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Review Article

Zoological Approaches for Biochemical Investigation of Viral Infections and COVID-19

Muhammad Iqbal[1], Asma Habib[2], Muhammad Zameel Khan[3*], Muhammad Khan[4], Kamran Ullah[5], Shah Masood Khan5, Haq Nawaz[5], Anwar Zaib Khan[5], Hilal Ahmad[5]

[1]Department Biological Science, University Government Girls, Degree College, Skardu, Pakistan
[2]Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan
[4]Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan
[5]Department of Zoology, Abdul Wali Khan University, Mardan, Pakistan

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***Corresponding author:** Muhammad Zameel Khan

Abstract

Biosensors for virus and bacterial detection and control have been developed over the last thirty years as a result of various biotechnological breakthroughs. With the exception of avian species, cell-lines offers certain advantages and are accessible for domestic animals. Current diagnostics rely on ELISA or RT-PCR, whereas these procedures frequently have limitations in terms of speed and sensitivity. Amplification techniques of nucleic acid are extremely beneficial for detecting viruses which are difficult and dangerous to culture, viruses that develop slowly in culture, and viruses with antigenic variants such as HCV (hepatitis C virus), Zika virus, dengue virus, EBV, influenza viruses, HIV, Ebola virus and coronavirus. Pathogen isolation and characterization, RT-PCR and sequencing-analyses are common. LAMP (Loop mediated isothermal amplification) has evolved into an important alternative for simplifying infectious illness diagnosis. Diagnostic assays are employed to determine present, functional SARS-CoV-2 infections. Diagnostic techniques can be antigen based for specific proteins on the virus's surface, or molecular based like LAMP, CRISPR and PCR. The gold-standard for the testing of COVID-19 is RT-PCR, that identifies SARS-CoV-2 genetic-information in nasopharyngeal specimens.

Keywords: ELISA, HCV, Zika virus, EBV, influenza viruses, RT-PCR, SARS-CoV-2.

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INTRODUCTION

Many of the animals virus-related diseases like Rift Valley fever, rabies, eastern and western, and the Venezuelan-encephalomyelitis are the zoonotic diseases and these are of enough human health significance to necessitate the care and the establishment of specific investigative labs. For instance, rabies diagnosis confirmation in the skunk which has bitten a child gives the basic information for post-exposure treatments of human-patient [1-3]. The early warnings and the confirmation of equestrian encephalomyelitis-virus epizootic permits applications of mosquitos control and the other actions like control of movement of the horses. The discharge of stable VOC (volatile organic compounds) from the tissues, which are found in exhaled air, feces, sweat and urine supports strong scientific data. The volatile is the name given to the VOCs which are discharged. VOCs are volatile at room temperature, have an odor, that can provide the signals required for real time detecting. Because of their accessibility, downsizing possibilities, and potential for real time analyses, biosensors as analytical devices are promising alternatives for quick and effective infectious diagnosis of diseases [4-7].

Biochemical investigation of viral infections and COVID-19

Biosensors for virus and bacterial detection and control have been developed over the last thirty years as a result of various biotechnological breakthroughs. Some evolving structures have resulted in the auspicious prototypes that attained quick pathogen recognition without demanding higher levels of sample manipulations which is extremely tiresome for the disease-ridden samples.

The best cell culture for primary isolation of an unidentified virus from the clinical samples is mostly

a matter of trial and error. Preliminary or low passage, homologue, mono-layer cell cultures produced from fetal tissues are likely the much more receptive substrate for isolating a wide range of viruses. Frequently the disease nature from which the samples are taken, suggests which viral infection is present and from this convenience the best cell culture can be chosen. With the exception of avian species, cell-lines offers certain advantages and are accessible for domestic animals [8-11].

Molecular approaches involving viral genomic material amplification are exceedingly specific and sensitive, give quick diagnoses, and facilitate the identification of many viruses at the same time. Amplification techniques of nucleic acid are extremely beneficial for detecting viruses which are difficult and dangerous to culture, viruses that develop slowly in culture, and viruses with antigenic variants. The nucleic acid amplifying assays are widely used to diagnose virus infections caused by a variety of viruses such as HCV (hepatitis C virus), Zika virus, dengue virus, EBV, influenza viruses, HIV, Ebola virus and coronavirus. There are presently many nucleic acid amplification methodologies are present for the laboratory diagnosis of virus infection all over the world [12-14].

MDSC (Myeloid derived suppressor cells) are juvenile myeloid cell types that can suppress the immune system. Myeloid derived suppressor cells were first linked to immuno-suppression in tumor micro-environments by a variety of pathways, including IL-10, induced Nitric oxide synthases (iNOS), arginase-1 and ROS. Myeloid derived suppressor cells have lately been linked to viral diseases including Hepatitis C virus (HCV), HIV and Hepatitis B virus (HBV). Immunosuppression mediated by MDSC populations has been linked to viral survival in various infection models, as well as the poorest clinical consequences. Thus far, these cell lines have only been analyzed in mice and humans' models, and it seems that there are primarily two phenotypic traits in both species: M-MDSC (monocytic MDSC) and PMN-MDSC (polymorphonuclear MDSC), which are phenotypically and morphologically similar to neutrophils and monocytes, respectively [15-17].

Virus infections are the major source of human diseases, but several of them involve molecular testing for definitive diagnosis. Current diagnostics rely on ELISA or RT-PCR, whereas these procedures frequently have limitations in terms of speed and sensitivity. Livestock disease surveillance is critical for diseases control and prevention, as well as a tool for gathering data for decision making on control and eradicating measures. Pathogen isolation and characterization, RT-PCR and sequencing-analyses are common. These techniques frequently focusing on a particular identified pathogen; however, analyzing several pathogens inside one or more hosts involves the use of several detection systems, personnel, and even laboratories. PCR is a validated tool for swiftly detecting and identifying identified pathogenic microbes as potential illness causes or as bioweapons. In March 2002, a traveller coming from Amazonas died as a result of yellow fever. The previously healthy 47-year-old man got coagulopathy, fever, pancytopenia, headache, hepatic and renal failure, and other symptoms [1, 7, 11].

The usage of molecular-beacons is another very typical detecting format. Molecular-beacons are the oligonucleotide markers with flank sequence of 5 to 7 nucleotides that are complementary with one another, as well as an intermediary sequence that is complementary to target region. The probe's ends are tagged with fluorescent and non-fluorescent quenched stain (DABCYL). The phrase "molecular-beacon" refers to how the complementary strands of a probe anneal to one another in the solution, making a stem like shape, while the intermediary sequence stays loop out and single stranded [18, 19].

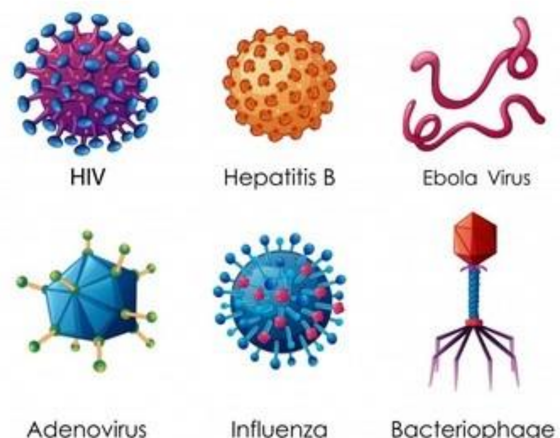

Fig 1: Shows the different types of viruses with structures

The shaker milling homogenization is a mechanically lysis, has repeatedly been demonstrated as a useful technique for tissues disruption, microbes and genetic specimens for down-stream molecular studies; even so, such down - stream usage frequently necessitate the use of extra cleansing, separation, or extraction method before all those assessments can be finished. Whereas shaker milling homogenization processes have still not been effectively used to diagnosis techniques, their capability to lyse the tissues and organisms significantly harder than a virus has been well documented. Researchers considered it was appropriate to investigate the potential of shaker milling homogenization for viruses' lysis to be employed for down-stream detection, given the rising demand for innovative viral diagnostic methodologies that minimize the time and resources necessary for accurate virus pathogen identification [20, 21].

LAMP (Loop mediated isothermal amplification) has evolved into an important alternative for simplifying infectious illness diagnosis. Furthermore, one benefit of Loop mediated isothermal amplification is that it enables easily readable of the end results using the visual identification. This process, however, should always be done carefully to remove contaminants and falsified findings. In diagnostic techniques, RT-PCR is indeed a fast and sensitive detection method. This method can amplify and detect even very few copies of certain nucleotide sequences in a range of materials, but it is dependent on some factors to produce trustworthy results, such as adequate collection of sample, transportation, processing and storage. It is used to identify viruses such as Astroviruses, Rotavirus, Adenovirus, and numerous gastrointestinal viruses obtained from feces. A significant disadvantage of this method is the requirement for a very well lab and experts to handle the investigation, that cannot alleviate the increasing demand for speedy tests during the pandemic conditions such as COVID-19 [22-24].

SARS-CoV-2 has very significant genomic variation in the gene location and sequences of nucleotide because to its fast pandemic scale dissemination. The results of SARS-CoV-2 genome-sequencing are periodically posted on GISAID databases. It is examined 220 SARS-CoV-2 genome-sequences on the GISAID and discovered 8 different recurring genomic alterations, implying that SARS-CoV-2 was changing and that multiple variants of SARS-CoV-2 may cohabit. Diagnostic assays are employed to determine present, functional SARS-CoV-2 infections. Diagnostic techniques can be antigen based (rapid-antigen), looking for specific proteins on the virus's surface, or molecular based (like LAMP, CRISPR and PCR), looking for viral protein's material specific to SARS-CoV-2. Nucleic acid amplification assays are another name for molecular assays that amplified genetic information (NAAT) [25-28].

The COVID-19 pandemic that began in 2019 has already been marked by the widespread usage of reverse transcription rt-PCR as a diagnosis method. Because many diseases are asymptomatic or pauci symptomatic, proper testing is critical. Early diagnostic identification of COVID-19 allows for improved management in the context of prompt hospitalization, the use of particular anti-viral medications like remdesivir, and infection-control practices. COVID-19 symptoms are very similar to those of ordinary flu. Coronaviruses only multiply RNA-genomes and subunit RNAs from the RNA templates and will not necessitate a DNA phase in their viral life cycle. The 3′ to 5′ exonuclease functionality of non - structural protein 14, which is specific to coronaviruses, allows proofreading, hence improving genome replicating fidelity. Unlike the other RNA-viruses that replicate in mistake, coronaviruses utilize Nsp-14 exonuclease, that is first documented proofread enzyme encoded by RNA virus and is most probably an adaptation to handle coronaviruses' enormous RNA-genomes. Coronaviruses mutate at a slower rate than some other RNA viruses, based on this proofreader capability. During the initial stages of the COVID-19 epidemic, a quick and easy method of collecting the patient samples was desperately needed to replace the traumatic nasopharyngeal swabs collection procedure. In this circumstance, the Rutgers Clinical Genomics Laboratory devised an RT-PCR based approach for detecting SARS-CoV-2 RNA in self-collected saliva samples. They created an assay-kit, that was marketed as the TaqPathTM COVID-19 combo-kit. This method reduced the danger of disease during samples taken by healthcare personnel. Patients' blood samples are required for protein-based testing such as LFA and IgG/IgM [29-31].

Despite the immense number of cases, diagnosis lab infrastructures in various areas, especially those who have limited funds, has been unable to keep up with the enormous wide range of clinical samples presented. Pooled-sample-testing (combining a number of observations in batches and processing the batch as a single specimen) has been suggested as a promising solution to this issue. Furthermore, because the genetic information is diluted as a result of pooling, the probability of falsified results rises. As a result, pooled-sample-testing should be performed only in environments with a lower incidence of cases. The gold-standard for the testing of COVID-19 is RT-PCR, that identifies SARS-CoV-2 genetic-information in nasopharyngeal specimens. While high reliability, RT-PCR diagnoses are difficult, time-consuming, and costly, and their widespread usage led to a lack of reagent required for collection of sample and extraction of viral-RNA in initial stages of pandemic[1, 9, 11, 19].

CONCLUSION

A range of research methods have been employed to study the epidemiology of the animal infectious illnesses, the most notable of which are animal pathogenic surveillance and pathogenic molecular epidemiological tracking. Over the duration of COVID-19 pandemic, the SARS-CoV-2 viruses evolved, resulted in genetic heterogeneity in populations of migrating virus strains. Genetic, antigen, and the serology assays are all adversely affected by the viral alterations due to intrinsic design peculiarities of each test.

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