Advancements in Virus Detection and Isolation: The Role of Chromatography in Virology Research and Health

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Abstract

Viral diseases, particularly those resulting from virus mutations, have become increasingly widespread, causing significant health, social, and economic impacts globally. Among these, seasonal influenza, driven by viral mutations, leads to chronic inflammation, epidemics, and pandemics. Effective management of viral infections requires accurate detection, reliable isolation methods, and timely diagnosis to control their spread. The COVID-19 pandemic has further highlighted the urgent need for advanced virology research and diagnostic approaches. This review aims to provide an overview of viral infections, explore existing virus isolation methods and diagnostic procedures, and emphasize the critical role of chromatography techniques in virology. Ongoing efforts to advance virology research are essential for mitigating viral mutations and improving public health and economic stability.

Significance | Chromatography enhances virus detection and isolation, enabling rapid diagnosis, precise identification, and improved virology research for global health security.

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1. Introduction

Viruses are pathogenic microorganisms that infect plants, animals, and humans, leading to significant health and economic consequences worldwide. Structurally, viruses consist of a nucleic acid core—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—enclosed within a protective protein coat called a capsid. These infectious agents lack the necessary cellular machinery for self-replication and must rely on host cells for propagation. Upon infection, viruses hijack the host's metabolic processes to replicate their genetic material and produce new viral particles. As a result, viral infections have emerged as a leading cause of human illnesses globally (Harsh & Tripathi, 2023). The capsid not only safeguards the viral genome but also plays a critical role in host cell entry, genome uncoating, and intracellular transport (Freire et al., 2015).

Historically, the identification of viruses in clinical and research settings relied on swine tissue culture, animal testing, and electron microscopy (Harsh & Tripathi, 2023). However, these traditional diagnostic methods were often time-consuming, expensive, and inconsistent. Advances in molecular biology have revolutionized virology by enabling the direct detection of viral nucleic acids in clinical samples through polymerase chain reaction (PCR) and other nucleic acid-based assays (Cassedy et al., 2021). Although these modern techniques have largely replaced older diagnostic methods, immunoassays remain essential for detecting viral antigens or host antiviral antibodies (Chen et al., 2015). Despite

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these advancements, there remains a need for more precise, rapid, and cost-effective diagnostic tools to ensure accurate viral detection and effective disease management. Early and accurate identification of viral infections is crucial for effective disease control. Timely diagnosis facilitates appropriate treatment selection, reduces healthcare costs, and improves patient outcomes. Moreover, accurate detection is instrumental in developing preventive measures, including vaccines and antiviral therapeutics. This review aims to provide a comprehensive discussion on viral characteristics and advancements in medical virus diagnostics.

2. Virus Background

Viruses, despite their minuscule size ranging from 20 to 400 nm, are among the most devastating infectious agents affecting human life. The discovery of viruses dates back to 1892 when Dmitri Ivanovsky identified the tobacco mosaic virus, marking the first recognition of these infectious entities as smaller than bacteria (Wei, 2014). Since then, the classification of viruses as either living or non-living organisms has remained a subject of debate due to their unique characteristics, which exhibit properties of both biological life and inanimate entities. This ambiguity presents a significant challenge for scientists in developing effective antiviral therapies. Throughout history, numerous viral epidemics have caused widespread mortality and posed persistent threats to global health. Viruses continue to emerge with increasing transmissibility and pathogenicity, leading to more severe and widespread outbreaks.

Viruses are obligate intracellular parasites, incapable of independent replication. Instead, they rely on host cells—such as human or animal cells—to complete their replication cycle. They utilize the host's ribosomal machinery to translate their genetic material and propagate within the infected cells (Alcami et al., 2002; Carty et al., 2021). Structurally, viruses are non-cellular entities encased in a protective protein coat called the capsid, which can either be enveloped with a lipid membrane containing protein spikes or non-enveloped. The viral core contains either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), which serves as the genetic blueprint for replication and infection (Figure 1).

In this review, we will explore key aspects of virology, including the interaction between viruses and the human immune system, virus classification, risks associated with viral mutations, and the role of sustainability in virological research. Additionally, we will discuss advanced chromatographic techniques and their significance in mitigating future pandemics.

3. Virus Types and Structure

All viruses share a common mechanism of survival: they invade host cells, replicate, and spread to new hosts. Viral transmission occurs rapidly, with newly derived viral strains emerging continuously through mutations (Carty et al., 2021; Rouse & Sehrawat, 2010). Some viral infections exhibit seasonal patterns, such as influenza A and B, while others cause lifelong diseases, including human immunodeficiency virus (HIV), hepatitis, and mumps (Weber et al., 2006; Alcami & Koszinowski, 2000) (Figure 3). Given the vast diversity of viruses and their rapid evolutionary changes, accurate classification is crucial for advancing virological research and improving diagnostic and therapeutic strategies (Valkenburg et al., 2011).

Viruses are classified based on several characteristics, including genome type, replication strategy, and host interactions. Common traits among viruses include their small size, genetic material composition, parasitic nature, and replication mechanisms (Louten, 2016). A significant breakthrough in viral classification was achieved by David Baltimore, who was awarded the Nobel Prize in 1975 for developing the Baltimore classification system based on viral genome types and replication strategies (Coffin & Fan, 2016). This system categorized viruses into seven groups:

- Double-stranded DNA (dsDNA) viruses
- Single-stranded DNA (ssDNA) viruses
- Double-stranded RNA (dsRNA) viruses
- Positive-sense single-stranded RNA (+ssRNA) viruses
- Negative-sense single-stranded RNA (-ssRNA) viruses
- Retroviruses (RNA viruses that reverse transcribe into DNA)
- Hepadnaviruses (partially double-stranded DNA viruses that use reverse transcription) (Dugga & Emerman, 2006; Chappell & Dermody, 2015).

The Baltimore classification remains a fundamental tool for virologists, enabling a systematic approach to studying virus behavior and developing antiviral treatments and vaccines. Understanding the classification of viruses aids in identifying potential therapeutic targets, preventing mutations, and controlling the spread of infections.

Structurally, most viruses exhibit a helical or icosahedral capsid arrangement, with some possessing a more complex architecture (Louten, 2016). The internationally accepted classification of viruses considers key parameters, including chemical composition, shape, size, structural components, genomic organization, and mutation patterns (Simmonds et al., 2017; Adams et al., 2015) (Figure 2, Figure 4)). These structural and genomic distinctions provide critical insights into viral pathogenesis and inform the development of targeted antiviral therapies.

In conclusion, viruses remain a dominant force in global health challenges, with their rapid evolution necessitating ongoing research and innovation in diagnostics and therapeutics. A deeper understanding of viral structure, classification, and replication strategies is essential for developing effective treatments and containment measures against viral diseases. Future advancements

in virology, including chromatography techniques, hold promise for better management and prevention of viral pandemics.

Viruses are responsible for numerous diseases, exhibiting pathogenicity similar to that of microbial infections. They have a high frequency of occurrence in most societies. Examples of viruses that infect only one species throughout life include Human Immunodeficiency Virus (HIV), measles virus, and Human Papillomavirus (HPV) (Alcami and Koszinowski, 2000). In contrast, zoonotic infections, such as Ebola, avian influenza, rabies, and parasitic zoonoses like toxoplasmosis, can transmit between animals and humans (Saéz et al., 2015; Hussein, 2023). Zoonotic viruses are particularly dangerous, causing hundreds of human fatalities and severe outbreaks in animal populations, potentially leading to pandemics or endemics (Hussein, 2023).

Viruses can be categorized based on infection duration and severity. Some viruses cause mild symptoms lasting between two and ten days (e.g., influenza), whereas others lead to severe complications (e.g., Ebola) or lifelong infections (e.g., HPV and HIV) (Saéz et al., 2015; Alcami and Koszinowski, 2000). Certain viruses, such as herpesviruses (HSV-6, HSV-7), can remain latent in the body and re-emerge when the immune system is compromised, often without initial symptoms (Cirone et al., 2007; Alibek et al., 2014). One of the most distinctive viral diseases is acquired immune deficiency syndrome (AIDS), caused by HIV, a retrovirus with unique properties and a complex life cycle, making the development of effective antiviral treatments challenging (Jiang et al., 2020).

Viral Transmission and Adaptation

Viruses can develop more aggressive traits when they infect new host species, resulting in severe symptoms and increased virulence. Examples include anthrax, tularemia, avian influenza, West Nile virus, and bovine spongiform encephalopathy (Ghasemzadeh and Namazi, 2015; Alcami and Koszinowski, 2000). Cross-species transmission often leads to pandemics, as seen with avian flu, swine flu, and other influenza strains (Walters, 2014; Kong et al., 2021).

Pandemics caused by viruses have historically led to significant mortality and economic disruption. Examples include:

- The 2009 swine flu (H1N1) pandemic, infecting 60 million people.
- The 1957 Asian flu, which resulted in over a million deaths.
- The 1918 Spanish flu, which killed more than 50 million individuals.
- The 1957 influenza A (H2N2) outbreak, leading to over a million deaths (Walters, 2014; Long et al., 2019; Sampath et al., 2021).

More recently, the COVID-19 pandemic (2019-2023) has had far-reaching effects on global economies, healthcare systems, and psychological well-being (Attiyah et al., 2020; Sampath et al., 2021). The spread of COVID-19 was influenced by different waves of infection, varying in intensity worldwide (Armenta-Castro et al., 2025).

3.1. Virus Mutation and Environmental Impact

The severity of COVID-19 and other RNA viruses arises from their high mutation rates, making them more adaptable and often more dangerous than DNA viruses. Environmental factors, including climate change, contribute to the increasing transmission of viruses from animals to humans (Long et al., 2019). Research has shown that environmental shifts and human population growth have exacerbated viral spillover events (Guihot et al., 2022; Meadows et al., 2023).

Despite these changes, the fundamental viral life cycle remains consistent:

- Attachment and penetration of the host cell.
- Synthesis of nucleic acids within the invaded cell.
- Replication and assembly of new viral particles.
- Release of new viral progeny.

3.2 Viral Structure and Morphology

Virus morphology and size play crucial roles in infection dynamics. Large, spike-bearing viruses like coronaviruses are difficult to detect and inhibit due to their structural complexity. Viral mutations generate new variants with altered characteristics, making pandemic preparedness challenging (Prasad and Schmid, 2012; Cueno and Imai, 2021).

A virus's structural components provide protection against environmental conditions. The outer envelope defines its appearance, with common shapes including:

- Helical (e.g., tubular structures with capsomers)
- Spherical (with or without protein spikes)
- Polyhedral
- Complex or mixed morphologies (Simmonds et al., 2017; Adams et al., 2015).

A thorough understanding of viral classification, transmission, and structural properties is essential in developing effective antiviral treatments and preventive measures. Ongoing research into virus-host interactions, mutation rates, and environmental factors will play a pivotal role in future pandemic control and mitigation efforts.

3.3 Seasonal Variation of Influenza Viruses

Influenza viruses exhibit seasonal variation, with peak activity observed at specific times of the year. This pattern is primarily influenced by factors such as temperature, humidity, and human behavior, which collectively contribute to the circulation of different influenza strains. As a result, new versions of the virus emerge annually due to antigenic drift and these seasonal fluctuations (Kong et al., 2021). The generation of new virus versions each year poses significant challenges for public health authorities, requiring continuous surveillance to monitor changes in circulating strains and assess their potential impact on human

health. Furthermore, vaccine development must account for the virus's evolving nature to ensure effective protection against the most prevalent strains (Smith et al., 2000).

There are four types of influenza viruses—A, B, C, and D—with influenza A being the most commonly encountered type (Paulonis, 2019). However, these viruses undergo substantial genetic changes, resulting in new viral subtypes each year, to which the human immune system may lack prior exposure. This contributes to the occurrence of seasonal epidemics and, occasionally, pandemics (Herbert & Panagiotou, 2022). Pandemic strains typically arise from type A, while type C causes milder flu, and type D affects cattle but does not transmit to humans (Sreenivasan et al., 2019). Influenza A is particularly concerning due to its ability to generate numerous subtypes, including those distinguished by hemagglutinin (H) and neuraminidase (N). Eighteen hemagglutinin subtypes (H1–H18) and eleven neuraminidase subtypes (N1–N11) have been identified (Wang et al., 2019; Sreenivasan et al., 2019). Moreover, around 130 different subtypes of influenza A, particularly those associated with avian species, highlight the virus's genetic diversity (Zhang et al., 2022).

3.4 The Sustainable Demand for Controlling Seasonal Viral Infections

The concept of sustainability, commonly addressed within healthcare, aligns well with the broader objectives of the health sector. This paper advocates for the application of this term specifically in the context of viral infection studies. Due to the constant mutation of viruses and the seasonal nature of influenza, which periodically leads to pandemics, epidemics, or localized outbreaks, sustainability provides a fitting framework. It effectively captures the ongoing, cyclical process of viral evolution, from historical outbreaks like cholera in 1883 to the current coronavirus pandemic of 2024. This sustainability concept underscores the continuous efforts by scientists over more than two decades to manage and mitigate the impact of seasonal viral infections.

4. Varieties of Virus Isolation Procedures

The success of virus isolation depends on several factors, including the method of sample collection, the stage of the viral infection, accurate sample handling, and proper storage conditions. The first step in the process involves selecting the appropriate sample type, which is based on the suspected virus and the pathogenesis of the infection. Common sample types include spinal cord and cerebrospinal fluid (CSF) for neurological diseases, broncho-alveolar lavage for respiratory diseases, nasal and oral swabs, and brain tissue for specific infections. The timing and location of sample collection are critical, with the optimal time being during the early phase of infection, when the virus concentration is highest. Fresh samples are preferred for virus isolation, as they yield more reliable results (Effio & Hubbuch, 2015). If viral transport media is unavailable, swabs should be placed immediately in a sterile tube containing saline to maintain sample integrity. It is important to note that fixed tissues are unsuitable for virus isolation.

Following sample collection, virus separation, purification, and identification are key steps in establishing an accurate diagnosis and determining an appropriate treatment plan. Virus isolation techniques are fundamental for separating the virus from a host organism or sample to study its properties, behavior, and potential impact. The isolation process is considered one of the most definitive methods for identifying viral infections, as it directly demonstrates the presence of the virus. This is essential for the development of vaccines and therapeutic treatments, as it provides a clear basis for understanding the virus's characteristics (Effio & Hubbuch, 2015).

4.1 Virus Isolation Methods: Key Techniques and Their Applications

Virus isolation is a crucial step in the diagnosis and study of viral infections, offering vital information for understanding the virus's characteristics, behavior, and impact. Several virus isolation methods are commonly employed by researchers and healthcare professionals, each with unique advantages and applications. Below is a detailed overview of these key techniques.

Cell Culture

Virus culture isolation, often referred to as the "gold standard," remains the most reliable and specific technique for virus identification. This method provides 100% specificity, offering definitive evidence of the presence of live virus particles in a controlled environment (Alexander et al., 2020). The technique requires living host cells for viral replication and can be performed in vivo (within living organisms or tissues) or in vitro (outside living organisms in cell cultures). Primary cell cultures, derived from human or animal organs and tissues through mechanical scraping or enzymatic methods, are typically used (Fehr & Perlman, 2015; Hematian et al., 2016). However, these cultures have limited lifespans and require regular subculturing and periodic dilution to maintain growth and prevent contact inhibition.

Molecular Techniques

Polymerase chain reaction (PCR) is an essential molecular technique for virus isolation. PCR allows the amplification of viral genetic material in a sample, enabling the identification of the virus even at low concentrations (Cassedy et al., 2021). This method is particularly useful for detecting viral RNA or DNA in clinical samples, offering high sensitivity and specificity for virus identification (Zhu et al., 2020).

Animal Models

Animal models are often used in virus isolation to study the pathogenicity and transmission dynamics of a virus. Researchers

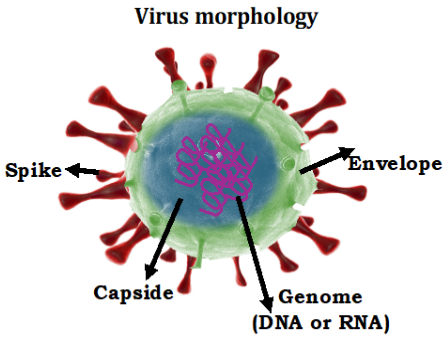

Figure 1. Virus morphology. The core of virus comprises DNA or RNA covered by protein coat.

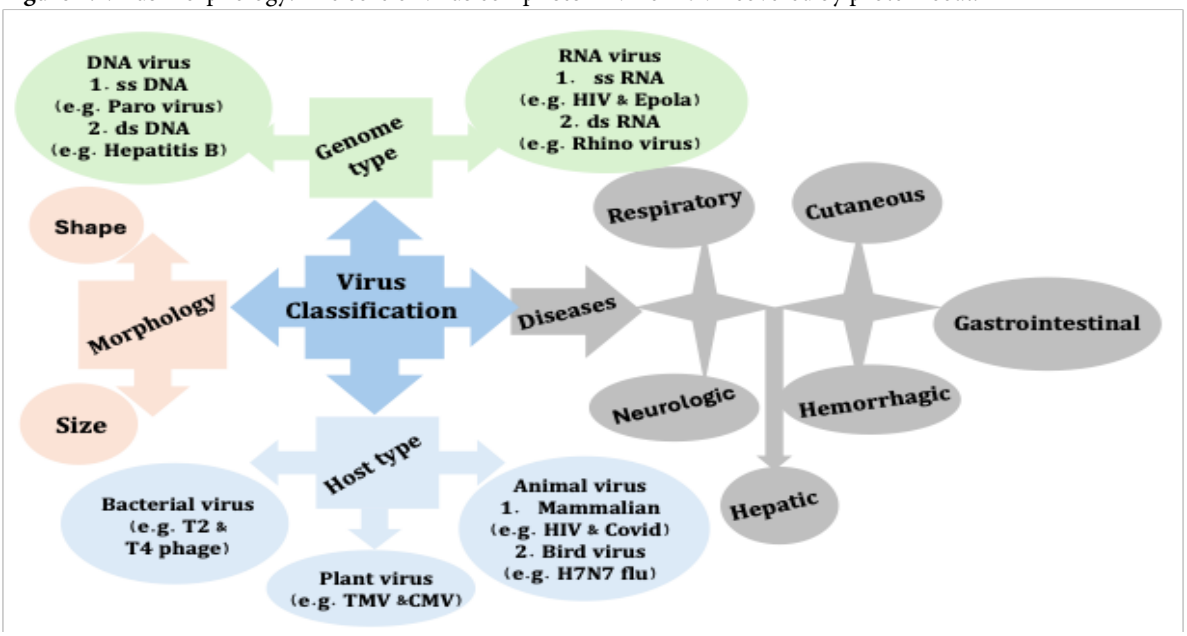

Figure 2. Virus basic international classification. Several virus categories demonstrate in the diagram based on diseases, genome type, host type, and virus morphology.

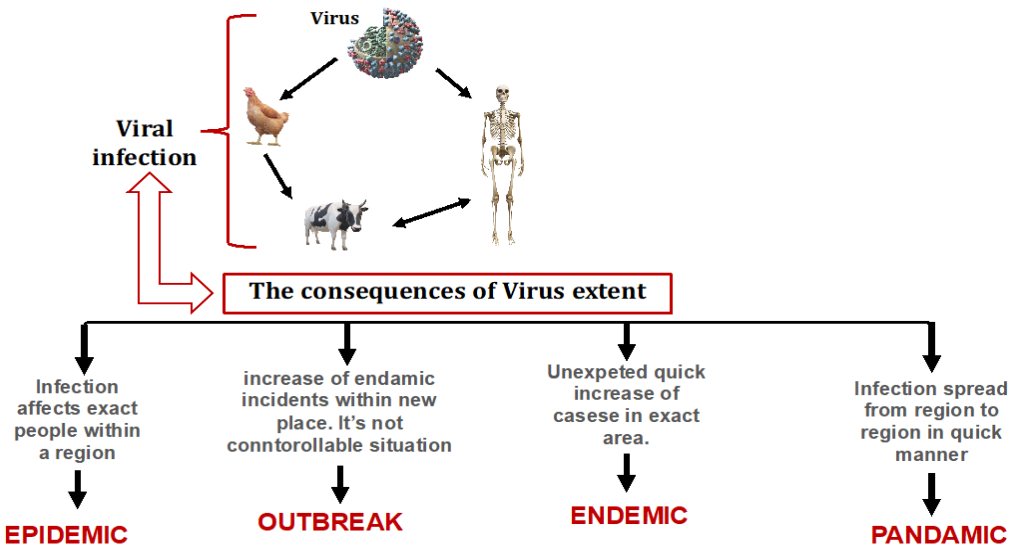

Figure 3. The consequences of viral infection. Virus transmission action between species. Virus transmits from animal to human or vies verse causing pandemic or endemic depend on virus extent degree and region.

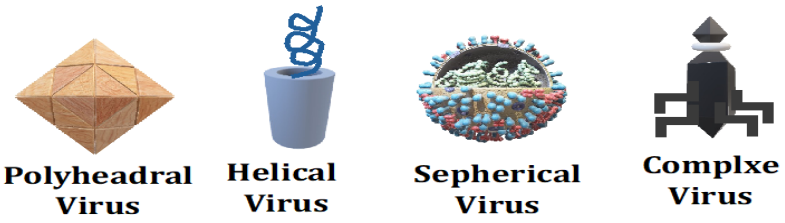

Figure 4. Virus variable shapes and size.

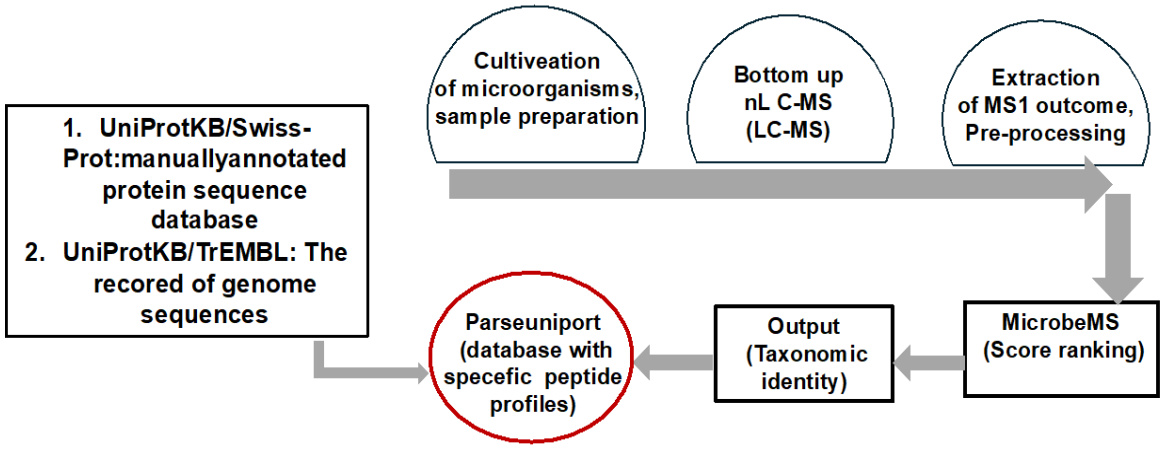

Figure 5. Shot gun proteomics applied for colony sample derived from flask microbial culture. UniProtKB/Swiss-Prot and UniProtKB/TrEMB used for subsequent matching process against bacteria library to identify the exact molecular weight and species. (Depect from: (Lasch et al., 2020).

inoculate animals with a suspected viral sample and monitor them for infection signs. This technique provides valuable insights into how the virus behaves in a living organism, which aids in understanding its virulence and spread.

Serological Assays

Serological assays detect antibodies produced by the immune system in response to a viral infection (Herbert & Panagiotou, 2022). These assays are particularly useful for identifying specific antibodies against a particular virus in a patient's blood sample, thus confirming previous or ongoing infections.

Immunofluorescence Assays

Immunofluorescence assays utilize fluorescently labeled antibodies to detect viral antigens in infected cells or tissues. This method allows for the localization of viruses within samples, providing visual confirmation of their presence through fluorescence signals (Cassedy et al., 2021). Immunofluorescence is widely used for rapid detection and is particularly effective for identifying viruses in tissue samples.

Electron Microscopy

Electron microscopy is a powerful tool for directly visualizing viruses. By using electron microscopes, researchers can observe viral particles in clinical samples, allowing for rapid identification based on their unique morphological features (Goldsmith & Miller, 2009). This method is particularly useful for identifying novel or previously uncharacterized viruses.

Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) technologies have revolutionized virus isolation by enabling high-throughput sequencing of viral genomes present in complex samples. NGS helps characterize novel viruses, track their evolution, and understand their genetic makeup (Sandybayev et al., 2022). This technique is invaluable for studying viral diversity and uncovering new viral strains.

Filtration and Centrifugation

Filtration and ultracentrifugation are commonly used to concentrate isolated viruses, especially influenza viruses and bacteriophages. Ultracentrifugation (>100,000 g) is a routine laboratory technique for virus separation, offering reliable and efficient results (Kutner et al., 2009; Sugita et al., 2011). Filtration, on the other hand, is an effective method for isolating and concentrating viruses with high separation quality (McNamara et al., 2018a; Corso et al., 2017; Busatto et al., 2018).

Chromatography Columns

Chromatography columns provide an excellent method for isolating viruses based on their molecular properties, including size, charge, and hydrophobicity. The development of chromatography techniques has enhanced the efficiency and quality of virus isolation, allowing for improved separation and purification (Baranyai et al., 2015). Various categories of column chromatography have been developed to optimize virus isolation, making this method particularly useful for studying viral proteins and other molecular components (Blom et al., 2014; Davis et al., 2019).

4.2 Classification of Virus Diagnosis: Methods and Techniques

Virus diagnosis is a critical component of healthcare, enabling the identification and study of viral infections. Various diagnostic methods are employed to detect viruses, each with its strengths and applications. These techniques can be broadly classified into biosensor and immunological approaches. Below is a comprehensive discussion of both, along with their specific diagnostic applications.

4.2.1 Biosensor Techniques

Biosensors are analytical devices that integrate biological components with physicochemical transducers, offering sensitive, specific, and rapid detection of analytes, including viruses. They generate a signal upon binding to the target analyte, making them invaluable for virus detection.

a. Electrochemical Biosensors

Electrochemical biosensors work by binding a target analyte to a bioreceptor. This interaction leads to a chemical reaction, adsorption, or other processes, which the transducer converts into an electrical signal detected by the instrument. These biosensors are highly specific and have been used for detecting a wide range of components, such as nucleic acids, enzymes, and proteins (Burrell et al., 2017; Pohanka, 2018). Electrochemical biosensors are widely used for viral detection due to their sensitivity, low cost, and ease of use (Imran et al., 2021).

b. Piezoelectric Biosensors

Piezoelectric biosensors, such as the quartz crystal microbalance (QCM), are commonly employed due to their excellent specificity and maneuverability. In these sensors, biomolecules are immobilized on a vibrating crystal surface, and the interaction with the target analyte causes a change in the oscillation frequency. A decrease in frequency indicates a greater binding response and, consequently, a higher mass. This method allows for highly sensitive and specific detection of viral proteins (Narita et al., 2021).

c. Optical Biosensors

Optical biosensors are among the most widely used types of biosensors. These sensors detect changes in optical behavior on the transducer surface when an analyte binds with a bioreceptor. Optical transducers such as surface plasmon resonance (SPR) sensors, surface-enhanced Raman scattering (SERS), and fluorophores are employed to monitor these binding events in real time. These biosensors are particularly useful for detecting viral antigens in clinical samples, allowing for label-free detection (Nandi et al., 2020).

4.2.2 Immunological Techniques

Immunological techniques are based on the detection of specific antibodies or antigens associated with viral infections. These methods are widely used due to their specificity, sensitivity, and ability to detect a variety of viral infections.

a. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a widely used immunological method for detecting viral antigens or antibodies in a sample. The technique involves the binding of an antigen to a specific antibody, followed by the addition of an enzyme-linked secondary antibody. When a chromogenic substrate is added, a color change occurs, which is directly proportional to the amount of antigen-antibody complex formed. ELISA is valued for its sensitivity, simplicity, and ability to provide rapid results, making it ideal for large-scale diagnostic screening (Harsh & Tripathi, 2023). Common types of ELISA include sandwich ELISA (antigen-capture) and indirect ELISA (antibody-capture) (Alhajj et al., 2024).

b. Western Blotting

Western blotting is a versatile technique used for the detection of viral proteins. Proteins from a sample are separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a membrane. The membrane is incubated with antibodies specific to viral proteins. A chromogenic substrate is applied, resulting in the appearance of colored bands corresponding to viral antigens. This technique is commonly used for confirming viral infections, particularly in proteomics research, due to its high specificity and ability to detect multiple viral strains (Yang et al., 2018; Xing et al., 2022).

c. Immunofluorescence Assay (IFA)

Immunofluorescence assays are used to detect viral antigens within infected cells or tissues. This method involves the use of antibodies labeled with fluorescent dyes, which bind to viral antigens. The presence of the viral antigen is detected by fluorescence microscopy. There are two types of IFA: direct and indirect. In the direct method, a labeled primary antibody binds directly to the target antigen, whereas in the indirect method, a labeled secondary antibody binds to a non-labeled primary antibody. The indirect method is more sensitive but requires additional steps (Kvinesdal et al., 1989; Im et al., 2019).

d. Lateral-Flow Immunoassay (LFIA)

The lateral-flow immunoassay (LFIA) is a rapid and simple diagnostic technique commonly used for detecting viral proteins and nucleic acids. LFIA typically uses labeled antibodies to bind to viral antigens in a sample, producing a color change at a test line that can be easily seen with the naked eye. The sandwich format, where the antibody captures the antigen between two lines, is commonly used for virus detection. LFIA is widely employed in field diagnostics, as it is portable, cost-effective, and provides results in a short amount of time (Sajid et al., 2015; Molina et al., 2013; Si et al., 2019).

5. Molecular Techniques in Virus Diagnosis

Molecular techniques are pivotal in the field of diagnostic virology due to their unparalleled sensitivity, specificity, and speed in detecting viral infections. These methods primarily focus on nucleic acid-based amplification, which enables the detection of viral genetic material even in very low quantities. Among the most widely used molecular techniques are polymerase chain reaction (PCR) and its derivatives, which have revolutionized the identification of viruses in clinical and research settings (Tesfaye et al., 2020).

5.1. Nucleic Acid-Based Amplification Techniques

Nucleic acid-based amplification methods are highly sensitive and specific, allowing for rapid diagnosis and the simultaneous detection of multiple viruses. These techniques are especially beneficial for detecting viruses that are difficult to culture, grow slowly, or exhibit antigenic variants. They also enable the identification of viruses that are otherwise challenging to isolate in laboratory settings, providing critical insights for both patient care and public health (Hodinka & Kaiser, 2013).

5.2 Real-Time Quantitative Polymerase Chain Reaction (qPCR)

Polymerase chain reaction (PCR) is a standard method for amplifying and analyzing DNA. The process involves extracting and purifying DNA, followed by amplification of a target sequence using a thermo-stable DNA polymerase and specific primers. After amplification, various methods such as gel electrophoresis, colorimetric techniques, or sequencing are employed to identify the amplified product. Real-time PCR, or quantitative PCR (qPCR), allows for the continuous monitoring of the amplification process, making it possible to quantify the viral load in a sample. Reverse transcription-PCR (RT-PCR), a variant of qPCR, is often used to amplify RNA viruses by converting their RNA into complementary DNA (cDNA) before amplification (Souf, 2016; Sundaramurthy et al., 2018). qPCR is particularly advantageous due to its sensitivity, speed, and ability to provide quantitative results, making it one of the most widely used molecular techniques for viral diagnostics.

5.3 Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is an amplification method that operates under constant temperature, eliminating the need for thermal cycling. LAMP uses a set of four to six primers that bind to the target DNA and initiate the polymerase-driven extension of the gene sequence. The amplification process is facilitated by the formation of stem-loop structures that serve as primers for further polymerization. LAMP can generate large quantities of DNA within a short period and is known for its high specificity and efficiency, making it suitable for field diagnostics where rapid and accurate results are needed (Notomi et al., 2000). This method has gained popularity for the detection of viruses in

resource-limited settings due to its simplicity and ability to operate without sophisticated laboratory equipment.

5.4 Recombinase Polymerase Amplification (RPA)

Recombinase polymerase amplification (RPA) is a relatively new nucleic acid amplification method that employs recombinase enzymes to locate target DNA regions. This technique uses a combination of recombinase proteins, single-stranded DNA binding proteins, and a strand-displacing polymerase to amplify the target DNA at a constant temperature, typically around 37–42°C. RPA is known for its rapid amplification and high sensitivity, making it ideal for detecting low-abundance viral DNA in clinical samples. The ability to amplify DNA without the need for thermal cycling makes RPA a useful tool in portable diagnostic devices (Lobato & O'Sullivan, 2018).

5.5 Helicase-Dependent Amplification (HDA)

Helicase-dependent amplification (HDA) is another isothermal amplification technique that uses a DNA helicase to unwind double-stranded DNA, enabling the amplification of the target sequence. HDA relies on flanking primers that bind to the unwound DNA strands, followed by amplification using a polymerase enzyme. This method offers several advantages, including faster amplification and a simplified assay design compared to traditional PCR. HDA does not require the high temperatures typically used in PCR for DNA denaturation, making it more energy-efficient. It also allows for the use of fluorescent dyes and lateral flow-based detection methods to visualize the amplified products (Kolm et al., 2019; Barreda-García et al., 2019).

5.6 Rolling Circle Amplification (RCA)

Rolling circle amplification (RCA) is designed to amplify circular DNA sequences and mimics the natural replication process of circular DNA. This method uses a single primer to initiate the replication of the circular DNA template, resulting in the generation of long, repetitive DNA sequences. RCA can be used for both DNA amplification and the detection of specific viral genomes. Although the standard RCA method does not provide exponential amplification, modifications such as hyper-branched RCA can be employed to enable faster, exponential amplification. RCA is particularly useful for detecting viruses with circular genomes and has been adapted for various diagnostic applications (Mohsen & Kool, 2016; Schweitzer & Kingsmore, 2001).

5.7 DNA Microarrays

DNA microarrays are high-throughput tools used to analyze gene expression and detect viral genomes. In DNA microarray diagnostics, fluorescently labeled viral nucleic acids are hybridized with a collection of oligonucleotide probes immobilized on a surface, such as glass or silicon. The probes are designed to specifically bind to viral sequences, and the fluorescence emitted upon binding is used to measure the interaction. DNA microarrays are highly versatile and can simultaneously detect multiple viruses in a single assay. This makes them particularly useful in viral surveillance and research, where large-scale screening is required (Bumgarner, 2013; Martínez et al., 2015).

6. The Involvement of Chromatography Procedures in Viral Infection Diagnosis and Treatment

Chromatography techniques have made significant strides in clinical research, particularly in the health care sector, where they have contributed to improved global health outcomes (Darie-Ion et al., 2022). These methods are essential for the identification and quantification of biological components due to their reliability, speed, and high accuracy. Chromatography is widely used in various omics-based disciplines, such as lipidomics, proteomics, peptidomics, metabolomics, and genomics, which have revolutionized medical and industrial science due to their sensitivity, reproducibility, and cost-effectiveness. These advancements have had notable applications in clinical research, including the study of cardiovascular, respiratory, and kidney diseases, as well as food and nutrition research.

In the realm of cardiovascular diseases, chromatography has played a pivotal role in the discovery of biomarkers for coronary vascular disease (CVD). Metabolomic and proteomic approaches have enabled the identification of metabolites and proteins related to CVD, offering valuable insights into the diagnosis, treatment, and prevention of heart disease (Lewis et al., 2008; Brindle et al., 2002). Significant atherosclerosis biomarkers that aid in prevention and improve patient health outcomes have been identified using chromatography, including studies that demonstrate the relationship between CVD and adipose tissue using liquid chromatography (LC) and gas chromatography-mass spectrometry (GC-MS) (Müller et al., 2021; Zhang et al., 2018; Sun et al., 2007; Griffin & Castro, 2019) (Figure 5).

While chromatography relies on chemical composition and molecular size to separate and analyze samples, its use in liquid chromatography-mass spectrometry (LC-MS) does not necessarily require the sample to be of the highest integrity or purity, unlike other techniques such as nuclear magnetic resonance (NMR) spectroscopy or structural biology methods (Hazu et al., 2022). This characteristic makes chromatography a promising tool for microbial pathogen detection and treatment, despite certain limitations when analyzing microorganisms. In contrast, techniques like spectroscopy and NMR require samples of much higher purity and integrity. Chromatography's adaptability, as evidenced in previous studies, has addressed some of these limitations, improving its utility for pathogen detection (Lasch et al., 2020; Lerer et al., 2021).

Chromatographic techniques have increasingly been employed in the study of microbial infections, including those caused by viruses, bacteria, and fungi. However, these methods are still underutilized

in viral pathogen detection and treatment. While researchers have yet to fully leverage chromatography in viral research, its potential is vast, and its application could greatly enhance virus diagnosis and therapeutic strategies (Robert et al., 2018; Mercuro et al., 2011). Several challenges remain, including time-consuming procedures and poor recovery rates for large molecular weight proteins, such as those found in viruses like coronavirus (Lasch et al., 2020; Lerer et al., 2021). These factors have hindered the widespread use of chromatography for viral studies, but ongoing adjustments to chromatographic conditions could help mitigate these issues.

Several chromatographic methods, including size-exclusion chromatography (SEC), ion-exchange chromatography (IEC), and affinity chromatography (AC), are already employed in viral infection studies. These methods have been especially effective in virus purification, with some types yielding excellent results while others offer less favorable outcomes (Mercuro et al., 2011; Brindle et al., 2002). The need for alternative diagnostic methods beyond PCR has prompted researchers to fine-tune chromatography techniques to better suit the unique properties of viruses, which has led to significant improvements in the quality of results. For example, chromatography has been successfully used to purify Adeno-associated virus (AAV) after optimizing several chromatographic factors (Bogdanovic et al., 2025).

Chromatography offers valuable advantages in the study, detection, and treatment of viral infections. Its ability to work with lower-purity samples and provide rapid results makes it a promising tool in the fight against viral diseases. As the methodology continues to evolve, it holds the potential to complement or even surpass traditional viral diagnostic methods, such as PCR, in certain clinical settings.

7. The Role of Chromatography in Viral Infection Research and Diagnosis

The application of chromatography in viral infection research and vaccine development has been growing significantly in recent years. A key recommendation for advancing seasonal influenza vaccine discovery using High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) was made by the Transfiguracion group in Canada in 2014. Their successful investigation into influenza viruses A and B demonstrated the effectiveness of this technique for viral research (Transfiguracion et al., 2014). The link between drug development and chromatography has been explored in previous studies, with evidence suggesting the value of chromatography in diagnosing and treating viral infections (Hazu et al., 2022; Enrico & Alessandro, 1996; Zhang et al., 2018).

Despite the advantages of liquid and gas chromatography in diagnosing viral infections, drug discovery, and industrial applications, membrane chromatography-based downstream processing platforms (MCBDPP) have proven to be the most compatible method for isolating high molecular weight proteins, such as the spike protein found in viruses (Ghosh, 2002; Chen et al., 2022). The use of MCBDPP has shown to achieve protein purification and separation with an efficiency of 95.5–99.7% for large molecules (molecular weight >250,000). This technique has been successfully applied in the purification of H1N1 and H7N9 influenza viruses, yielding high-quality results (Ghosh, 2002; Chen et al., 2023). However, some studies, such as those by Tan and colleagues in 2015, reported suboptimal results when chromatography was used to isolate large proteins like hepatitis B virus-like particles. About 50% of these proteins were lost during the chromatographic process, a finding that was consistent with similar issues encountered with coronavirus proteins in 2021 (Weber et al., 2006; Tan et al., 2015; Lerer et al., 2021).

Techniques such as MALDI-TOF-MS and HPLC-MS have been widely used in microorganism studies, though challenges remain, particularly in terms of the time-consuming nature of LC-MS in detecting strain-specific peptides. To overcome this issue, the bottom-up proteomics approach linked with Swiss-Prot and TrEMB, accessible via the UniProt Knowledgebase, has been employed to enhance efficiency and accuracy (Boulund et al., 2017; Zougman et al., 2014; Lasch et al., 2020). The bottom-up proteomics method has proven to be a highly effective and fast system, producing results in less than two minutes per sample. Lasch and colleagues (2020) recommended LC-MS fingerprinting as a significant, reliable, and reproducible system for analyzing microorganisms. In a published study, they demonstrated a workflow for bottom-up proteomics, identifying 39 peaks from a test set of 19 different pathogens in just 38 to 40 minutes (Zougman et al., 2014; Lasch et al., 2020).

In India, a research group highlighted the powerful diagnostic potential of chromatography for COVID-19 detection. They advocated for alternative diagnostic methods to real-time reverse transcription-polymerase chain reaction (rRT-PCR) to address shortages in PCR supplies during the pandemic (Gupta et al., 2021). Their chromatography-based approach demonstrated high specificity (99.6%) and adequate sensitivity (81.8%) for diagnosing SARS-CoV-2, showcasing the method's reliability and potential in viral diagnostics (Gupta et al., 2021; Mistry et al., 2022). Chromatography and mass spectrometry tools, combined with resins, have been instrumental in maintaining the quality of downstream chemical analysis, particularly for peptides derived from proteins of varying lengths (Hazu et al., 2022).

Several chromatography methods have been successfully employed in COVID-19 research, including Spray Mass Spectrometry (PS-MS), HPLC-MS/MS, Thin Layer Chromatography (TLC) densitometry, and Micellar Liquid Chromatography (MLC), all yielding valuable results (Khalil et al., 2023). However, challenges arose due to the large size of the spike protein in SARS-CoV-2,

which deeply interacts with the absorbent surface of membrane adsorbers and packed-bed ion-exchange chromatography columns, leading to lower virus recovery rates. In one study, virus recovery rates were improved from 15% to 33% when packed-bed chromatography was used. Furthermore, the use of Capto™ Core 700 resin resulted in over 85% protein recovery for the rVSV-S virus, both with and without ultrafiltration phases (Lerer et al., 2021; Guihot et al., 2022).

Chromatography, particularly HPLC-MS and its various forms, has become an essential tool in viral infection research and diagnostics. It holds great promise for future applications in viral vaccine development and infection management, especially in light of its ability to purify high molecular weight proteins, such as the spike proteins of viruses like influenza and SARS-CoV-2.

Chromatography, particularly Liquid Chromatography-Mass Spectrometry (LC-MS), has demonstrated its capability in diagnosing viral infections, including the detection of SARS-CoV-2. Spicka et al. (2021) showed that LC-MS could effectively diagnose the coronavirus using patient skin swabs, highlighting its potential for non-invasive diagnostic approaches. Additionally, the detection of COVID-19 biomarkers from exhaled breath, derived from positive PCR samples, was successfully achieved using Gas Chromatography-Mass Spectrometry (GC-MS) (Ibrahim et al., 2021; Service, 2022). These findings underscore the evolving role of chromatography in viral diagnostics, further emphasizing its versatility in identifying viral markers across different sample types.

One of the most influential chromatography techniques is Reversed Phase Chromatography-Mass Spectrometry (RPC-MS), which has been widely utilized in various fields, including food analysis, vaccine development, and drug discovery. In 2024, Peruri et al. reported a successful application of RPC-MS in the purification, identification, and quantification of virus-like particles (VLPs). Their research demonstrated the high sensitivity and accuracy of RPC-MS, making it a powerful tool for viral research and related biomedical applications (Peruri et al., 2024).

Metabolomics, a critical subset of chromatography techniques, plays a crucial role in understanding the metabolic mechanisms underlying diseases, treatments, and drug effects. Both LC-MS and GC-MS are fundamental in identifying and investigating metabolic changes associated with various health conditions. For example, in a study conducted by Chen et al. (2023), the serum metabolites of COVID-19 patients were analyzed, revealing significant fluctuations compared to control samples. Specifically, eight metabolites remained unchanged, eighteen metabolites were decreased, and fifty-three metabolites increased. Notably, the study identified the elevated levels of lactic acid, 1-monopalmitin, and cholesterol as important biomarkers of inflammation in COVID-19 patients. These findings highlight the potential of chromatography techniques to detect disease-specific metabolic alterations and their value in understanding disease mechanisms (Chen et al., 2023).

The importance of chromatography in viral disease research cannot be overstated. It offers a method that delivers speed, accuracy, and consistent results, making it indispensable in the scientific and healthcare sectors. Although certain limitations related to virus investigations remain, such as challenges with large protein recovery and virus purification, previous studies have presented solutions that enhance the efficiency of chromatography in viral research. As demonstrated in the studies reviewed here, continuous advancements in chromatography technology and methodology are addressing these challenges, making it an even more reliable and sustainable approach for studying viral infections.

Chromatography has proven to be an essential tool in the diagnosis, investigation, and treatment of viral diseases. It provides reliable and reproducible results, helping to bridge the gaps in knowledge and offering a means to control viral mutations and their spread. The future of viral research will undoubtedly benefit from the ongoing development and refinement of chromatography techniques, offering new avenues for diagnostics, therapeutic development, and disease management.

8. Conclusion

In conclusion, virus classification plays a crucial role in advancing research and understanding viral mutations, aiding in the timely and accurate detection of infections. While nucleic acid-based diagnostics offer high sensitivity and selectivity, immunoassays remain valuable due to their simplicity and affordability, though they face accessibility challenges in resource-limited regions. Chromatographic techniques, particularly column chromatography, emerge as promising solutions for cost-effective virus detection and isolation. However, no single technology currently addresses all detection needs. Continued development of innovative approaches, combining traditional biological methods and advanced technologies, is essential to bridge gaps in virology detection, isolation, and mutation studies.

Author contributions

The author has written the entire manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Adams, MJ., Hendrickson, RC., Dempsey, DM., and Lefkowitz, EJ. (2015). Tracking the changes in virus taxonomy. Arch Virol, PubMed PMID: 25708839.

Alcami, A., and Koszinowski, UH. Viral mechanisms of immune evasion. (2000). Molecular Medicine Today, V6.

Alcami, A., Ghazal, P., & Yewdell, J, W. (2002). Viruses in control of the immune system. EMBO, 3 (10), 927–932.

Alexander, A., Dolskiy, I, V., Grishchenko and Dmitry, V, Y. (2020). Cell Cultures for Virology: Usability, Advantages, and Prospects. Int. J. Mol. Sci, 21, 7978.

Alhajj, M., Zubair, M., Farhana, A. (2024). Enzyme Linked Immunosorbent Assay. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 32310382

Alibek, K., Baiken, Y., Kakpenova, A., Mussabekova, A., Zhussupbekova, S., Akan, M., and Sultankulov, B. (2014). Implication of human herpesviruses in oncogenesis through immune evasion and suppression. Infectious Agents and Cancer, 9(3). http://www.infectagentscancer.com/content/9/1/3.

Armenta-Castro, A. , Rosa, O,D,L., , Aguayo-Acosta, A., Oyervides-Muñoz, M, A., Flores-Tlacuahuac, A., Parra-Saldívar, R., and Sosa-Hernández, J, E. (2025). Interpretation of COVID-19 Epidemiological Trends in Mexico Through Wastewater Surveillance Using Simple Machine Learning Algorithms for Rapid. Viruses, 17, 109. https://doi.org/10.3390/v17010109

Attiyah, S, M., Elsayed, H, M., Al-Mughales, J, A., Moharram, A, B., Abdel-Fattah, M. (2020). Critical cases of COVID-19 patients can be predicted by the biomarkers of complete blood count. Indian Journal of Science and Technology,13(48):4739–4745.

Baranyai, T., Herczeg, K., Onódi, Z., Voszka, I., Módos, K., Marton, N., Nagy, G., Mäger, I., Wood, M, J., Andaloussim S., Pálinkás, Z., Kumar, V., Nagy, P., Ferdinandy, P., Giricz, Z. (2015). Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. PLoS One, 10(12). https://doi.org/10.1371/journal.pone.0145686.

Barreda-García, S., Miranda-Castro, R., de-los-Santos-Álvarez, N., Miranda Ordieres, A. J., and Lobo-Castañón, M. J. (2018). Helicase-dependent isothermal amplification: a novel tool in the development of molecular based analytical systems for rapid pathogen detection. Anal Bioanal Chem, 410, 679–693. doi:10.1007/s00216-017-0620-3

Blom, H., Åkerblom, A., Kon, T., Shaker, S., van der, Pol, L., Lundgren, M. (2014). Efficient chromatographic reduction of ovalbumin for egg- based influenza virus purification. Vaccine, 32(30):3721–3724. https://doi.org/10.1016/j.vaccine.2014.04.033

Bogdanovic, A., Donohue, N., Glennon, B., McDonnell, S., Whelan, J. (2025). Towards a Platform Chromatography Purification Process for Adeno-Associated Virus (AAV). Biotechnology Journal, 20,1. https://doi.org/10.1002/biot.202400526.

Boulund, F., Karlsson, R., Gonzales-Siles, L., Johnning, A., Karami N, Al- Bayati, O., Åhrén, C., Moore, ERB., and Kristiansson, E. (2017). Typing and characterization of bacteria using bottom-up tandem mass spectrometry proteomics. Mol Cell Proteomics, 16, 1052–1063.

Brindle, JT., Antti, H., Holmes, E., Tranter, G., Nicholson, JK., Bethell, HWL., Clarke, S., Grainger, DJ. (2002). Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomic. Nat Med, 8:1439-44.

Bumgarner, R. (2013). DNA microarrays: Types, Applications, and their future. Curr Protoc Mol Biol, 1(22).

Burrell, C, J., Howard, C, R., Murphy, F, A. (2017). Laboratory Diagnosis of Virus Diseases. Fenner and White's Medical Virology, 135–54. doi: 10.1016/B978-0-12-375156-0.00010-2. Epub 2016 Nov 11. PMCID: PMC7149825.

Busatto, S., Vilanilam, G., Ticer, T., Lin, WL., Dickson, D, W., Shapiro, S. (2018). Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid. Cells, 7(12). https://doi.org/10.3390/cells7120273

Carty, M., Guy, C., BowieM AG. (2021). Detection of Viral Infections by Innate Immunity. Biochemical Pharmacology, 183 (11) 4316.

Cassedy, A., Parle-McDermott, A., O'Kenned, R. (2021). Virus Detection: A Review of the Current and Emerging Molecular and Immunological Methods. Frontiers in Molecular Biosciences, 8. doi: 10.3389/fmolb.2021.637559

Chappell, J, D., and Dermody, T, S. (2015). Biology of Viruses and Viral Diseases. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 1681–1693.e4. doi: 10.1016/B978-1-4557-4801-3.00134-X.

Chen, J., Yu, B., Cong, H., Shen, Y. (2022). Recent development and application of membrane chromatography. Analytical and Bioanalytical Chemistry, 415, 45–65 https://doi.org/10.1007/s00216-022-04325-8.

Chen, X., Gu, X.,Yang, J., Jiang, Z. (2023). Gas Chromatography–Mass Spectrometry Technology: Application in the Study of Inflammatory Mechanism in COVID-19 Patients. Jianjun Deng Chromatographia, 86, 175–183. https://doi.org/10.1007/s10337-022-04222-3.

Chen, Y., Chan, K, H., Kang Y. et al. (2015). "A sensitive and specific antigen detection assay for Middle East respiratory syndrome coronavirus." Emerging Microbes and Infections, 4(4), e26.

Cirone, M., Lucania, G., Bergamo, P., Trivedi, P., Frati, L., Faggioni, A. (2007). Human herpesvirus 8 (HHV-8) inhibits monocyte differentiation into dendritic cells and impairs their immunostimulatory activity. Immunol Lett, 113(1), 40–46.

Coffin, J, M., and Fan, H. (2016). The Discovery of Reverse Transcriptase. Annual Review of Virology, 3, 29-51.

Corso, G., Mäger, I., Lee, Y., Görgens, A., Bultema J, Giebel, B., Wood, M, J, A., Nordin, J, Z., Andaloussi, S, E, L. (2017). Reproducible and scalable puri- fication of extracellular vesicles using combined bind-elute and size exclusion chromatography. Sci Rep, 7(1), 11561. https://doi.org/10. 1038/s41598-017-10646-x

Cueno, ME., and Imai, K. (2021). Structural Comparison of the SARS CoV 2 Spike Protein Relative to Other Human-Infecting Coronaviruses. Frontiers in Medicine, 7. doi: 10.3389/fmed.2020.594439.

Darie-Ion, L., Whitham, D., Jayathirtha, M., Rai, Y., Neagu, A., Costel, C., and Brîndus, A, P. (2022). Applications of MALDI-MS/MS-Based Proteomics in Biomedical Research. Molecules, 27, 6196. https://doi.org/10.3390/molecules27196196.

Davis, C, N., Phillips, H., Tomes, J, J., Swain, M, T., Wilkinson, T, J., Brophy, P, M., Morphew, R, M. (2019). The importance of extracellular vesicle purification for downstream analysis: A comparison of differential centrifugation and size exclusion chromatography for helminth pathogens. PLoS Negl Trop Dis, 13(2), e0007191. https://doi.org/10.1371/ journal. pntd.0007191

Dugga, N, K., and Emerman, M. (2006). Evolutionary conflicts between viruses and restriction factors shape immunity. Nature Reviews Immunology, 12, 687–695.

Effio, C, L., Hubbuch, J. (2015). Next generation vaccines and vectors: de- signing downstream processes for recombinant protein-based virus- like particles. Biotechnol J, 10(5), 715–727. https://doi.org/10.1002/ biot.201400392

Enrico, T., and Alessandro, B. (1996). High-performance liquid chromatography and identification of mycobacteria. Reviews in Medical Microbiology, 7(4), 207-220.

Fehr, A, R., and Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol, 1282,1–23. doi: 10.1007/978-1-4939-2438-7_1.

Freire, J, M., Santos, N, C., Veiga, A, S., Da Poian, A, T., and Castanho, M, A, R, B. (2015). Rethinking the capsid proteins of enveloped viruses: multifunctionality from genome packaging to genome transfection. FEBS J, 282, 2267–2278. doi:10.1111/febs.13274.

Ghasemzadeh, I., and Namazi, S, H. (2015). Review of bacterial and viral zoonotic infections transmitted by dogs. J Med Life, 8(4), 1–5.

Ghosh, R. (2002). Protein separation using membrane chromatography: opportunities and challenges. J Chromatogr A, 952(1–2), 13–27. https://doi.org/10.1016/s0021-9673(02) 00057-2.

Goldsmith, C, S., and Miller, S, E. (2009). Modern Uses of Electron Microscopy for Detection of Viruses. Clinical Microbiology Reviews, 22(4), 552–563. doi:10.1128/CMR.00027-09

Griffin, J, L., and Castro, C. (2019). NMR-Based Metabolomics in Cardiac Research. Methods Mol Biol, 2037, 189-94. In book: NMR-Based Metabolomics, Methods and Protocols. DOI: 10.1007/978-1-4939-9690-2_11.

Guihot, A., Litvinova, E, L., Autran, B, A., Debr, P. (2022). Cell-Mediated Immune Responses to COVID-19 Infection. Frontiers in Immunology, 11.

Gupta, A., Khurana, S., Das, R., Srigyan, D., Singh, A., Mittal, A., Singh, P., Soneja, M., Patidar, D., Lathwal, A., Dar, L., Mathur, B., & Wig, N. (2021). Rapid chromatographic immunoassay-based evaluation of COVID-19: A cross-sectional, diagnostic test accuracy study & its implications for COVID-19 management in India. Indian J Med Res,153, 126-131. DOI: 10.4103/ijmr.IJMR_3305_20

Harsh, A., and Tripathi, P. (2023). Medical viruses: diagnostic techniques. Virol J, 20, 143. https://doi.org/10.1186/s12985-023-02108-w

Hazu, M., Ahmed, A., Curry, E., Hornby, D, P., Gjerdeb, D, T. (2022). Cell Chromatography: Biocompatible Chromatographic Separation and Interrogation of Microbial Cells. Cell Chromatography, 10(5) 10.1128/spectrum.02450-22 1.

He, Q., Chong, K, H., Chng, H, H., Leung, B., Ling, A, E., Wei, T., Ahn, H, J., Chong, C, K., Dias, R, F., Nam, H, W. (2016). Western blot detection of human anti-Chikungunya virus antibody with recombinant envelope 2 protein. Korean J Parasitol, 54, 239–41.

Hematian, A., Sadeghifard, N., Mohebi, R., Taherikalani, M., Nasrolahi, A., Amraei, M., Ghafourian, S. (2016). Traditional and Modern Cell Culture in Virus Diagnosis. Osong Public Health and Research Perspectives, 7(2), 77-82.

Herbert, J, A., and Panagiotou, S. (2022). Immune Response to Viruses. Encyclopedia of Infection and Immunity. 429–444. doi: 10.1016/B978-0-12-818731-9.00235-4.

Hodinka. R, L., and Kaiser, L. (2013). "Point-counterpoint: is the era of viral culture over in the clinical microbiology laboratory?" Journal of Clinical Microbiology, 51(1), 2–8.

Hussein, H, A. (2023). Brief review on Ebola virus disease and one health approach. Heliyon, 9 e19036.

Ibrahim, W., Cordell, L, R., Michael, J., Richardson, M., Carr, L., Dasi, A, S, D., Greening, N, J. (2021). Diagnosis of COVID-19 by exhaled breath analysis using gas chromatography–mass spectrometry. ERJ Open Research, 7. 00139. DOI: 10.1183/23120541.00139-2021.

Im, K., Mareninov, S., Diaz, M, P., Yong, W, H. (2019). An introduction to performing immunofluorescence staining. Methods Mol Biol, 1897, 299.

Imran, S., Ahmadi, S., Kerman, K. (2021). Electrochemical biosensors for the detection of SARS-CoV-2 and other viruses. Micromachines, 12, 174.

Jiang, C., Lian, X., Gao, C., Sun, X., Joshua, M., Milush, J., Bertagnolli, LN., Sweet, SE., Varriale, JA., Peter, D. (2020). Distinct viral reservoirs in individuals with spontaneous control of HIV-1. Nature, 585, 261–267.

Khalil, A, H., Hassanein, N, A., and El-Yazbi, A, F. (2023). Recent analytical methodologies for the determination of anti-covid-19 drug therapies in various matrices: a critical review. RSC Adv, 13, 13224.

Kolm, C., Martzy, R., Führer, M., Mach, R. L., Krska, R., Baumgartner, S. (2019). Detection of a microbial source tracking marker by isothermal helicase dependent amplification and a nucleic acid lateral-flow strip test. Sci. Rep, 9, 393. doi:10.1038/s41598-018-36749-7.

Kong, H., Burke, D, F., da Silva Lopes, T, J., Takada, K., Imai, M., Zhong, G., Hatta, M., Fan, S., Chiba, S., Smith, D. (2021). Plasticity of the Influenza Virus H5 HA. Protein Mbio, 12, e03324-20.

Kutner, R, H., Zhang, X, Y., Reiser, J. (2009). Production, concentration, and titration of pseudotyped HIV-1-based lentiviral vectors. Nat Protoc, 4(4):495–505. https://doi.org/10.1038/nprot.2009.22.

Kvinesdal, B, B., Nielsen, C, M., Poulsen, A, G., Hojlyng, N. (1989). Immunofluorescence assay for detection of antibodies to human immunodeficiency virus type 2. J Clin Microbiol, 1989;27:2502.

Lasch, P., Schneider, A., Blumenscheit, C., and Doellinger, J. (2020). Identification of Microorganisms by Liquid Chromatography-Mass Spectrometry (LC-MS1) and in Silico Peptide Mass Libraries. Mol Cell Proteomics, 19(12) 2125–2138 2125.

Lerer, E., Oren, Z., Kafri, Y., Adar, Y., Toister, E., Cherry, L., Lupu, E., Epstein, E., Natan, N., and Makovitzk., A. (2021). Highly Efficient Purification of Recombinant VSV-ΔG-Spike Vaccineagainst SARS-CoV-2 by Flow-Through Chromatography. Bio Tech, 10(4). https://doi.org/10.3390/biotech10040022.

Lobato, I, M., and O'Sullivan, C, K. (2018). Recombinase polymerase amplification: basics, applications, and recent advances. Trends Anal Chem, 98, 19–35. doi:10. 1016/j.trac.2017.10.015

Long, JS., Mistry, B., Haslam, SM., and Barclay, WS. (2019). Host and Viral Determinants of Influenza A Virus Species Specificity. Nat. Rev. Microbiol, 17 (2), 67–81. doi: 10.1038/s41579-018-0115-z.

Louten, L. (2016). Virus Structure and Classification. Essential Human Virology, 19–29. doi:10.1016/B978-0-12-800947-5.00002-8

Martínez, M, A., Soto-Del Río, M, L, D., Gutiérrez, R, M, Chiu, C, Y, Greninger, A, L., Contreras, J, F. (2015). DNA microarray for detection of gastrointestinal viruses. J Clin Microbiol, 53:136.

McNamara R, P., Caro-Vegas, C, P., Costantini, L, M., Landis, J, T., Griffith, J, D., Damania, B, A., Dittmer, D, P. (2018a). Large-scale, crossflow-based isolation of highly pure and endocytosis-competent extracellular vesicles. J Extracell Vesicles, 7(1):1541396. https://doi.org/10. 1080/20013078.2018.1541396

Meadows, A, J., Stephenson, N., Madhav, N, K., Oppenheim, B. (2014). Historical trends demonstrate a pattern of increasingly frequent and severe spillover events of high-consequence zoonotic viruses. BMJ Glob Health, 8e. 012026. doi:10.1136/bmjgh-2023-012026

Mercuro, G., Bassareo, P, P., Deidda, M., Cadeddu,C., Barberini, L., Atzori, L. (2011). Metabolomics: A new era in cardiology. Journal of Cardiovascular Medicine, 12(11), DOI:10.2459/JCM.0b013e32834a658f.

Mistry, P., Barmania, F., Mellet, J., Peta, K., Strydom, A., Viljoen, Gordon, S., and Michael, S. (2022). SARS-CoV-2 Variants, Vaccines, and Host Immunity. Frontiers in Immunology, 12.

Mohsen, M, G., and Kool, E, T. (2016). The discovery of rolling circle amplification and rolling circle transcription. Acc. Chem. Res, 49, 2540–2550. doi:10.1021/acs.accounts.6b00417.

Molina, D, M., Jafari, R., Ignatushchenko, M., Seki, T., Dan, C., Sreekumar, L., Cao, Y., Nordlund, P. (2013). Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. Science, 341, 84–87

Müller, J., Bertsch, T., Volke, J., Schmid, A., Klingbeil, R., Metodiev, Y., Behnes, M. (2021). Narrative review of metabolomics in cardiovascular disease. J Thorac Dis, 3(4):2532-2550 | http://dx.doi.org/10.21037/jtd-21-22.

Nandi, S., Mondal, A., Roberts, A., Gandhi, S. (2020). Biosensor platforms for rapid HIV detection. Adv Clin Chem, 98:1–34.

Narita, F., Wang, Z., Kurita, H., Li, Z., Shi, Y., Jia, Y. (2021). A review of piezoelectric and magnetostrictive biosensor materials for detection of COVID-19 and other viruses. Adv Mater, 33, 2005448. https://doi.org/10.1002/adma. 202005448.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Res, 28, e63. doi:10.1093/nar/28.12.e63.

Paulonis, J, J. (2019). The Antigenic Shift or Drift of the Influenza Virus. Journal of the Washington Academy of Sciences, 105(1), 7-12.

Peruri, V., Bhattacharya, S., Rathore, A, S. (2024). Reversed phase high performance liquid chromatography for monitoring type-16 human papilloma virus like particles. Journal of Chromatography, 5.

Pohanka, M. (2018). Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications. Mater, 11, 448.

Prasad, B, V, V., and Schmid, M, F. (2012). Principles of virus structural organization. Adv Exp Med Biol, 726, 17–47. doi: 10.1007/978-1-4614-0980-9_3.

Robert, W., McGarrah, M, D., Scott, B., Zhang, C, G., Shah, S, H., and Christopher, B. (2018). Cardiovascular Metabolomics. Newgard Circ Res, 122(9), 1238–1258. doi:10.1161/CIRCRESAHA.117.311002.

Rouse, B, R., and Sehrawat, S. (2010). Immunity and immunopathology to viruses: what decides the outcome? Nat Rev Immunol, 10 (7), 514–526. doi: 10.1038/nri2802.

Saéz, A, M., Weiss, S., Nowak, K., Lapeyre, V., Zimmermann, F., Düx. A., Merkel, K., Nitsche, A., Calvignac-Spencer, S., Leendertz, F, H. (2015). Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med, 7(1),17-23. doi: 10.15252/emmm.201404792.

Sajid, M., Kawde, A. N., and Daud, M. (2015). Designs, formats, and applications of lateral flow assay: a literature review. J Saudi Chem Soc, 19, 689–705. doi:10.1016/j.jscs.2014.09.001

Sampath, S., Khedr, A., Qamar, S., Tekin, A., Singh, R., Green, R., Kashyap, R. (2021). Pandemics Throughout the History. Cureus, 13(9), e18136. DOI 10.7759/cureus.1813.

Sandybayev, V., Beloussov, V., Strochkov, V., Solomadin, M., Granica, J., and Yegorov, S. (2022). Next Generation Sequencing Approaches to Characterize the Respiratory Tract Virome. Microorganisms, 10, 2327. doi.org/10.3390/microorganisms10122327

Schweitzer, B., and Kingsmore, S. (2001). Combining nucleic acid amplification and detection. Curr Opin Biotechnol, 12, 21–27. doi:10.1016/S0958-1669(00)00172-5.

Service, R, F. (2022). Bad news for Paxlovid? Coronavirus can find multiple ways to evade COVID-19 drug. Science, 8, 377(6602):138-139. doi: 10.1126/science.add8037.

Si, J., Li, J., Zhang, L., Zhang, W., Yao, J., Li, T. (2019). A signal amplification system on a lateral flow immunoassay detecting for hepatitis e-antigen in human blood samples. J Med Virol, 91(7), 1301–1306.doi:10.1002/ jmv.25452.

Simmonds, P., Adams, M, J., Benkő, M., Breitbart, M., Brister, J, R., Carstens, E, B., Davison, A, J., Delwart E, Gorbalenya A, E, Sullivan, M, B., Suttle, C, A., Zerbini, F, M. (2017). Consensus statement: Virus taxonomy in the age of metagenomics. Nat Rev Microbiol, 15(3),161-168. PMID: 28134265.

Smith, V, P., Bryant, N, A., and Alcamí, A. (2000). Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. J Gen Virol, 81, 1223–1230.

Souf , S. (2016). “Recent advances in diagnostic testing for viral infections.” Bioscience Horizons, 9, 1–11. https://doi.org/10.1093/biohorizons/hzw010.

Spick, M., Longman, K., Frampas, C., Lewis, H., Costa, C., Walters, D, D., Stewart, A., Wilde, M., Greener, D., Evetts, G., Trivedi, D., Barran, P., Pitt, A., Bailey, M. (2021). Changes to the sebum lipidome upon COVID-19 infection observed via rapid sampling from the skin. EClinicalMedicine, 33, 100786. doi: 10.1016/j.eclinm.2021.100786.

Sreenivasan, C, C., Thomas, M., Kaushik, R, S., Wang, D., Li, F. (2019). Influenza A in Bovine Species: A Narrative Literature Review. Viruses, 11(6): 561.doi: 10.3390/v11060561.

Sugita, Y., Noda, T., Sagara, H., Kawaoka, Y. (2011). Ultracentrifugation de- forms unfixed influenza A virions. J Gen Virol, 92(Pt 11), 2485– 2493. https://doi.org/10.1099/vir.0.036715-0

Sun, G., Yang, K., Zhao, Z., Guan, S., Han, X., and Gross, R, W. (2007). Shotgun Metabolomics Approach for the Analysis of Negatively Charged Water-Soluble Cellular Metabolites from Mouse Heart Tissue. Anal Chem, 79(17), 6629–6640.

Sundaramurthy, R., Dhodapkar, R., Kaliaperumal, S., and Harish, B. N. (2018). “Investigational approach to adenoviral conjunctivitis: comparison of three diagnostic tests using a Bayesian latent class model.” The Journal of Infection in Developing Countries, 12 (1), 043–051.

Tan, W, S., Lee, M., Chan, E., Tey, B, T. (2015). Negative chromatography purification of hepatitis B virus-like particles using poly (oligo (ethylene glycol) methacrylate) grafted cationic adsorbent. J Chromatogr A, 1415, 161-5. doi: 10.1016/j.chroma.2015.08.056.

Tesfaye, Y., Khan, F., and Gelaye, E. (2020). Molecular characterization of foot-and-mouth disease viruses collected from Northern and Central Ethiopia during the 2018 outbreak. Vet World, 13(3), 542–548. doi: 10.14202/vetworld.2020.542-548.

Transfiguracion, J., Manceur, A, P., Petiot, E., Thompson, C, M., Kamen, A, A. (2014). Particlequantification of influenza viruses by high performance liquid chromatography. Vaccine, 33(1), 78-84 https://doi.org/10.1016/j.vaccine.2014.11.027.

Valkenburg, S, A., Rutigliano, J, A., Ellebedy, A, H., Doherty, P, C., and Kedzierska, K. (2011). Immunity to seasonal and pandemic influenza A viruses. Microbes Infect, 13(5), 489–501. doi:10.1016/j.micinf.2011.01.007.

Walters, M, J. (2014). Birds, Pigs, and People: The Rise of Pandemic Flus. Seven Modern Plagues. 151–173. doi: 10.5822/978-1-61091-466-6_7.

Wang, W., Lin, C., Chang, M, R., Urbina, A, N., Assavalapsakul, W., Wang, S. (2019). The role of galectins in virus infection - A systemic literature review, J Microbiol Immunol Infect, 53(6), 925-935. https://doi.org/10.1016/j.jmii.2019.09.005.

Wang, Y., Song, T., Li, K., Jin, Y., Yue, J. (2019). Different Subtypes of Influenza Viruses Target Different Human Proteins and Pathways Leading to Different Pathogenic Phenotypes. Biomed Res Int, 4794910. doi: 10.1155/2019/4794910.

Weber, R., Sabin, C, A Friis-Møller, N., Reiss, P., El-Sadr, W, M., Kirk, O., Lundgren, J, D. (006). Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med, 166(15), 1632-4.

Wei, Y. (2014). Dmitri Iosifovich Ivanovsky - Founders of Virology. Journal of Applied Virology, 3(1),1.

Xing, W., Li, L., Zhangm J., Ma, C., Xue, X., Ye, S., et al. (2022). Design of a chimaeric anti gen and its use in the detection of IgG antibodies against rubella virus. Virol J, 19,1–6.

Yang, M., Zhang, Y., Xie, X., Yue, N., Li, J., Wang, X., Han, C., Yu, J., Liu,b, Y., and Li, D.(2018). Barley stripe mosaic virus γb Protein Subverts Autophagy to Promote Viral Infection by Disrupting the ATG7-ATG8 Interaction. The Plant Cell, 30, 1582–159.

Zhang, B., Li, M., Huang, J., Zeng, Q., Zhu, Q., Xu, S., and Chen, H. (2022). H1N1 Influenza A Virus Protein NS2 Inhibits Innate Immune Response by Targeting IRF7. Viruses, 14(11), 2411; https://doi.org/10.3390/v14112411.

Zhang, L., Wei, T., Li, Y., Li, J., Fan, Y., Huang, F., Liu, B., Zhao, D., Shen, J., Qi. L. (2018). Functional Metabolomics Characterizes a Key Role for N-Acetylneuraminic Acid in Coronary Artery Diseases. Circulation, 137:1374-90.

Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabečná, M., Neužil, P. (2020). PCR past, present and future. Biotechniques, 69(4), 317-325. doi: 10.2144/btn-2020-0057.

Zougman, A., Selby, P, J., and Banks, R, E. (2014). Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis. Proteomics, 14, 1006–1010.