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REVIEW ARTICLE

Advances in Viral Diagnostics: From Conventional Methods to Molecular and Genomic Technologies

Qader Abdel SORAN[1], Ahmed Farhan SHALLAL[2], Ahmed Ahmed SYAMAND[3], Nazem Qader IBRAHIM[4]

ABSTRACT

Viral infections continue to pose a significant global public health threat due to their high transmissibility, genetic diversity, and capacity to cause widespread epidemics. Consequently, accurate, rapid, and scalable diagnostic techniques are essential for effective patient management, outbreak control, and epidemiological surveillance. This paper critically examines the evolution of viral diagnostic methodologies, from traditional laboratory techniques to advanced molecular and genomic technologies. Conventional approaches, including virus isolation, microscopy, and serological assays such as ELISA, remain widely used because of their cost-effectiveness and accessibility. However, these methods are limited by long turnaround times, reduced sensitivity during the early stages of infection, and a limited ability to detect emerging variants. Molecular techniques, including polymerase chain reaction (PCR), real-time PCR, and nucleic acid amplification tests (NAATs), offer high sensitivity and specificity, enabling rapid detection of viral nucleic acids. In addition, next-generation sequencing (NGS) allows comprehensive genomic analysis, facilitating mutation detection, pathogen identification, and transmission tracking. Emerging biosensor-based diagnostics represent a promising approach for rapid, portable, and point-of-care testing, particularly in resource-limited settings. Comparative analysis indicates that no single diagnostic modality is universally optimal; rather, the choice of technique depends on the clinical context, available infrastructure, cost considerations, and specific diagnostic objectives.

Keywords: viral infection, viral culture, ELISA, PCR, RT-PCR, NAAT, NGS, Biosensors.

1. Medical Laboratory Science, College of Science, University of Raparin, Rania, 46012, Sulaymaniyah, Iraq 2. College of Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan Region – Iraq 3. Nursing Department, Rwandz Private Technical Institute, Soran, Erbil, Kurdistan Region, Iraq. 4. Department of Physics, College of Science, University of Raparin, Rania, 46012, Sulaymaniyah, Iraq.

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Correspondance to: Ahmed SYAMAND
E-mail: syamandahmed30@gmail.com

1. INTRODUCTION

Viral infections pose a significant challenge to global public health due to their swift transmission, genetic variability, and tendency to instigate epidemics and pandemics. The COVID-19 pandemic has highlighted the urgent necessity for precise, rapid, and dependable diagnostic tools to facilitate effective clinical decision-making and public health measures [1, 2]. Furthermore, chronic viral infections such as human immunodeficiency virus and viral hepatitis are associated with substantial morbidity and mortality worldwide [3, 4]. Viruses are acellular infectious agents consisting of nucleic acid (DNA or RNA) encased in a protein capsid and, in certain instances, a lipid envelope. They possess no autonomous metabolic function and depend entirely on host cells for replication. Their elevated mutation rates and genetic diversity render early detection and precise identification very difficult [5-7]. Traditional diagnostic techniques, including virus culture, serological assays, and microscopy, have been commonly utilised owing to their accessibility and comparatively cheap expense. Viral culture is labour-intensive and may fail to detect certain viruses. However, there are significant drawbacks to these methods, such as long turnaround times and reduced sensitivity in early infection due to late antibody production [8-10]. However, serology is crucial for immune response assessment, epidemiological research, and retrospective diagnosis [11]. The

creation of molecular diagnostic technologies, especially polymerase chain reaction, has substantially improved the sensitivity and specificity of detecting viruses. PCR allows amplification of viral genetic material, thereby allowing accurate diagnosis even at low levels of virus [12-14].Advancements in molecular diagnostics have developed real-time PCR (RT-PCR) and NAATs that enable rapid and quantitative detection of viral genetic material [15, 16]. Viral diagnostics has recently experienced a transformation due to NGS and biosensor technologies, facilitating high-throughput genomic analysis and rapid point-of-care diagnosis [17, 18]. Meanwhile, biosensors offer fast, portable, cost-effective diagnostic solutions and are particularly appropriate for point-of-care testing and resource-limited situations [19, 20]. However, all these diagnostic modalities have their own intrinsic advantages and restrictions in terms of sensitivity, cost, infrastructure requirements, and technical complexity. No single method is universally good, and the choice of diagnostic instruments relies on the clinical context and available resources [21, 22]. This paper aims to provide a comparative and analytical overview of traditional and new viral diagnostic technologies, focusing on their clinical applications, limits, and future prospects.

2. LITERATURE SEARCH STRATEGY

Search Strategy

A systematic literature search was done to locate relevant peer-reviewed articles on traditional and advanced viral diagnostic modalities. The search strategy employed a combination of Medical Subject Headings (MeSH) and free-text keywords, including “viral diagnostics,” “viral detection,” “viral culture,” “serological assays,” “ELISA,” “polymerase chain reaction (PCR),” “real-time PCR (RT-PCR),” “nucleic acid amplification tests (NAATs),” “next-generation sequencing (NGS),” and “biosensor-based diagnostics.” Boolean operators (AND, OR) were used to combine search phrases to improve retrieval accuracy. The articles considered were those published in the English language between 2000 and 2025. The search was focused on research assessing the diagnostic principles, sensitivity, specificity, clinical applications, advantages, limits, and developing technologies in viral diagnostics.

Databases Consulted

A literature review was undertaken in the following electronic databases: PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect. A comprehensive search of these databases was performed to discover relevant peer-reviewed articles linked to conventional and current viral diagnostic methods.

Inclusion and Exclusion Criteria

The inclusion criteria were peer-reviewed studies on both traditional and novel viral diagnostic technologies, such as viral culture, serological tests, PCR, RT-PCR, NAATs, next-generation sequencing (NGS), and biosensor-based diagnostics. Eligible studies described diagnostic principles, analytical performance, sensitivity, specificity, clinical utility, epidemiological applications, technological developments, or comparisons of technologies for viral detection. It comprised original research articles, systematic reviews, and clinically relevant laboratory studies published in English between 2000 and 2025. Exclusion criteria were duplicate publications, conference abstracts, editorials, articles not published in English, studies not related to viral diagnostics, studies that only focused on non-viral pathogens, and studies not providing sufficient methodological or scientific information for diagnostic evaluation.

3. IMPORTANCE OF VIRAL DIAGNOSIS IN PUBLIC HEALTH

Virus-related infections or viral contamination are among the primary causes of illness that kill hundreds of thousands of people worldwide every year. The fact that millions of people suffer from various diseases indicates that these medical problems have not yet been resolved [23]. It is critical to identify the causative viral pathogens quickly and accurately to choose the best course of action, prevent epidemics, save lives, and minimize the needless use of antibiotics [24]. Certain viruses cause chronic infections so frequently that their presence in the normal flora of most humans masks any immunologic imprint they may have left behind or their possible role in disease. Therefore, to examine this essential component of our biology, a reinterpretation of chronic viral infection is required, one that paves the way for novel and intriguing research. Humans harbor a diverse range of persistent viral infections, whether they are good or bad [25]. A process of viral infection that is neither in equilibrium nor in a state of dynamic, metastable equilibrium is called an acute viral infection. Until the infection is eradicated, the host dies, or it turns chronic, both the virus and the host undergo constant change during an acute infection. A series of cytosolic sensors expressed by innate immune cells is used to identify viral infections; the immune response to viral infections is determined by a complicated relationship between the pathogen and the host. [26].There is still a serious risk to health from viral infection. This was not thought to be the case in the past. Many people gained hope that infectious illnesses could be simply eliminated with the development of antibiotics and vaccinations. Such optimism was effectively dashed by the recovery and spread of established diseases, such as West Nile and Ebola hemorrhagic fevers, as well as the appearance of new illnesses like acquired immune deficiency syndrome, severe acute respiratory syndrome, and avian influenza [27].

Antiviral medications, which stop viruses from replicating, are now widely accessible. A lot of viruses, such as HIV, are highly mutable and give rise to resistant strains that are uncontrollable by previously effective medications. Additionally, as global trade in products and people grows, viruses that were previously restricted to specific geographical areas can now spread quickly throughout the world [4]. Considering the difficulties each of these situations poses, new tactics to combat potentially fatal viruses must be developed. The mechanisms underlying host-virus interactions, including the host immune system's antiviral defenses and the viruses defense mechanisms that allow them to elude host immune reactions, need to be better understood to accomplish this [28].

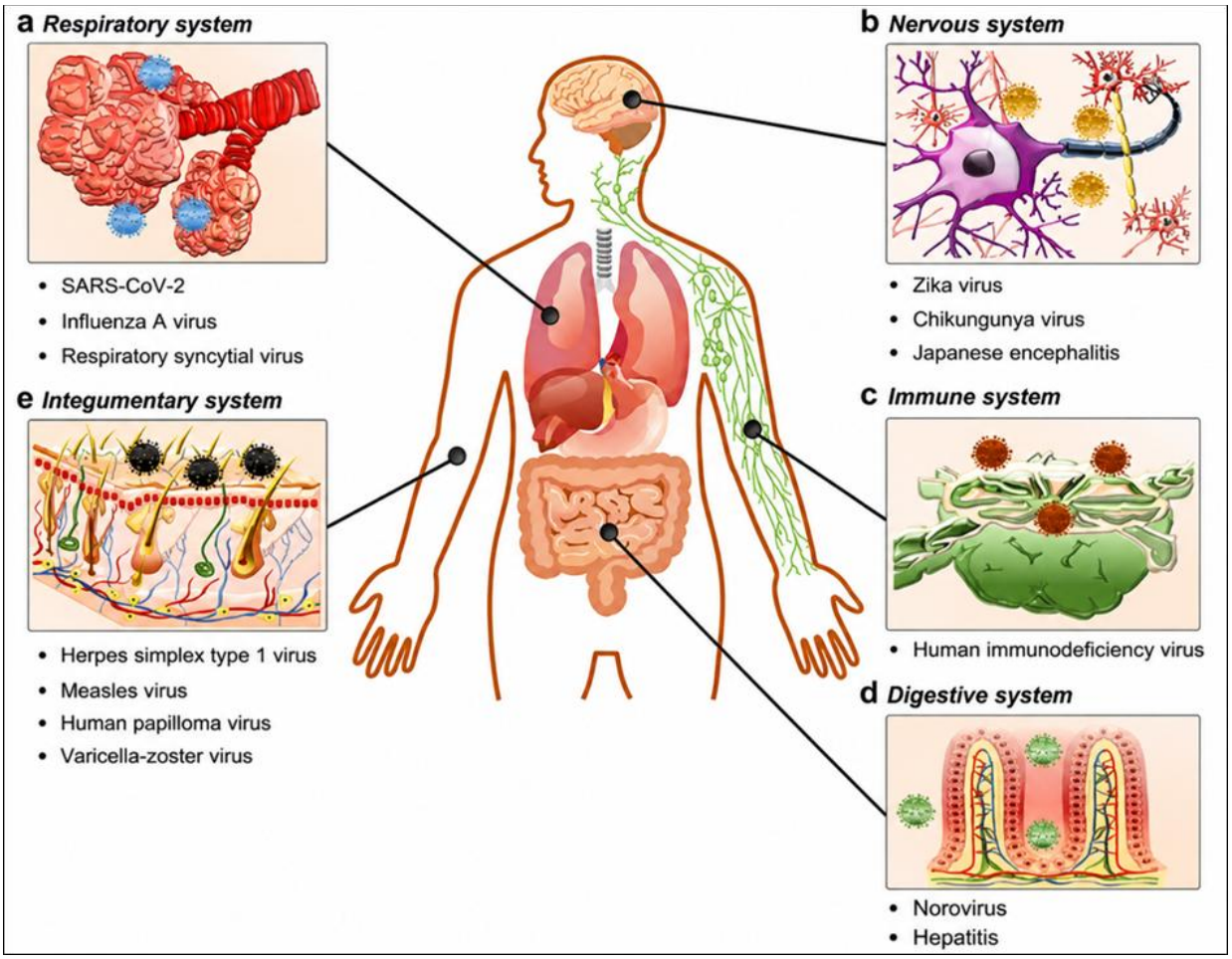

Figure 1. Main systems of the human body impacted by viral infections. The figure shows the organ systems most commonly affected by the various human viruses, including the respiratory system (SARS-CoV-2, influenza A virus, and respiratory syncytial virus), nervous system (Zika virus, chikungunya virus, and Japanese encephalitis virus), immune system (human immunodeficiency virus), digestive system (Norovirus and hepatitis viruses), and integumentary system (Herpes simplex virus type 1, measles virus, human papillomavirus, and varicella-zoster virus) [29].

4. CLASSIFICATION OF VIRAL DIAGNOSTIC METHODS

Conventional Diagnostic Methods

Overview of Conventional Diagnostic Methods

Microscopy, culture, and serology are examples of conventional techniques for pathogen detection. Microscopy is a basic, user-friendly, and adaptable technique. Serology serves as the foundation for the diagnosis of certain diseases, such as syphilis. When it comes to diagnosing numerous microorganisms, including Mycobacterium tuberculosis, culture remains the gold standard. Conventional detection techniques are low-cost but time-consuming. In many situations, the sensitivity of microscopy is limited, and

interpretations can be subjective [30]. However, there is a chance that the culture will become contaminated by commensal flora and that its viability may be compromised while being transported. The need for recuperating sera and the possibility of false-positive results from organism cross-reactions are the primary drawbacks of serology. The use of molecular techniques, which provide a growth-independent strategy, has increased during the past 20 years [31]. It is especially suited for organisms that are difficult to cultivate (like Bordetella pertussis, HIV, HBV, and Treponema pallidum), slow-growing (like Mycobacterium tuberculosis), dangerous to cultivate (like Coxiella burnetii), and unculturable (like Bordetella pertussis). Molecular techniques encompass both amplification-based and non-amplification techniques, such as probe-based hybridization. In diagnostics, nucleic acid amplification techniques (NAATs) have cemented their place inexorably. Some of these systems include branch DNA technology (signal amplification), ligase chain reaction (LCR) for probe amplification, transcription-based amplification (target amplification), loop-mediated isothermal amplification for isothermal amplification, and PCR for polymerase chain reaction [21].

These techniques are well known for their sensitivity and for providing information on the tested microorganisms in both qualitative and quantitative forms. These methods, however, are costly and take a long time to assay. The most popular nucleic acid amplification test, PCR, is capable of identifying a single copy of a target DNA sequence, making it possible to identify a single pathogenic bacterium. It is encouraging because it amplifies the target rather than the signal to detect the organism, which reduces the possibility of false-positive results. As a result, PCR detection of pathogens offers specificity, sensitivity, speed, accuracy, and the ability to detect minute amounts of target nucleic acid in a sample, which makes it superior to culture and other standard methods [8].

Viral Culture

Viral culture involves growing viruses in a controlled environment, such as a laboratory dish, to identify the specific virus causing an infection. This process takes a lot of time and may take several days or weeks to produce results, but it can provide valuable information about this virus and its characteristics [32]. The "gold standard" for testing these pathogens has been established as viral culture; however, this approach is typically sluggish, requiring up to 14 days to yield results. Fast results are obtained from viral antigen detection by IF, but certain viruses are frequently not detected by this method, necessitating additional viral culture confirmation. The proportion of positive results can be increased by combining the two methods, but despite clinical and epidemiological suspicions of viral infection, a sizable portion of specimens have been reported to remain negative. [33]. Adults with acute respiratory syncytial virus (RSV) infections can be challenging to diagnose due to the low sensitivity of antigen detection and viral culture. To diagnose RSV in adults suffering from respiratory illnesses, a newly created single-tube nested reverse transcription-PCR was compared to viral culture and serology using an enzyme immunoassay [34]. During respiratory illnesses, nasal swab samples were taken from five groups of subjects: individuals with chronic heart and lung disease, individuals with healthy aging parents, residents of nursing homes, and adults admitted to the hospital in the winter with cardiopulmonary conditions. 995 samples were negative using all three tests, while 117 samples were positive using at least one of the methods. A total of 1,112 samples were tested. Due to positive serology and/or culture results, 110 patients were regarded as true positives. 43 (39%) of these were culture positive, while 80 (73%) were PCR positive. Because of the negative culture and serology, seven PCR results were deemed to be false positives. Overall, 73% of the RT-PCR samples had sensitivity, and 99% had specificity. RT-PCR is a highly effective technique for identifying adult cases of acute RSV infection, according to the data presented [35]. While virus isolation on cell culture substrate is probably the most specific approach, it's also the slowest and least sensitive—it can take anywhere from seven to twenty-one days and multiple passages to produce detectable viruses [36].

Serological Tests

Serological tests detect the presence of antibodies or antigens in a patient's blood serum to diagnose a viral infection. Such as enzyme immunoassays (ELAs) and enzyme-linked immunosorbent assays (ELISA) are commonly used serological tests to detect specific antibodies or antigens. These tests are often used to diagnose infections like HIV, hepatitis, and other viral illnesses [37]. To assess a person's resistance or susceptibility to recurrent infection, serological testing is crucial. Although there are other effective and specific techniques, such as RT-PCR, serological test kits are very useful in assessing and determining the production of antibodies against the virus and the likelihood that an individual will develop sustained immunity that will result in herd immunity [38]. Even though direct viral identification via antigen or nucleic acid-based assays is currently the focus of clinical virology, antibody detection is still vital to the diagnosis and treatment of many viral illnesses. This is especially true for the measles, mumps, and rubella viruses; the arboviruses; the parvovirus B19; the hepatitis viruses A–E, the herpesviruses; and the human immunodeficiency virus types 1 and 2 (HIV-1 and -2) [39]. Viral serology performance is helpful in the diagnosis of recent or persistent viral infections, in assessing an individual's or population's immunity to a particular virus, and in validating the immune response to immunization. In a hospital context, a quick assessment of immune status may help avoid the needless transmission of some viruses to patients or healthcare personnel who lack immunity. Before transplantation, blood products, organ donors, and recipients should be screened for virus-specific antibodies to prevent the spread of blood-borne viruses to people who are highly susceptible to serious illness [40]. In some situations, the only way to diagnose a viral infection in a patient is also through the identification of virus-specific antibodies in their serum. Many viruses are

challenging to detect through alternative means or to grow in culture. It might be challenging or impossible to obtain the right specimens for direct detection assays or cultures [41]. Additionally, specimens may be gathered too late in the illness for direct viral detection. Serology may help establish a causal relationship, even though the role of the identified virus in the current disease process is uncertain. The usefulness of this method is that it is highly accurate, quick, low-cost, specific, and applicable, and it has more ability in antibody and antibody reactions [42].

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a very sensitive molecular technique for the amplification and identification of specific DNA or RNA sequences in clinical samples. It allows for the detection of viral genetic material even at extremely low concentrations, which makes it an important tool in current diagnostic virology. Kary Mullis, an American biochemist, invented the polymerase chain reaction in 1984. Mullis won the Japan Prize in 1993 and the Nobel Prize for the discovery of PCR. The basic premise of PCR is simple [12]. PCR is a chain reaction, as the name suggests; two are created from one DNA molecule. resulting in four copies, eight copies, and so on. Molecular building blocks of DNA can be joined to form lengthy molecular strands by specialized proteins called polymerases, which are responsible for this continuous doubling [43]. A supply of nucleotides made up of the four bases adenine (A), thymine (T), cytosine (C), and guanine (G) is necessary for polymerases to perform their function. In addition, they require a template DNA molecule to act as a basis for the construction of the new strand and a tiny DNA fragment called the primer, to which nucleotides are added. The enzymes will create identical replicas of the templates if these three components are provided [44]. PCR is a method used to obtain numerous copies of a specific nucleic acid strand. It is a technique for amplifying a specific DNA segment in a targeted manner. This method amplifies and detects viral DNA or RNA in a patient's sample, allowing for rapid and accurate diagnosis [45]. PCR tests are widely used for diagnosing viral infections such as hepatitis, herpesvirus, and SARS-CoV-2 (COVID-19). In the last ten years, molecular diagnostics have become essential procedures due to progressions in PCR technology and other DNA signal and target amplification techniques [46]. Conceptually straightforward, these methods are extremely focused, sensitive, and fully automatable [47]. The most developed of these technologies, polymerase chain reaction (PCR), is becoming more widely used in routine diagnostic laboratory settings, undergraduate and high school education, and research laboratories in various forms [48]. PCR-based methodologies have been extensively utilised for the diagnosis of viral infections, including hepatitis viruses, herpesviruses, and SARS-CoV-2. PCR exhibits enhanced sensitivity, specificity, and expedited turnaround time relative to traditional diagnostic methods [49].Cost and occasionally the availability of sufficient test sample volume are the main constraints on the use of PCR in diagnostic laboratories. To address these drawbacks and enhance the diagnostic potential of PCR, a variation known as multiplex PCR has been reported [50].

Modern methods

Real-Time PCR

Quantitative PCR (qPCR), commonly referred to as real-time PCR (RT-PCR), is a potent molecular biology method for the identification and measurement of particular DNA or RNA sequences [51]. RT-PCR is a popular and very useful technique for diagnosing viruses because it can positively and specifically identify viruses in clinical samples. There are numerous uses for it, from identifying sudden viral infections to tracking the amount of virus in individuals receiving antiviral medication [52]. With RT-PCR, as opposed to conventional PCR, target DNA or RNA can be amplified and quantified simultaneously, yielding useful data on viral load and aiding in the precise diagnosis of viral infection. The viral diagnosis was transformed by RT-PCR because it provided rapid delivery, high sensitivity, and specificity [53]. The aforementioned characteristics render it an indispensable instrument for diagnosing viruses in clinical labs, public health organizations, academic establishments, and the global healthcare system. Ongoing advancements in real-time PCR technology also keep improving its efficacy, adaptability, and suitability for viral analysis and diagnosis [54]. This introduction aims to provide a comprehensive review of the concepts, methods, and clinical applications underlying RT-PCR for viral detection. It will go into the technical details of real-time PCR and the advantages of it being higher than conventional PCR techniques, as well as how it has changed the surveillance and detection of viruses [55]. Furthermore, it will demonstrate the important role of RT-PCR in the global fight against viral diseases and how it benefits the treatment of patients, healthcare worldwide, vaccination production, and virology [56]. Moreover, real-time PCR diminishes the risk of contamination post-amplification, as no additional handling is necessary following the amplification process. However, the procedure entails many drawbacks, including elevated costs, the requirement for specialised equipment, and reliance on expert personnel [57].

Nucleic Acid Amplification Tests

Nucleic acid amplification tests (NAATs) are very sensitive and specific techniques to detect viral genetic material. These techniques allow for quick and precise identification of viruses directly from clinical samples [58]. These assays have revolutionised viral diagnostics, allowing for fast and reliable identification of viral nucleic acids straight from patient samples. However, precise and timely detection of viral agents is still difficult due to a range of variables, despite providing a clear indication of the presence of viruses [59].

NAATs are used as an integral part of various diagnostic methods, including nucleic acid sequence-based amplification, loop-mediated isothermal amplification, real-time NAATs, including RT-PCR, PCR, and other highly sophisticated techniques [60]. These methods use unique primers and probes to identify and quantify viral nucleic acid sequences, making it possible to detect even small numbers of genes from viruses in biological samples or clinical samples [61]. NAATs are highly regarded for their ability to detect minuscule amounts of viral genetic material in clinical samples, facilitating early infection identification. These processes provide rapid, accurate, and precise results, making them essential instruments in clinical diagnostics and public health monitoring [62]. A notable characteristic of NAATs is their ability to identify active infections by directly detecting viral nucleic acids, in contrast to serological diagnostics, which depend on the immune response [63]. The development of NAATs has changed the nature of virus screening, powerful tools for charity physicians, laboratories, and public health professionals to combat infectious diseases [64]. The ongoing improvement and modification of NAAT technologies continue to initiate novelty in viral diagnostics, leading to enhanced patient care, boosted observation and epidemiological studies, and the expansion of targeted therapeutic interferences and vaccination approaches [65].

Next-Generation Sequencing

Next-Generation Sequencing (NGS) has emerged as a transformative technology in the field of viral diagnosis, providing a powerful and wide-ranging approach to identifying and characterization viral pathogens with unique detail and specificity [66]. This innovative approach enables researchers and clinicians to rapidly sequence and analyze viral genes, advancing our understanding of viral infections and providing valuable insights into viral diversity, evolution, transmission, and drug resistance [67]. In contrast to conventional diagnostics that focus on specific viral genes or proteins, next-generation sequencing (NGS) provides exceptional accuracy by sequencing entire viral genomes, whether RNA or DNA, directly from clinical samples with minimal risk [68]. The capacity to identify infections with high precision enhances diagnostic accuracy and may lead to the discovery of novel or emerging pathogens, including genetically modified or previously unrecognised mutant viruses. This method employs bioinformatics tools for the interpretation of sequencing data, facilitating the identification and description of bacterial species, subtypes, and genetic diversity [69]. NGS provides critical information for monitoring viral epidemics, distribution mechanisms, and determining the efficacy of public health treatments in providing the most comprehensive genetic segregation of pathogens in the 21stcentury [70]. In addition, NGS technology is essential to identify drug-resistant mutations and monitor changes in the viral genome that may affect the efficacy of antiviral drugs [71]. In the clinical setting, NGS-based viral diagnostics exhibit the potential to increase the accuracy and speed of viral identification, particularly in situations where traditional diagnostic methods may yield confusing results or not in a satisfactory manner [17].

To further improve the capacity to identify possible risks to public well-being and carry out focused response plans, real-time genetic tracking of viral isolates is made possible by the use of NGS in epidemics and pandemic studies [72]. These advancements are paving the way for the use of NGS in typical clinical settings and pointing the way toward customized and accurate methods of treating infections caused by viruses. In summary, NGS represents a paradigm shift in viral diagnosis, offering unparalleled insights into viral genomics, epidemiology, and pathogenesis [73]. By harnessing the power of NGS, researchers and healthcare professionals are poised to develop the detection and investigation, ushering in a new era of precision virology and public health response capabilities [18].

Biosensor-Based Diagnostics

Biosensors are analytical devices that detect specific viral targets by combining biological recognition elements (e.g., antibodies, enzymes, or nucleic acids) with physicochemical detection methods [20]. These devices provide fast, sensitive, and portable diagnostic solutions, making them extremely appropriate for point-of-care testing. The ability and specificity to detect highly sensitive organisms have made biosensors a very attractive tool for the detection of pathogens, as shown in Figure 2 [74]. The ability to rapidly and efficiently identify samples is one of the major advantages of biological diagnostics. This can lead to faster diagnosis of infection and faster implementation of treatment [75]. Furthermore, biosensors are low-cost, simple, and portable. Another advantage is that they are especially helpful in resource-limited environments and medical situations where resources are rare. Biosensors can be used in a variety of ways, including optical, electrical, and microfluidic platforms [76]. Such as optical biosensors,biosensors which combine light with a biologically detectable substance to generate a signal for the presence of a target pathogen [19].

In contrast, biosensors with electrochemical properties rely on the measurement of electrical impulses resulting from sensing events, providing a flexible and scalable approach [77]. At the same time, microfluidic biosensors are small liquid systems that can rapidly process samples, making them particularly useful for point-of-care research [78].The versatility enables the development and optimization of biosensing technologies for various pathogenic organisms, providing the ability to treat microbial threats and emerging infectious diseases identified in advanced care settings. The value is greater [79]. Biosensors have the benefit of being portable and easy to use, making them applicable in resource-limited environments [80].

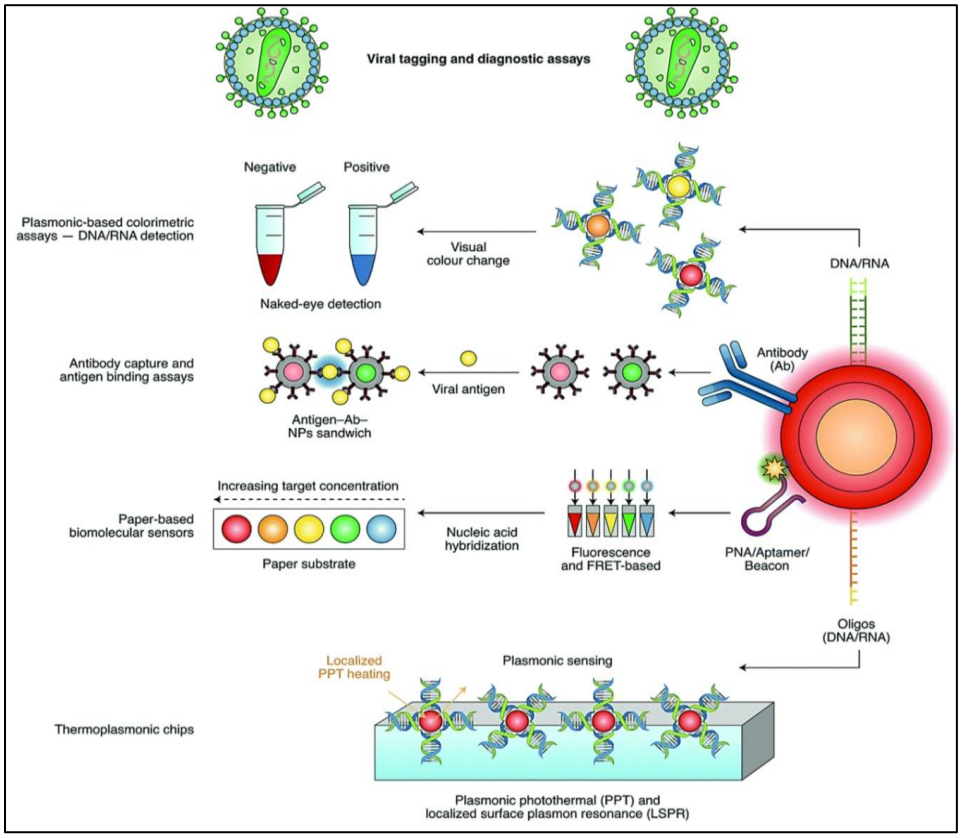

Figure 2. Schematic representation of biosensing based on plasmonic nanotechnology for viral tagging and diagnostic purposes. The figure shows plasmonic colorimetric DNA/RNA biosensing assays via visible colour change, antibody–antigen nanoparticle sandwich biosensors, paper-based biomolecular biosensors, fluorescence and Förster resonance energy transfer-based biosensing systems, and thermoplasmonic chips using localised surface plasmon resonance and plasmonic photothermal effects for rapid, sensitive, and selective viral nucleic acid and antigen detection [81].

5. COMPARATIVE ANALYSIS OF DIAGNOSTIC APPROACHES

Each diagnostic approach has its own advantages and disadvantages depending on clinical and operational needs. Traditional techniques are inexpensive and broadly available but suffer from lengthier turnaround times and less sensitivity, especially during early infection phases [82, 83].

PCR and NAATs are highly sensitive, specific, and quick detection methods and are hence the method of choice in many clinical contexts. However, these techniques demand sophisticated laboratory infrastructure and specialized manpower [63, 84]. NGS provides a complete set of genetic information and high-resolution analysis but is limited by its expensive cost and challenging data interpretation [85]. On the contrary, biosensors offer fast, decentralised and point-of-care diagnostics but still require additional validation for reliability, as shown in Table 1 [86, 87]. Consequently, no singular diagnostic approach is ideal for all applications. A comprehensive approach that amalgamates many technologies can enhance diagnostic precision, efficiency, and clinical decision-making.

6. CHALLENGES AND FUTURE PERSPECTIVES

Viral diagnostics still confront several obstacles despite major progress. These include high costs, restricted availability in low-resource settings, and the necessity for specialised technical competence [92, 93]. Also, false positive and false negative results are an issue, especially in high-throughput testing situations [94, 95]. Another key difficulty is the rapid evolution of viral genomes that may limit the efficiency of existing diagnostic techniques and require continual upgrades [96, 97].

Table 1. Comparative Overview of Conventional and Modern Viral Diagnostic Methods.

Method Type	Technique	Sensitivity/ Specificity	Time	Cost Level	Best Use Case	Key Limitations	Ref.
Conventional	Viral Culture	Moderate/ High	Slow	Low	Virus isolation; research applications	Time-consuming; low sensitivity	[88]
	Serology (ELISA)	Moderate/ Moderate	Fast	Low	Immune response detection; epidemiological surveillance	Not useful in early infection	[9]
	PCR	High / High	Moderate	Medium–High	Gold-standard clinical diagnosis	Risk of contamination; requires skilled personnel	[89]
Modern	RT-PCR	Very High / Very High	Very fast	High	Early infection detection; large-scale screening	Expensive; technical complexity	[10]
	NAATs	Very High / Very High	Fast	High	Mutation analysis; advanced research	Requires specialized equipment and trained staff	[22]
	NGS	Ultra-high	Slow–Moderate	Very High	Whole-genome sequencing; novel pathogen detection	High cost; complex bioinformatics analysis	[90, 91]
	Biosensors	Variable	Very fast	Low–Medium	Point-of-care and rapid diagnostics	Limited validation; possible false results	[19]

Future advancements are anticipated to solely concentrate on the amalgamation of artificial intelligence, automation, and digital health technologies. AI-driven systems enhance data processing, pattern recognition, and diagnostic precision, whereas portable diagnostic gadgets facilitate real-time, point-of-care monitoring [98, 99]. In addition, the confluence of genetics and diagnostics will enable personalised treatment and precision virology, increasing patient outcomes and global health preparedness.

7. CONCLUSION

Diagnostic methods for viruses have evolved from conventional, labour-intensive techniques to advanced molecular and genomic technologies. Traditional methods such as viral culture and serology remain useful in some clinical and epidemiological settings, but molecular methods including PCR, NAATs and NGS are now the mainstay of modern diagnostic practice because of their high sensitivity, specificity, and rapid detection. Biosensors and other developing technologies have promise for speedy point-of-care diagnostics, particularly in resource-constrained environments. Each diagnostic procedure possesses distinct advantages and disadvantages, and no singular method is adequate in all circumstances. Consequently, a multifaceted diagnostic strategy employing many methodologies is essential to improve diagnostic precision and public health intervention. Future initiatives will concentrate on automation, the integration of artificial intelligence, and the development of portable diagnostic platforms, thereby enhancing the efficacy of viral diagnostics and global health readiness.

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