ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

The Laboratory Investigations and Diagnosis of Viral Diseases

Nancy Verma[1]*, Neeraj Grover[2], Kanika Bhalla Prabhat[3], Shreya Singh[4]

[1] PG 3rd [y]ear STudent,
Department of Oral and Maxillofacial Pathology and Oral Microbiology,
Santosh Dental College and Hospital, Santosh Deemed to be University,
Ghaziabad, Uttar Pradesh, India.
[2] Professor & Head of Department,
Department of Oral and Maxillofacial Pathology and Oral Microbiology,
Santosh Dental College and Hospital, Santosh Deemed to be University,
Ghaziabad, Uttar Pradesh, India.
[3,4] Reader, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Santosh Dental College and Hospital, Santosh Deemed to be University,
Ghaziabad, Uttar Pradesh, India.
Email- [1] nancy.verma0305@gmail.com

ABSTRACT:

The proper management of viral infections entails accurate diagnosis of viral diseases and their modes of transmission; as a result, appropriate treatment modalities are applied. Additionally, it improves clinicians' abilities to decide on the best course of action for patients, monitor disease progression, and avoid overusing antibiotics. Understanding the pathogen also aids in understanding infection control and the success rate of antiviral therapies, both of which affect the prognosis of patients. For early intervention of a particular viral condition, specific nucleic acid and serological testing for various viral infections is crucial. However, these tests should be complemented by the continuing use of serology in cases of chronic infections or viruses with brief viremia. This article gives a quick overview of recent advancements in diagnostics that could soon increase our ability to provide a definitive diagnosis. Clinical specimens must always be tested in the lab for the presence of virus, viral antigens, or particular antibodies and specificity in order to make an accurate virus diagnosis.

Keywords: virus infection, viral diagnosis, immunoassays virus isolation, PCR, nucleic acid amplification.

INTRODUCTION:

Viruses are also known as dynamic nucleoprotein assemblies that are able to spread between cells and specific organisms as well as within cells with supported growth. 1 Initially, viruses were thought of as filterable disease-causing entities. 2 The word "virus," which means "slimy liquid" or "poison," has Latin origins. They are also known as tiny, straightforward

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

infectious agents that can reproduce exclusively in the living cells of bacteria, plants, or mammals. As they are not free living, meaning they cannot reproduce on their own and cannot share a metabolic body with a host, 3 viruses should not even be considered to be other species.4

They are also viewed as mobile genetic elements, primarily of cellular origin, and distinguished by a long co evolution of virus and host. Viruses are small, obligate intracellular parasites that consist of either an "RNA or DNA genome" surrounded by a virus coded protein coat that serves as protection.5

When viruses were first discovered, there were only a few tools available to classify or identify them as microbiological agents because there were no powerful enough microscopes to see viral agents. There were no available probes to mark an infectious agent, such as antibodies or nucleic acids.6

Properties of virus particles include the following:

i. Virus particles are paradigms to understand structure function relationships in bio macromolecular assemblies and biological machines.

ii. Virus particles constitute excellent models to understand and learn to manipulate molecular self-assembly.

iii. Knowledge of virus structure, dynamics and properties is essential to understand the life cycles of viruses.1

Origin of virus

The main features of virus as follows:

i. They can replicate only within a host cell.

ii. They generally are quite smaller in size with a diameter of less than 200 nanometers (nm).

iii. virus doesn't contain ribosomes, which help in formation of protein in a cell.7

Diagnostic virology

The aetiology of viral infections must be ascertained using diagnostic testing, which are crucial. Assays are employed in direct diagnostic procedures to check for the presence of the virus, whereas indirect diagnostic methods check for the infection's effects. [8]

There are several different types of diagnostic immunoassays, including western blots, enzyme linked immunosorbent tests, lateral flow immunoassays, and agglutination reactions. The PCR or nucleic acid hybridization principles serve as the foundation for highly sensitive and specific viral nucleic acid detection assays.9

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

STEPS IN THE LABORATORY TECHNIQUES FOR THE DIAGNOSIS OF VIRAL INFECTIONS:

Electron microscope has been used as an efficient tool for direct detection of viruses through visualization and counting of the viral particles in body fluids, stools or histopathologic samples.

Lab diagnosis of viral infection starts with the collection of specimens.11

Specimen collection

Mainly the organs get infected like skin for cutaneous lesions, direct secretions from respiratory & Gastro intestinal tract which require respiratory or GI system involvement. Virus gets enter in the body through the respiratory or GI tract, by mucous and gastric secretions.1

Transportation & storage of specimen

In a sterile, leak proof container, all samples should be delivered to the lab. There should be little time between collecting the material and inoculating it. Specimens are kept chilled until they are ready for additional processing. If the samples will be kept for a very long time (weeks or months), they should be kept at 70°C.

Cell culture

Cell culture is one of the most popular methods for isolating viruses using cell lines. it was first described by Weller and Enders in 1948.

VARIOUS METHODS OF DETECTING VIRUS:

1. Antigen detection

Antigen detection methods are particularly useful for viruses that grow slowly or are labile, making recovery in culture difficult. Commonly used for RSV, influenza and para influenza viruses, and adenovirus in respiratory specimen.4

2. Nucleic acid detection

Nucleic acid testing is a molecular technology that detects viral DNA or RNA. The detection of specific DNA and RNA from viruses is mainly performed by PCR. These molecular techniques are commonly used to obtain high sensitivity and specificity.5

3. Western Blotting Technique

Blotting techniques consist of antigen detection on the surface of a membrane. The dot blot, or slot blot, is a technique which can be used for the detection of viral antigens from a sample. This method is used as it is cost effective and small sample size is required.1

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

4. Serology

Serological assays are used as an adjunct with nucleic acid detection assays. It is used to identify the source of infection with the identification of transmission pattern analysis, patient contact studies and the identification of asymptomatic cases.4

5. Polymerase Chain Reaction

Dr. Kary Mullis was the first who discovered the PCR assay. PCR is a simple, elegant, enzymatic assay, which allows for the amplification of a specific DNA fragment from a complex pool of DNA, PCR can be performed using source DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes.10

Primer, nucleotide, template DNA, and DNA polymerase must all be present for a PCR assay to work. The DNA polymerase is a crucial enzyme that joins different nucleotides to create the PCR result. Adenine, thymine, cytosine, and guanine (A, T, C, and G), the four bases contained in DNA, are included in the nucleotides. which serve as the building pieces that the DNA polymerase uses to form the end product of the PCR. The particular DNA product to be amplified is determined by the primers used in the process. The target DNA that is to be identified and amplified is complementary to the short DNA fragments known as primers. These act as a foundational point for the DNA polymerase to expand upon.10

TYPES OF PCR:

I. Real time PCR

Real time PCR has revolutionized the diagnosis of human pathogenic viruses in clinical laboratories. Thus, real time PCR plays an important role in the detection, quantification, and typing of viral pathogens in diagnostic methods related to viruses.11

II. Digital PCR

In contrast to qPCR, the digital PCR (dPCR) uses an alternative method that is not dependent upon the determination of the amplification cycle that the reporter dye signal exceeds a threshold.8

III. Reverse Transcription PCR (RT PCR).

RT PCR was designed to amplify RNA targets. In this technique, reverse transcriptase (RT) is used to convert viral RNA targets into complementary DNA (cDNA), and then the resulting cDNA is amplified by conventional PCR. Since its development, RT PCR has been used for the diagnosis of human infection by RNA viruses.12

6. Transcription Based Amplification Methods

Transcription based amplification method includes nucleic acid sequence-based amplification (NASBA) and transcription mediated amplification (TMA). They are isothermal

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

amplification methods. The entire amplification process is carried out at the temperature of 41°C. 10

7. Enzyme Linked Immunosorbent Assay

In ELISA, enzyme conjugated antibody is utilized to detect the presence of specific antiviral antibody or viral antigen in human specimens. In positive sample, the reaction between an enzyme conjugated with an antibody and colorless chromogenic substrate leads to the formation of a colorful product. In the absence of antigen/antibody in the clinical specimen, no color is produced. The intensity of the color is directly proportional to the amount of antigen antibody complex formed.11

8. Metagenomic sequencing

Metagenomic sequencing is most appropriate for diagnostic sequencing of unknown or poorly characterized viruses, PCR amplicon sequencing works well for short viral genomes and low diversity in primer binding sites, and target enrichment works for all pathogen sizes but is particularly advantageous for large viruses and for viruses that have diverse but well characterized genomes.8

Diagnosis of Covid 19

“Diagnosis tests is an essential step in a reliable diagnosis. SARS CoV 2 infection can be detected in a variety of clinical specimens, such as nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, and bronchoalveolar lavage. The median duration of SARS CoV 2 shedding in respiratory samples is 24 (IRQ, 18–31) days in survivors, but shedding can last for up to 39 days”.10

Preventive Measures

Hand washing using plain (non-antimicrobial) soaps or hand rubbing with alcohol based hand sanitizers are frequent hand hygiene techniques used in healthcare and community settings. Face covering also act as a mechanical barrier for protection against several infections.2

CONCLUSION:

Clinical microbiology is changing as a result of recently discovered viral diagnostic techniques, which may also help to lower the incidence of major infectious diseases.

In normal practise and the adoption of new techniques in the poor world and the endemic areas, good quality diagnosis has a cost that only wealthy countries can afford.

REFERENCES:

1. David J Rowlands et al. A Brief History of Virology, Editor(s): Dennis H. Bamford, Mark Zuckerman, Encyclopedia of Virology,Academic Press, 2021;23:3 13.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

2. Gelderblom HR, et al. Structure and Classification of Viruses. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX). University of Texas Medical Branch at Galveston; 1996;21(1):34 39.
3. Jennifer L, et al. Virus Structure and Classification, Editor(s): Jennifer Louten, Essential Human Virology, Academic Press, 2016;41:19 29.
4. Krupovic Mart, Dolja Valerian V, Koonin Eugene V. Plant viruses of the Amalgaviridae family evolved via recombination between viruses with double stranded and negative strand RNA genomes. Biology Direct , 2015;10(1):21 32.
5. Matue mg. Introduction: the structural basis of virus function. Subcell biochem. 2013;68:3 51.
6. Nasir A, Kim KM, Caetano Anollés G et al. Viral evolution: Primordial cellular origins and late adaptation to parasitism. Mob Genet Elements. 2012;2(5):247 252.
7. Oldstone MBA et al. History of Virology. Encyclopedia of Microbiology. 2014;608 612.
8. Rossmann MG, et al. Structure of viruses: a short history. Q Rev Biophys. 2013 May;46(2):133 80.
9. Suman M, et al. ACS Infect. Dis. Publication Date:2020;64(9): 2319–2336.
10. Wagner, Robert R. et al."virus". Encyclopedia Britannica, 12 Nov. 2020, December 2021;24:5 9.
11. Wessner, D. R.et al. The Origins of Viruses. Nature Education. 2010;3(9):3 7.
12. Wang Shick R, et al. Molecular Virology of Human Pathogenic Viruses, Academic Press, 2017;34(2):31 45.

Figures

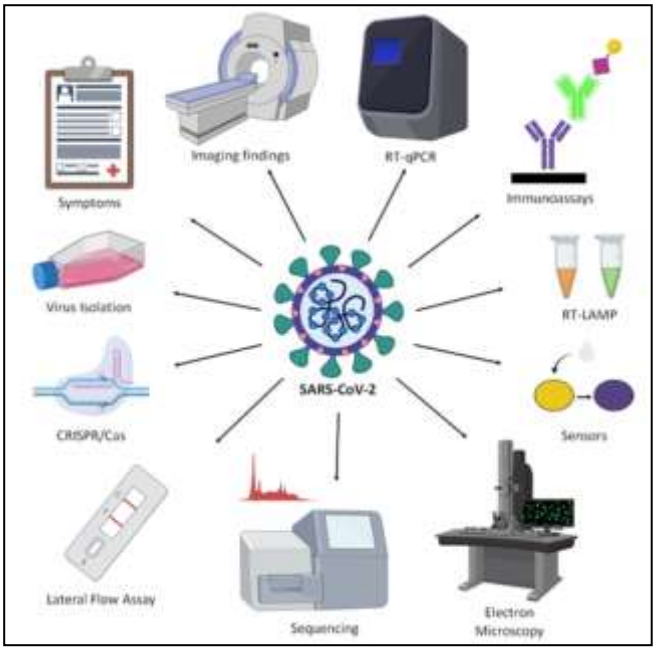

Figure :1 Covid 19 Diagnostic approaches (Suman M et al., 2020)

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11, S Iss 2, 2022**

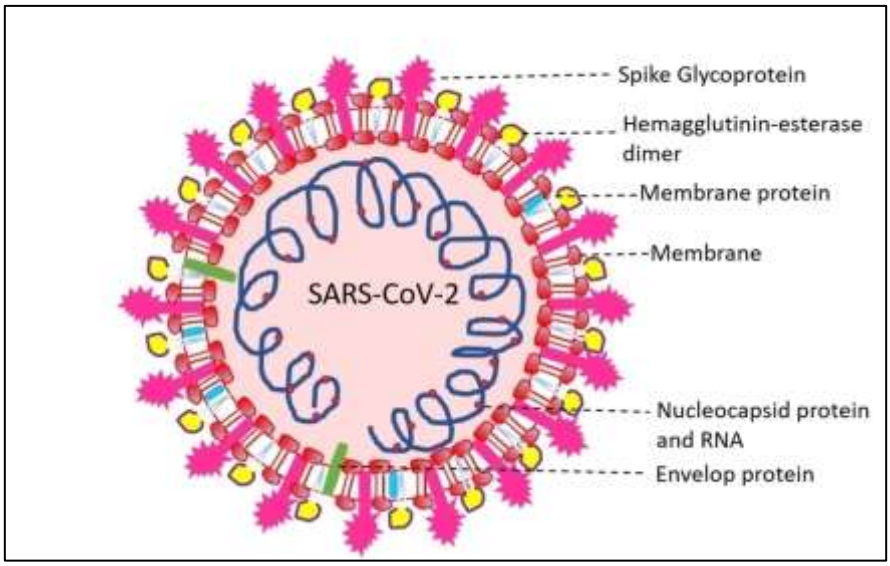

Figure :2 Viral Surface (Soumita M et al., 2021)

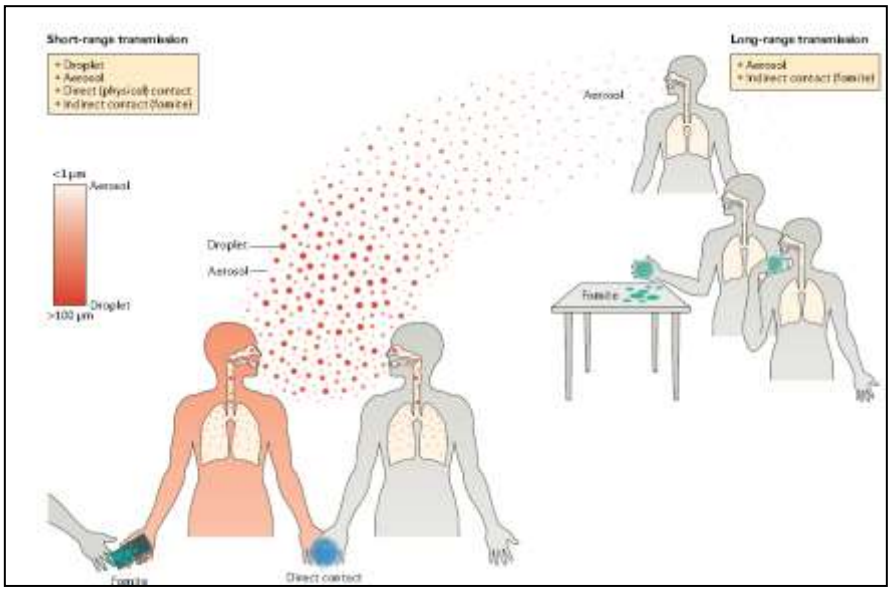

Figure :3 Mode of Transmission of virus (Leung et al., 2021)