stable materials can be used to separate components according to their passage speed **(Smith, 2013).**

The technique steps involve preparing the chromatography column, then introducing the biological sample into it. The sample is passed through the column under specific conditions, allowing the components to separate based on their interaction with the fixed material inside the column. The separated components are then detected using measuring devices such as UV or fluorescence detectors **(Snyder et al., 2011).**

In the diagnostic steps, a sample such as blood serum is collected and prepared by filtering out impurities. The prepared sample is then passed through a chromatography machine to isolate antigens or proteins specific to the pathogen. Finally, the results are analyzed to determine the presence of the pathogen **(Hage et al., 2012).**

Chromatography techniques, especially when coupled with mass spectrometry or immunodetection systems, have contributed to molecular diagnostics by enabling precise separation and identification of nucleic acid derivatives, microbial metabolites, and molecular markers of infection.

In viral hepatitis, liquid chromatography combined with mass spectrometry **(LC-MS)** was employed to detect viral peptides and specific nucleoside analogs from the ***HBV*** and ***HCV*** genomes, enabling accurate viral genotyping and treatment monitoring **(Lu et al., 2015).**

In bacterial infections, gas chromatography-mass spectrometry **(GC-MS)** was used to identify unique fatty acid methyl esters **(FAME)** or volatile organic compounds **(VOCs)** produced by pathogens such as ***Mycobacterium tuberculosis***, serving as metabolic biomarkers linked to specific genomic pathways **(Butler & Guthertz, 2001).**

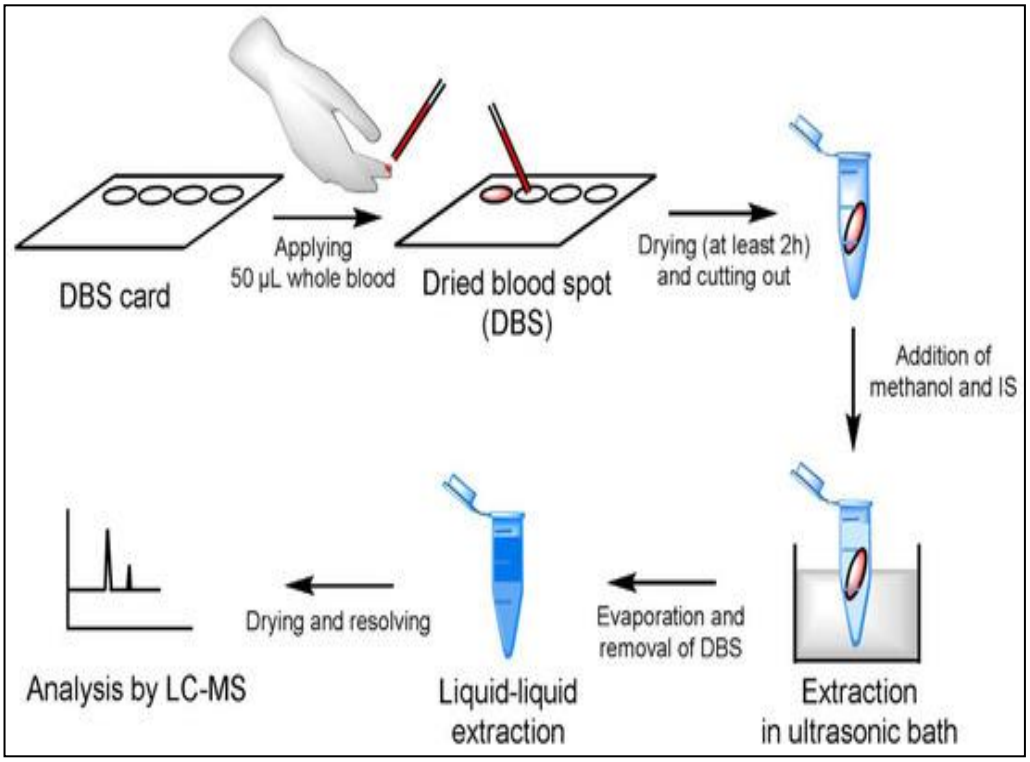

Figure 16. DBS extraction and sample purification workflow **(Câmara et al., 2022).**

II.6.2 Immunofluorescence

The technique was developed in the 1940s and was first used in 1942 by Albert Coons, since then it has been used to detect antigens and viruses within cells or tissues **(Coons, 1961).** Immunofluorescence technique relies on the use of antibodies labeled with a fluorescent dye that bind to specific antigens within the sample, when exposed to a specific light they emit a visible light signal under a fluorescence microscope **(Schutzbank, McGuire, & Scholl, 2009).**

The technique steps start with preparing a slide containing cells or a sample, followed by the application of a fluorescently labeled antibody. After allowing binding, the slide is washed to remove any non-specific interactions. The sample is then mounted and observed under a fluorescence microscope **(Im et al., 2019).**

For the diagnostic steps, a biological sample is first collected—such as cerebrospinal fluid, a swab, or tissue. An antibody specific to the target pathogen is applied to the sample. The presence of the pathogen is detected through the emitted light observed under the microscope, and the results are interpreted based on the fluorescence pattern **(Arbache et al., 2014).**

Immunofluorescence has been widely used in molecular diagnostics to detect pathogen-specific antigens or host cell markers with high spatial resolution. In respiratory infections, direct fluorescent antibody tests targeting the ***nucleoprotein*** of influenza and respiratory syncytial viruses enabled rapid detection of viral antigens in epithelial cells **(Kuypers et al., 2006).** In parasitic diagnostics, immunofluorescence assays using **probes** against ***Cryptosporidium*** and ***Giardia*** surface antigens provided specific identification of protozoa in stool samples, correlating with molecular signatures encoded by species-specific genes **(Tobie & Coatney, 1961).** In autoimmune diagnostics, indirect immunofluorescence was applied to detect autoantibodies against nuclear antigens such as **dsDNA** and ***Sm*** proteins, associated with systemic lupus erythematosus, thereby linking antibody presence to genes involved in nuclear structure and regulation **(Chen, Chen, & Yu, 2025).** Furthermore, in sexually transmitted infections, fluorescent **probes** against ***Chlamydia trachomatis*** major outer membrane protein **(MOMP)**, encoded by the ***ompA*** gene, facilitated direct detection in clinical specimens **(Salari & Ward, 1979).**

These applications underscore the utility of immunofluorescence as a bridge between protein expression and underlying genetic markers in pathogen detection **(Stadler et al., 2013).**

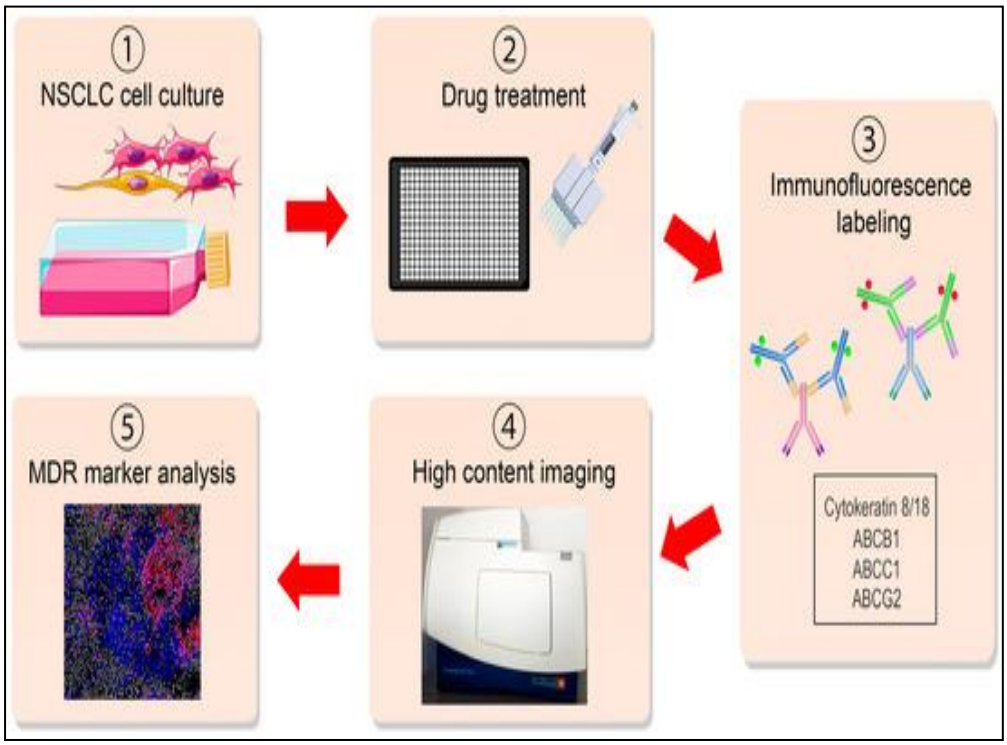

Figure 17. Immunofluorescence assay for MDR in NSCLC cells **(Dinić et al., 2023).**

II.6.3 Western Blot

Harry Towbin developed the technique in 1979, and it began to be used directly in virology and immunology to identify specific proteins like antibodies or antigens **(Sule, Rivera, & Gomes, 2023).** Western blot is based on separating proteins according to molecular weight using electrophoresis (SDS-PAGE), then transferring them to a membrane and identifying the target protein using a specific antibody **(Mahmood & Yang, 2012).**

The technique steps begin with extracting protein from the sample, followed by separating the proteins using gel electrophoresis. The separated proteins are then transferred from the gel onto a membrane through a process called blotting. A primary antibody specific to the target protein is applied, followed by a secondary antibody that is labeled with an enzyme. Afterward, a substrate is added to produce a visible signal indicating the presence of the target **(Taylor & Posch, 2014)**.

For the diagnostic steps, a biological sample is first collected, and proteins are isolated from it. The Western blot technique is then performed as previously described. The appearance of a band indicates the presence of an antigen or antibody, and the location and intensity of the band are analyzed to identify the pathogen **(Zöller, Cremer, & Faulde, 1993).** Western blot has been instrumental in molecular diagnostics by enabling the detection of pathogen-specific proteins that are direct products of gene expression. In HIV diagnosis, it was used to confirm the presence of antibodies against viral proteins such as **gp120**, **gp41**, and **p24**, each encoded by key HIV genes **(env, gag).** This test served as the gold standard for confirmatory HIV testing for decades **(Jackson et al., 1990).**

In Lyme disease, Western blot detection of antibodies against Borrelia burgdorferi proteins such as **OspC** and **p41** products of bacterial **ospC** and **flaB** genes provided essential diagnostic specificity in late-stage infection **(Rose et al., 1991).**

For hepatitis C, antibodies targeting structural and non-structural proteins, particularly core, **NS3**, and **NS5A**, were detected to confirm infection and guide molecular subtyping, directly reflecting expression from the HCV genome **(Yeh et al., 1994).** Western blot also supported research into **prion** diseases through detection of misfolded **PrP** proteins, which are translated from the host **PRNP** gene **(Nicholson et al., 2007).** These applications link protein-level detection directly to gene expression, making Western blot a crucial bridge between genotype and phenotype in molecular diagnostics.

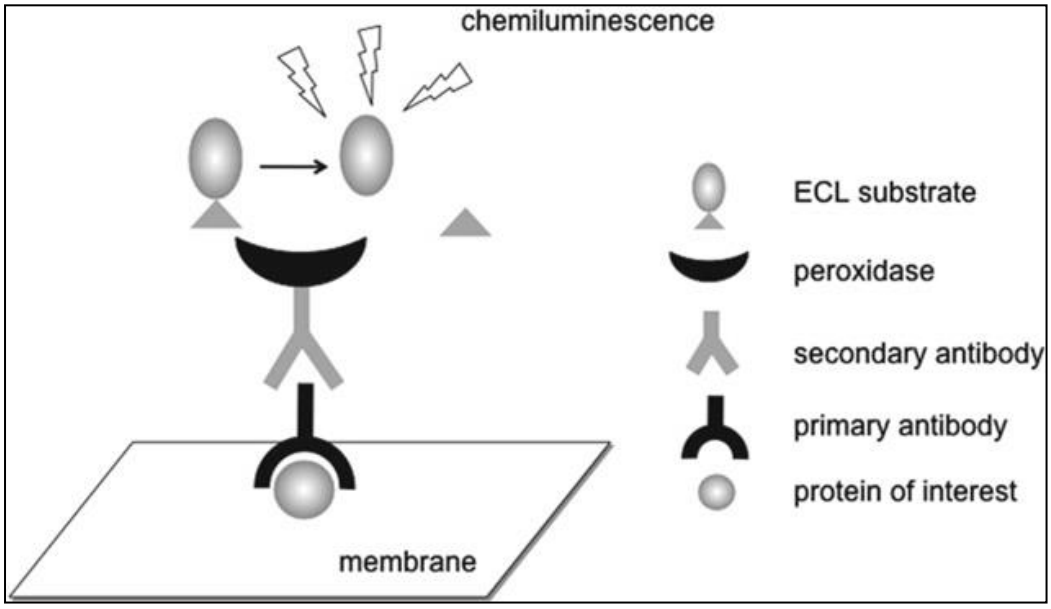

Figure 18. Detection of proteins on Western blot membrane **(Hirano, 2012).**

II.6.4 Advantages and limitations of proteomics and antigen detection techniques

Chromatography-based methods, immunofluorescence, and Western blot are key tools for protein separation, localization, and identification, offering high specificity and structural insight. These techniques are widely used in both research and diagnostics. However, they require skilled operation, are time-consuming, and may lack sensitivity for low-abundance targets, especially in complex biological samples **(Goldis et al., 2023).**

Conclusion

The dynamic evolution of molecular microbiological diagnostics has significantly advanced the precision, speed, and depth of pathogen detection. However, as highlighted in the preceding discussion, current diagnostic tools, despite their individual strengths, often operate in isolated frameworks that limit their accessibility, integration, and scalability especially in resource-constrained settings. The future of this field lies not in the competition among techniques but in their convergence. A hybrid diagnostic model that strategically combines the amplification power of PCR, the field-ready simplicity of LAMP, the genomic accuracy of NGS and CRISPR systems, and the functional insight of proteomic assays could offer unparalleled diagnostic efficiency.

Artificial intelligence is poised to play a transformative role by enabling adaptive, automated decision-making based on real-time data interpretation and epidemiological trends. This evolution will not only streamline diagnostic workflows but also reduce reliance on specialized expertise and costly infrastructure. Moreover, prioritizing affordability and portability is crucial to ensure equitable access to these innovations, particularly in low-income regions where infectious diseases remain a major public health threat.

In this context, the envisioned next-generation platforms compact, intelligent, and cost-effective represent more than technical advancements; they embody a paradigm shift toward inclusive, globalized precision diagnostics. As we confront future outbreaks and antimicrobial resistance challenges, integrating these technologies into public health strategies will be vital. Ultimately, the transition from fragmented methodologies to unified, smart, and accessible systems marks the essential path forward in molecular microbiological diagnostics.

Bibliographic references

Ahlquist, P. (2002). RNA-dependent RNA polymerases, viruses, and RNA silencing. Science, 296(5571), 1270-1273.

Ai, J. W., Zhou, X., Xu, T., Yang, M., Chen, Y., He, G. Q., ... & Zhang, W. H. (2019). CRISPR-based rapid and ultra-sensitive diagnostic test for Mycobacterium tuberculosis. Emerging microbes & infections, 8(1), 1361-1369.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2014). Molecular biology of the cell (6th ed.). Garland Science.

Amann, R. I., Ludwig, W., & Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological reviews, 59(1), 143-169.

Ampoot, W., Chujan, S., Kiattanaphon, A., & Sistayanarain, A. (2025). A diagnostic target in the 3'untranslated region of hepatitis C virus genome. Molecular Biology Reports, 52(1), 1-5.

Arbache, S. T., Nogueira, T. G., Delgado, L., Miyamoto, D., & Aoki, V. (2014). Immunofluorescence testing in the diagnosis of autoimmune blistering diseases: overview of 10-year experience. Anais brasileiros de dermatologia, 89(6), 885-889.

Aslam, B., Basit, M., Nisar, M. A., Khurshid, M., & Rasool, M. H. (2016). Proteomics: technologies and their applications. Journal of chromatographic science, 1-15.

Bachman, J. (2013). Reverse-transcription PCR (rt-PCR). In Methods in enzymology (Vol. 530, pp. 67-74). Academic Press.

Baranwal, A., Aralappanavar, V. K., Behera, B. K., Bansal, V., & Shukla, R. (2022). Current approaches and prospects of nanomaterials in rapid diagnosis of antimicrobial resistance. Nano-Strategies for Addressing Antimicrobial Resistance: Nano-Diagnostics, Nano-Carriers, and Nano-Antimicrobials, 75-108.

Bartlett, J. M., & Stirling, D. (2003). A short history of the polymerase chain reaction. PCR protocols, 3-6.

Behjati, S., & Tarpey, P. S. (2013). What is next generation sequencing?. Archives of Disease in Childhood-Education and Practice, 98(6), 236-238.

Bernard, H. U., Chan, S. Y., Manos, M. M., Ong, C. K., Villa, L. L., Delius, H., ... & Wheeler, C. M. (1994). Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. Journal of Infectious Diseases, 170(5), 1077-1085.

Brosius, J., & Raabe, C. A. (2016). What is an RNA? A top layer for RNA classification. RNA biology, 13(2), 140-144.

Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., ... & Chiu, C. Y. (2020). CRISPR–Cas12-based detection of SARS-CoV-2. Nature biotechnology, 38(7), 870-874.

Buonavoglia, A., Leone, P., Prete, M., Solimando, A. G., Guastadisegno, C., Lanave, G., ... & Racanelli, V. (2021). Epstein–Barr Virus in Salivary samples from systemic Lupus Erythematosus patients with oral lesions. Journal of Clinical Medicine, 10(21), 4995.

Bustin, S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. Journal of Molecular Endocrinology, 25(2), 169-193.

Bustin, S. A., & Nolan, T. (2004). Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. Journal of Biomolecular Techniques, 15(3), 155-166.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., ... & Wittwer, C. T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry, 55(4), 611-622.

Butler, W. R., & Guthertz, L. S. (2001). Mycolic acid analysis by high-performance liquid chromatography for identification of Mycobacterium species. Clinical microbiology reviews, 14(4), 704-726.

Call, D. R., Borucki, M. K., & Loge, F. J. (2003). Detection of bacterial pathogens in environmental samples using DNA microarrays. Journal of microbiological methods, 53(2), 235-243.

Câmara, J. S., Martins, C., Pereira, J. A., Perestrelo, R., & Rocha, S. M. (2022). Chromatographic-based platforms as new avenues for scientific progress and sustainability. Molecules, 27(16), 5267.

Cao, Y., Tian, Y., Huang, J., Xu, L., Fan, Z., Pan, Z., ... & Ren, F. (2024). CRISPR/Cas13-assisted carbapenem-resistant Klebsiella pneumoniae detection. Journal of Microbiology, Immunology and Infection, 57(1), 118-127.

Cardenas, K. S. M. (2021). Molecular methods for the detection of infectious diseases: bringing diagnostics to the point-of-care (Doctoral dissertation, Imperial College London).

Chen, J., Chen, M., & Yu, X. (2025). Fluorescent probes in autoimmune disease research: current status and future prospects. Journal of Translational Medicine, 23(1), 411.

Chen, X., Zhang, J., Pan, M., Qin, Y., Zhao, H., Qin, P., ... & Yang, Z. (2021). Loop-mediated isothermal amplification (LAMP) assays targeting 18S ribosomal RNA genes for identifying P. vivax and P. ovale species and mitochondrial DNA for detecting the genus Plasmodium. Parasites & vectors, 14(1), 278.

Chiara, M., D'Erchia, A. M., Gissi, C., Manzari, C., Parisi, A., Resta, N., ... & Pesole, G. (2021). Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. Briefings in Bioinformatics, 22(2), 616-630.

Chiu, C. Y., & Miller, S. A. (2019). Clinical metagenomics. Nature Reviews Genetics, 20(6), 341-355.

Chiu, R. W., Lo, Y. D., & Wittwer, C. T. (2015). Molecular diagnostics: a revolution in progress.

Choudhuri, S. (2004). Microarrays in biology and medicine. Journal of biochemical and molecular toxicology, 18(4), 171-179.

Clementi, M., Menzo, S., Bagnarelli, P., Manzin, A., Valenza, A., & Varaldo, P. E. (1993). Quantitative PCR and RT-PCR in virology. PCR methods and applications, 2, 191-191.

Cobb, M. (2017). 60 years ago, Francis Crick changed the logic of biology. PLoS biology, 15(9), e2003243.

Coffin, J. M., & Fan, H. (2016). The discovery of reverse transcriptase. Annual review of virology, 3(1), 29-51.

Collard, P. J., & Collard, P. (1976). The development of microbiology. CUP Archive.

Conn, G. L., & Draper, D. E. (1998). RNA structure. Current opinion in structural biology, 8(3), 278-285.

Coons, A. H. (1961). The beginnings of immunofluorescence. The Journal of Immunology, 87(5), 499-503.

Copley, S. D., Smith, E., & Morowitz, H. J. (2007). The origin of the RNA world: co-evolution of genes and metabolism. Bioorganic chemistry, 35(6), 430-443.

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., ... & Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3), 2000045.

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., ... & Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3), 2000045.

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., ... & Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3), 2000045.

Crouse, D. T. (2007). X-ray diffraction and the discovery of the structure of DNA. A tutorial and historical account of James Watson and Francis Crick's use of x-ray diffraction in their discovery of the double helix structure of DNA. Journal of Chemical Education, 84(5), 803.

Damin, F., Galbiati, S., Gagliardi, S., Cereda, C., Dragoni, F., Fenizia, C., ... & Chiari, M. (2021). CovidArray: a microarray-based assay with high sensitivity for the detection of Sars-Cov-2 in nasopharyngeal swabs. Sensors, 21(7), 2490.

Dey, P. (2023). Sanger sequencing and next generation gene sequencing: Basic principles and applications in pathology. In Basic and advanced laboratory techniques in histopathology and cytology (pp. 247-261). Singapore: Springer Nature Singapore.

Dinić, J., Podolski-Renić, A., Dragoj, M., Jovanović Stojanov, S., Stepanović, A., Lupšić, E., ... & Pešić, M. (2023). Immunofluorescence-based assay for high-throughput analysis of multidrug resistance markers in non-small cell lung carcinoma patient-derived cells. Diagnostics, 13(24), 3617.

Dreaden, E. C., Alkilany, A. M., Huang, X., Murphy, C. J., & El-Sayed, M. A. (2012). The golden age: gold nanoparticles for biomedicine. Chemical Society Reviews, 41(7), 2740-2779.

Drosten, C., Günther, S., Preiser, W., Van Der Werf, S., Brodt, H. R., Becker, S., ... & Doerr, H. W. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. New England Journal of Medicine, 348(20), 1967-1976.

Ellis, T. N., Hofer, J. M., Timmerman-Vaughan, G. M., Coyne, C. J., & Hellens, R. P. (2011). Mendel, 150 years on. Trends in plant science, 16(11), 590-596.

Erken, R., Loukachov, V., van Dort, K., van den Hurk, A., Takkenberg, R. B., de Niet, A., ... & Kootstra, N. (2022). Quantified integrated hepatitis B virus is related to viral activity in patients with chronic hepatitis B. Hepatology, 76(1), 196-206.

Ewis, A. A., Zhelev, Z., Bakalova, R., Fukuoka, S., Shinohara, Y., Ishikawa, M., & Baba, Y. (2005). A history of microarrays in biomedicine. Expert review of molecular diagnostics, 5(3), 315-328.

Fiore, A., Treglia, I., Ciccaglioni, G., Ortoffi, M. F., & Gattuso, A. (2023). Application of a Loop-Mediated Isothermal Amplification (LAMP) assay for the detection of listeria monocytogenes in cooked ham. Foods, 12(1), 193.

Fraga, D., Meulia, T., & Fenster, S. (2008). Real-time PCR. Current protocols essential laboratory techniques, (1), 10-3.

Fraga, D., Meulia, T., & Fenster, S. (2008). Real-time PCR. Current protocols essential laboratory techniques, (1), 10-3.

Furutani, S., Furutani, N., Kawai, Y., Nakayama, A., & Nagai, H. (2022). Rapid DNA sequencing technology based on the sanger method for bacterial identification. Sensors, 22(6), 2130.

Gannon, F. (2002). Molecular biology—what's in a name?. EMBO reports.

Garcia-Garcia, S., Cortese, M. F., Rodriguez-Algarra, F., Tabernero, D., Rando-Segura, A., Quer, J., ... & Rodriguez-Frias, F. (2021). Next-generation sequencing for the diagnosis of hepatitis B: current status and future prospects. Expert Review of Molecular Diagnostics, 21(4), 381-396.

Garibyan, L., & Avashia, N. (2013). Polymerase chain reaction. Journal of investigative dermatology, 133(3), 1-4.

Garrido-Cardenas, J. A., Garcia-Maroto, F., Alvarez-Bermejo, J. A., & Manzano-Agugliaro, F. (2017). DNA sequencing sensors: an overview. Sensors, 17(3), 588.

Gellrich, M. M., Schmidt, D., & Gellrich, M. M. (2013). History of the slit lamp. In The Slit Lamp: Applications for Biomicroscopy and Videography (pp. 189-210). Berlin, Heidelberg: Springer Berlin Heidelberg.

Giljohann, D. A., Seferos, D. S., Daniel, W. L., Massich, M. D., Patel, P. C., & Mirkin, C. A. (2020). Gold nanoparticles for biology and medicine. Spherical nucleic acids, 55-90.

Goldis, R., Kaplan, B., Kukuy, O., Arad, M., Magen, H., Shavit-Stein, E., ... & Livneh, A. (2023). Diagnostic challenges and solutions in systemic amyloidosis. International Journal of Molecular Sciences, 24(5), 4655.

Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. Nature Reviews Genetics, 17(6), 333-351.

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., ... & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. Science, 356(6336), 438-442.

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., ... & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. Science, 356(6336), 438-442.

Guardino, R. F. (2005). Early history of microbiology and microbiological methods. Encyclopedia of rapid microbiological methods, 1, 1-27.

Hage, D. S., Anguizola, J. A., Bi, C., Li, R., Matsuda, R., Papastavros, E., ... & Zheng, X. (2012). Pharmaceutical and biomedical applications of affinity chromatography: recent trends and developments. Journal of pharmaceutical and biomedical analysis, 69, 93-105.

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. Genomics, 107(1), 1-8.

Hille, F., & Charpentier, E. (2016). CRISPR-Cas: biology, mechanisms and relevance. Philosophical transactions of the royal society B: biological sciences, 371(1707), 20150496.

Hirano, S. (2012). Western blot analysis. Nanotoxicity: methods and protocols, 87-97.

Houpikian, P., & Raoult, D. (2002). Traditional and molecular techniques for the study of emerging bacterial diseases: one laboratory's perspective. Emerging infectious diseases, 8(2), 122.

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. Human immunology, 82(11), 801-811.

Huber, D., Von Voithenberg, L. V., & Kaigala, G. V. (2018). Fluorescence in situ hybridization (FISH): History, limitations and what to expect from micro-scale FISH?. Micro and Nano Engineering, 1, 15-24.

Hutchins, J. R. (2014). What's that gene (or protein)? Online resources for exploring functions of genes, transcripts, and proteins. Molecular Biology of the Cell, 25(8), 1187-1201.

Im, K., Mareninov, S., Diaz, M. F. P., & Yong, W. H. (2019). An introduction to performing immunofluorescence staining (pp. 299-311). Springer New York.

Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. Journal of bacteriology, 200(7), 10-1128.

Ivashko, M., Burmei, S., Yusko, L., Chaikovska, T., & Boyko, N. (2023). Microbiological diagnostics: From traditional to molecular genetic methods: A literature review.

Jackson, J. B., MacDonald, K. L., Cadwell, J., Sullivan, C., Kline, W. E., Hanson, M., ... & Osterholm, M. T. (1990). Absence of HIV infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. New England Journal of Medicine, 322(4), 217-222.

Ji, H., Enns, E., Brumme, C. J., Parkin, N., Howison, M., Lee, E. R., ... & Paredes, R. (2020). Deep sequencing reveals rare HIV-1 drug resistance mutations at low frequencies in treatment-naive individuals. Journal of Antimicrobial Chemotherapy, 75(5), 1305-1313.

Jobin, J., Veeraraghavan, B., & Vasudevan, K. (2019). Metagenomic next-generation sequencing in clinical microbiology. Indian journal of medical microbiology, 37(2), 133-140.

Jouanguy, E., Lamhamedi-Cherradi, S., Altare, F., Fondanèche, M. C., Tuerlinckx, D., Blanche, S., ... & Casanova, J. L. (1997). Partial interferon-γ receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guérin infection and a sibling with clinical tuberculosis. Journal of Clinical Investigation, 100(11), 2658-2664.

Jung, R., Soondrum, K., & Neumaier, M. (2000). Quantitative pcr.

Jyothy, A., Sabu, S. T., & Dharan, S. S. (2020). REAL-TIME PCR OR QUANTITATIVE PCR (QPCR)–A REVOLUTION IN MODERN SCIENCE. World Journal of Pharmaceutical Research, 9(11), 249-264.

Kaarj, K., & Yoon, J. Y. (2023). Loop-Mediated Isothermal Amplification on Paper Microfluidic Chips for Highly Sensitive and Specific Zika Virus Detection Using Smartphone. In Clinical Applications of Nucleic Acid Amplification (pp. 307-323). New York, NY: Springer US.

Kaattari, I. M., Rhodes, M. W., Kaattari, S. L., & Shotts, E. B. (2006). The evolving story of Mycobacterium tuberculosis clade members detected in fish. Journal of Fish Diseases, 29(9), 509-520.

Kaittanis, C., Naser, S. A., & Perez, J. M. (2007). One-step, nanoparticle-mediated bacterial detection with magnetic relaxation. Nano Letters, 7(2), 380-383.

Karnath, L. (2019). Remembering Dr. Kary Mullis—Inventor of PCR. Molecular Frontiers Journal, 3(02), 101-102.

Kaushik, S., & Singh, A. (2023). Biomolecules: From Genes to Proteins. Walter de Gruyter GmbH & Co KG.

Kessler, N., Ferraris, O., Palmer, K., Marsh, W., & Steel, A. (2004). Use of the DNA flow-thru chip, a three-dimensional biochip, for typing and subtyping of influenza viruses. Journal of clinical microbiology, 42(5), 2173-2185.

Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., ... & Montefiori, D. C. (2020). Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell, 182(4), 812-827.

Kornberg, A., & Baker, T. A. (2005). DNA replication. University Science Books.

Krause, M. (1995). Transcription and translation. Methods in Cell Biology, 48, 483-512.

Kulski, J. (2016). Next-generation sequencing—an overview of the history, tools, and “Omic” applications. Next generation sequencing-advances, applications and challenges, 3-60.

Kuypers, J., Wright, N., Ferrenberg, J., Huang, M. L., Cent, A., Corey, L., & Morrow, R. (2006). Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. Journal of clinical microbiology, 44(7), 2382-2388.

Leavitt, S. (2004). Deciphering the genetic code: Marshall Nirenberg. Office of NIH History.

Leung, H. Y., Yeung, M. H. Y., Leung, W. T., Wong, K. H., Tang, W. Y., Cho, W. C. S., ... & Wong, S. C. C. (2022). The current and future applications of in situ hybridization technologies in anatomical pathology. Expert Review of Molecular Diagnostics, 22(1), 5-18.

Levieux, D. (2007). Immunodiagnostic Technology and its Applications. Advances in Food Diagnostics, 211-227.

Liehr, T. (2017). Fluorescence in situ hybridization (FISH). Springer Berlin Heidelberg.

Lin, B., Han, C., Li, J. H., & Wang, R. (2024). Evaluation of the performance of a qPCR-based assay for HIV-1 viral load determination. PloS one, 19(12), e0315579.

Liu, S., Li, B., Liang, Q., Liu, A., Qu, L., & Yang, J. (2020). Classification and function of RNA–protein interactions. Wiley Interdisciplinary Reviews: RNA, 11(6), e1601.

Lu, R., Wu, X., Wan, Z., Li, Y., Zuo, L., Qin, J., ... & Zhang, C. (2020). Development of a novel reverse transcription loop-mediated isothermal amplification method for rapid detection of SARS-CoV-2. Virologica Sinica, 35(3), 344-347.

Lu, Y., Huang, C., Gao, L., Xu, Y. J., Chia, S. E., Chen, S., ... & Ong, C. N. (2015). Identification of serum biomarkers associated with hepatitis B virus-related hepatocellular carcinoma and liver cirrhosis using mass-spectrometry-based metabolomics. Metabolomics, 11, 1526-1538.

Mahmood, T., & Yang, P. C. (2012). Western blot: technique, theory, and trouble shooting. North American journal of medical sciences, 4(9), 429.

Manos, M. M., Ting, Y., Wright, D. K., Lewis, A. J., Broker, T. R., & Wolinsky, S. M. (1989). Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells, 7(1), 209-214.

McNeil, S. E. (2005). Nanotechnology for the biologist. Journal of leukocyte biology, 78(3), 585-594.

McPartlin, D. A., & O'Kennedy, R. J. (2014). Point-of-care diagnostics, a major opportunity for change in traditional diagnostic approaches: Potential and limitations. Expert review of molecular diagnostics, 14(8), 979-998.

Men, A. E., Wilson, P., Siemering, K., & Forrest, S. (2008). Sanger DNA sequencing. Next Generation Genome Sequencing: Towards Personalized Medicine, 1-11.

Metzker, M. L. (2010). Sequencing technologies—the next generation. Nature Reviews Genetics, 11(1), 31-46.

Miege, M. N. (1982). Protein types and distribution. In Nucleic Acids and Proteins in Plants I: Structure, Biochemistry and Physiology of Proteins (pp. 291-345). Berlin, Heidelberg: Springer Berlin Heidelberg.

Mikhailovich, V., Lapa, S., Gryadunov, D., Sobolev, A., Strizhkov, B., Chernyh, N., ... & Mirzabekov, A. (2001). Identification of rifampin-resistant Mycobacterium tuberculosis strains by hybridization, PCR, and ligase detection reaction on oligonucleotide microchips. Journal of clinical microbiology, 39(7), 2531-2540.

Moitra, P., Alafeef, M., Dighe, K., Frieman, M. B., & Pan, D. (2020). Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles. ACS nano, 14(6), 7617-7627.

Morange, M. (2000). A history of molecular biology. Harvard University Press.

Morange, M. (2021). A history of biology. Princeton University Press.

Moss, P. (2019). History and development of molecular biology. Molecular hematology, 353-362.

Moss, P. (2019). History and development of molecular biology. Molecular hematology, 353-362.

Moter, A., & Göbel, U. B. (2000). Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. Journal of microbiological methods, 41(2), 85-112.

Myhrvold, C., Freije, C. A., Gootenberg, J. S., Abudayyeh, O. O., Metsky, H. C., Durbin, A. F., ... & Sabeti, P. C. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. Science, 360(6387), 444-448.

Newton, C. R., Graham, A., & Ellison, J. S. (1997). PcR (p. 192). Oxford, UK: BIOS Scientific Publishers.

Nicholson, E. M., Kunkle, R. A., Hamir, A. N., Lebepe-Mazur, S., & Orcutt, D. (2007). Detection of the disease-associated isoform of the prion protein in formalin-fixed tissues by Western blot. Journal of Veterinary Diagnostic Investigation, 19(5), 548-552.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Research, 28(12), e63-e63.

Olby, R. (2006). The molecular revolution in biology. In Companion to the history of modern science (pp. 503-520). Routledge.

Olm, M. R., Butterfield, C. N., Copeland, A., Boles, T. C., Thomas, B. C., & Banfield, J. F. (2017). The source and evolutionary history of a microbial contaminant identified through soil metagenomic analysis. MBio, 8(1), 10-1128.

Ou, C. Y., Kwok, S., Mitchell, S. W., Mack, D. H., Sninsky, J. J., Krebs, J. W., ... & Schochetman, G. (1988). DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science, 239(4837), 295-297.

Park, J. W. (2022). Principles and applications of loop-mediated isothermal amplification to point-of-care tests. Biosensors, 12(10), 857.

Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., & Frey, J. (2005). Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. Journal of clinical microbiology, 43(5), 2291-2302.

Pinkel, D., Straume, T., & Gray, J. W. (1986). Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences, 83(9), 2934-2938.

Plomin, R., & Crabbe, J. (2000). DNA. Psychological bulletin, 126(6), 806.

Poppert, S., Essig, A., Marre, R., Wagner, M., & Horn, M. (2002). Detection and differentiation of chlamydiae by fluorescence in situ hybridization. Applied and environmental microbiology, 68(8), 4081-4089.

POUR-EL, A. K. I. V. A. (1981). Protein functionality: classification, definition, and methodology.

Prober, J. M., Trainor, G. L., Dam, R. J., Hobbs, F. W., Robertson, C. W., Zagursky, R. J., ... & Baumeister, K. (1987). A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides. Science, 238(4825), 336-341.

Prusiner, S. B. (1984). Prions. Scientific American, 251(4), 50-59.

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. Nature biotechnology, 35(9), 833-844.

Razonable, R. R., Inoue, N., Pinninti, S. G., Boppana, S. B., Lazzarotto, T., Gabrielli, L., ... & Schmid, D. S. (2020). Clinical diagnostic testing for human cytomegalovirus infections. The Journal of infectious diseases, 221(Supplement_1), S74-S85.

Rich, A. (1993). DNA comes in many forms. Gene, 135(1-2), 99-109.

Rose, C. D., Fawcett, P. T., Singsen, B. H., Dubbs, S. B., & Doughty, R. A. (1991). Use of Western blot and enzyme-linked immunosorbent assays to assist in the diagnosis of Lyme disease. Pediatrics, 88(3), 465-470.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., ... & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239(4839), 487-491.

Salari, S. H., & Ward, M. E. (1979). Early detection of Chlamydia trachomatis using fluorescent, DNA binding dyes. Journal of Clinical Pathology, 32(11), 1155-1162.

Sánchez-Carvajal, J. M., Galán-Relaño, Á., Ruedas-Torres, I., Jurado-Martos, F., Larenas-Muñoz, F., Vera, E., ... & Luque, I. (2021). Real-Time PCR validation for Mycobacterium tuberculosis complex detection targeting IS 6110 directly from bovine lymph nodes. Frontiers in Veterinary Science, 8, 643111.

Sauvagère, S., & Siatka, C. (2023). CRISPR-Cas:‘the multipurpose molecular tool’for gene therapy and diagnosis. Genes, 14(8), 1542.

Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science, 270(5235), 467-470.

Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science, 270(5235), 467-470.

Schleif, R. (2023). Genetics and molecular biology. The Johns Hopkins University Press.

Schochetman, G., Ou, C. Y., & Jones, W. K. (1988). Polymerase chain reaction. The Journal of infectious diseases, 158(6), 1154-1157.

Schutzbank, T. E., McGuire, R., & Scholl, D. R. (2009). Immunofluorescence. Clinical virology manual, 77-88.

Shafer, R. W., Rhee, S. Y., Pillay, D., Miller, V., Sandstrom, P., Schapiro, J. M., ... & Bennett, D. (2007). HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. Aids, 21(2), 215-223.

Simmonds, P., Bukh, J., Combet, C., Deléage, G., Enomoto, N., Feinstone, S., ... & Widell, A. (2005). Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology, 42(4), 962-973.

Sleator, R. D., Shortall, C., & Hill, C. (2008). Metagenomics. Letters in applied microbiology, 47(5), 361-366.

Smith, I. (Ed.). (2013). Chromatography. Elsevier.

Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2011). Introduction to modern liquid chromatography. John Wiley & Sons.

Speicher, M. R., & Carter, N. P. (2005). The new cytogenetics: blurring the boundaries with molecular biology. Nature Reviews Genetics, 6(10), 782-792.

Stadler, C., Rexhepaj, E., Singan, V. R., Murphy, R. F., Pepperkok, R., Uhlén, M., ... & Lundberg, E. (2013). Immunofluorescence and fluorescent-protein tagging show high correlation for protein localization in mammalian cells. Nature methods, 10(4), 315-323.

Sule, R., Rivera, G., & Gomes, A. V. (2023). Western blotting (immunoblotting): history, theory, uses, protocol and problems. Biotechniques, 75(3), 99-114.

Tanner, N. A., & Evans Jr, T. C. (2014). Loop-mediated isothermal amplification for detection of nucleic acids. Current protocols in molecular biology, 105(1), 15-14.

Taylor, S. C., & Posch, A. (2014). The design of a quantitative western blot experiment. BioMed research international, 2014(1), 361590.

Telenti, A., Imboden, P., Marchesi, F., Matter, L., Schopfer, K., Bodmer, T., ... & Cole, S. (1993). Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. The Lancet, 341(8846), 647-651.

Thwala, L. N., Ndlovu, S. C., Mpofu, K. T., Lugongolo, M. Y., & Mthunzi-Kufa, P. (2023). Nanotechnology-based diagnostics for diseases prevalent in developing countries: current advances in point-of-care tests. Nanomaterials, 13(7), 1247.

Tobie, J. E., & Coatney, G. R. (1961). Fluorescent antibody staining of human malaria parasites. Experimental Parasitology, 11(2-3), 128-132.

Tolochko, N. K. (2009). History of nanotechnology. Encyclopedia of Life Support Systems (EOLSS).

Travers, A., & Muskhelishvili, G. (2015). DNA structure and function. The FEBS journal, 282(12), 2279-2295.

Valencia, C. A., Pervaiz, M. A., Husami, A., Qian, Y., Zhang, K., Valencia, C. A., ... & Zhang, K. (2013). Sanger sequencing principles, history, and landmarks. Next Generation Sequencing Technologies in Medical Genetics, 3-11.

Valencia, C. A., Pervaiz, M. A., Husami, A., Qian, Y., Zhang, K., Valencia, C. A., ... & Zhang, K. (2013). Sanger sequencing principles, history, and landmarks. Next Generation Sequencing Technologies in Medical Genetics, 3-11.

Valones, M. A. A., Guimarães, R. L., Brandão, L. A. C., Souza, P. R. E. D., Carvalho, A. D. A. T., & Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Brazilian Journal of Microbiology, 40, 1-11.

Varmus, H. (1987). Reverse transcription. Scientific American, 257(3), 56-65.

Veselinyová, D., Mašlanková, J., Kalinová, K., Mičková, H., Mareková, M., & Rabajdová, M. (2021). Selected in situ hybridization methods: principles and application. Molecules, 26(13), 3874.

Voelkerding, K. V., Dames, S. A., & Durtschi, J. D. (2009). Next-generation sequencing: from basic research to diagnostics. Clinical chemistry, 55(4), 641-658.

Volpi, E. V., & Bridger, J. M. (2008). FISH glossary: an overview of the fluorescence in situ hybridization technique. Biotechniques, 45(4), 385-409.

Walker, T. M., Kohl, T. A., Omar, S. V., Hedge, J., Elias, C. D. O., Bradley, P., ... & Peto, T. E. (2015). Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. The Lancet infectious diseases, 15(10), 1193-1202.

Wang, D., Coscoy, L., Zylberberg, M., Avila, P. C., Boushey, H. A., Ganem, D., & DeRisi, J. L. (2002). Microarray-based detection and genotyping of viral pathogens. Proceedings of the National Academy of Sciences, 99(24), 15687-15692.

Wang, H., Zhang, W., & Tang, Y. W. (2022). Clinical microbiology in detection and identification of emerging microbial pathogens: past, present and future. Emerging Microbes & Infections, 11(1), 2579-2589.

Wang, K., Xu, B. F., Lei, C. Y., & Nie, Z. (2021). Advances in the integration of nucleic acid nanotechnology into CRISPR-Cas system. Journal of Analysis and Testing, 5, 130-141.

Weil, H., & WILLIAMS, T. I. (1951). Early history of chromatography. Nature, 167(4257), 906-907.

Weiss, L. M., Chen, Y. Y., Liu, X. F., & Shibata, D. (1991). Epstein-Barr virus and Hodgkin's disease. A correlative in situ hybridization and polymerase chain reaction study. The American journal of pathology, 139(6), 1259.

Whitford, D. (2013). Proteins: structure and function. John Wiley & Sons.

Wilson, M. R., Sample, H. A., Zorn, K. C., Arevalo, S., Yu, G., Neuhaus, J., ... & Chiu, C. Y. (2019). Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. New England Journal of Medicine, 380(24), 2327-2340.

Wilson, W. J., Strout, C. L., DeSantis, T. Z., Stilwell, J. L., Carrano, A. V., & Andersen, G. L. (2002). Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. Molecular and cellular probes, 16(2), 119-127.

Wong, Y. P., Othman, S., Lau, Y. L., Radu, S., & Chee, H. Y. (2018). Loop-mediated isothermal amplification (LAMP): a versatile technique for detection of micro-organisms. Journal of applied microbiology, 124(3), 626-643.

Xiong, D., Dai, W., Gong, J., Li, G., Liu, N., Wu, W., ... & Tang, G. (2020). Rapid detection of SARS-CoV-2 with CRISPR-Cas12a. PLoS biology, 18(12), e3000978.

Yang, S., & Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. The Lancet Infectious Diseases, 4(6), 337-348.

Yanofsky, C. (1967). Structural relationships between gene and protein. Annual Review of Genetics, 1(1), 117-138.

Yeh, C. T., Han, C. M., Lo, S. Y., Ou, J. H., Fan, K. D., Sheen, I. S., ... & Liaw, Y. F. (1994). Early detection of anti-HCc antibody in acute hepatitis C virus (HCV) by western blot (immunoblot) using a recombinant HCV core protein fragment. Journal of Clinical Microbiology, 32(9), 2235-2241.

Yoshiba, T., Saga, Y., Urabe, M., Uchibori, R., Matsubara, S., Fujiwara, H., & Mizukami, H. (2019). CRISPR/Cas9-mediated cervical cancer treatment targeting human papillomavirus E6. Oncology letters, 17(2), 2197-2206.

Zhang, C. Y., Yeh, H. C., Kuroki, M. T., & Wang, T. H. (2005). Single-quantum-dot-based DNA nanosensor. Nature materials, 4(11), 826-831.

Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabečná, M., & Neužil, P. (2020). PCR past, present and future. Biotechniques, 69(4), 317-325.

Zöller, L., Cremer, J., & Faulde, M. (1993). Western blot as a tool in the diagnosis of Lyme borreliosis. Electrophoresis, 14(1), 937-944.

Abstract

This thesis provides a bibliographic synthesis of the development of molecular biology and its impact on microbiological diagnostics. It begins with a simplified overview of molecular biology's foundations, then outlines key diagnostic techniques such as PCR, sequencing, and FISH, along with newer tools like CRISPR, microarrays, nanotechnology, and proteomics. These methods offer greater accuracy and speed but face limitations like cost and access. The thesis ends with recommendations to enhance their integration into clinical practice and support their future advancement in diagnostic microbiology.

Keywords: molecular biology, microbiological diagnosis, PCR, sequencing, CRISPR, FISH, emerging technologies

Résumé

Ce mémoire est une synthèse bibliographique sur l'évolution de la biologie moléculaire et son application dans le diagnostic microbiologique. Il présente brièvement les bases de cette discipline, puis décrit plusieurs techniques diagnostiques comme la PCR, le séquençage, la FISH, ainsi que des outils récents tels que CRISPR, les puces à ADN, la nanotechnologie et la protéomique. Ces méthodes offrent une meilleure précision et rapidité, mais rencontrent des limites liées au coût et à l'accessibilité. Le mémoire propose enfin des recommandations pour renforcer leur usage en pratique clinique.

Mots-clés : biologie moléculaire, diagnostic microbiologique, PCR, séquençage, CRISPR, FISH, technologies émergentes

ملخص :

هذه المذكرة هي دراسة مرجعية تستعرض تطور علم البيولوجيا الجزيئية ودوره في التشخيص الميكروبيولوجي. تبدأ بشرح مبسط لأساسيات هذا العلم ونشأته، مع توضيح أهم مكوناته. ثم تنتقل إلى عرض مجموعة من التقنيات الجزيئية المستعملة في التشخيص، مثل تقنية تضخيم الحمض النووي، وتقنية تحديد تسلسل المادة الوراثية، وتقنية التهجين الضوئي، إضافة إلى أدوات حديثة مثل أنظمة التعديل الوراثي، الشرائح الدقيقة، تكنولوجيا الذرات الدقيقة، والأساليب المعتمدة على تحليل البروتينات. وتُختتم المذكرة بتحديد الفوائد والمعوقات وتقديم توصيات لتوسيع استخدامها في المجال الطبي.

الكلمات المفتاحية :

التشخيص المجهري الحيوي، علم الأحياء الجزيئي، تفاعل البوليميراز المتسلسل، تسلسل الحمض النووي، التهجين الموضعي المتألق، أنظمة كريسبر-كاس للتعديل الوراثي، الشرائح الدقيقة، تقنية النانو، علم البروتينات، التوهج المناعي، لطخة البروتين الغربية، الدقة، السرعة، التقنيات الحديثة.