Molecular Diagnostic Methods For Viral Infections

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ABSTRACT: Viral diagnostic tests are categorized as direct diagnosis, indirect examination (virus isolation), and serology. Molecular testing is often done to detect potent viral markers resulting from any gene or chromosomal modifications during viral replication in the host. The clinical specimen is tested specifically in direct analysis for the presence of virus particles, virus antigens or viral nucleic acids. Molecular diagnosis of viruses can be achieved from clinical samples like blood, hair, skin, amniotic fluid, or other tissue. Molecular biology techniques are considered to be the standard and confirmatory protocols in the analysis of viral DNA, RNA and protein. Molecular viral diagnosis are considered to be more specific and sensitive in preliminary viral screening and can pave the way for rapid diagnosis. However, the procedures are costly and involve automated bio-instruments to perform the same. This review thus highlights the various molecular diagnostic methods used in diagnostic virology with their limitations and challenges.

KEYWORDS: Nucleic acid amplification techniques; Molecular diagnosis; Viral infections; mortality; Serology.

1. INTRODUCTION:

Molecular diagnosis is a set of techniques used to examine biological markers in the genome and proteome by applying molecular biology to medical research – the genetic code of the organism and how their cells express their genes as proteins (Marickar, Geetha, and Neelakantan 2014). Molecular biology techniques are standard methods used in molecular biology, biochemistry, genetics, and biophysics that typically include DNA, RNA, protein, and lipid manipulation, and analysis (Schrijver 2011). In medicine, a laboratory test in a sample of tissue, blood, or other body fluid which checks for certain genes, proteins, or other molecules.Molecular testing often tests for any gene or chromosome modifications that can

cause or affect the risk of developing a particular disease or condition, such as cancer (Ashwin and Muralidharan 2015). Molecular diagnostics is a more active tool for detecting lower levels of infectious agents and thereby providing earlier than previously possible the ability to diagnose infections (Stoler 2001). Buccal smears, amniotic fluids and many other tissues will have loads of viruses which can be rapidly detected by the molecular methods (Selvakumar and Np 2017, Shahana and Muralidharan 2016, Vaishali and Geetha 2018).

Molecular diagnostics (MDx) has taken a prominent position and demonstrated benefits for routine identification, fingerprinting, and epidemiological study of infectious microorganisms in the clinical diagnostic laboratory (Morgan et al. 2005). Firstly, MDx minimizes the cultivation requirement, which reduces the time needed to diagnose morphology and biochemistry (Schrijver 2011). Science advances into a nuclear microscope that offers detailed analysis of bacterial genomes (e.g. transcription, translation, replication, methylation, and nuclear DNA folding) and is readily used in clinical microbiology to replace traditional pathogens characterization studies (Ramesh 2017).

Viruses being obligate intracellular parasites require live cells for its replication and yields viral progeny with many inclusion particles in the host cells (Volovitz, Faden, and Ogra 1988). Viruses can not replicate without a host 's support (Pratha and Geetha 2017). Rapid detection of the viral particles in the host cells is essential for expediting the treatment process in the affected individuals (Marickar, Geetha, and Neelakantan 2014). Diagnostic tests can usually be divided into 3 categories: (1) direct diagnosis, (2) indirect exam (virus isolation), and (3) serology. The clinical specimen is tested specifically in direct analysis for the presence of virus particles, virus antigens or viral nucleic acids including oral mucosal specimens (Ashwin and Muralidharan 2015). However, the molecular methods have over-ruled the conventional methods due to its sensitivity and specificity. This review thus throws highlights on the molecular diagnostic methods available to detect various virus mediated diseases.

Need for early detection of viral markers:

Viruses cause infectious diseases that are familiar, such as common cold, flu, warts and new viruses can emerge suddenly like the recent CoV viruses. They also cause serious diseases like HIV / AIDS, smallpox and Ebola. They invade living, human cells and replicate using those cells to create other viruses like themselves. Antigen detection is done with strategies starts with basic staining to fluorescent antibody (FA), staining of the immunoperoxidase, and EIA (M, Geetha, and Thangavelu 2019, Monfared et al. 2019). Progressively the application of serological and molecular methodologies were implemented to detect the viral markers. With the emergence of more drug resistant strains including fungi and bacteria (Shahzan et al. 2019), co-infection can also occur in virus infected patients, thus urging the need for differential diagnosis too.

Recently methodologies like polymerase chain reaction, mass spectrometry and next-generation sequencing, were implemented for viral diagnosis (Rodríguez-Lázaro and Hernández 2016). These powerful tools combine speed and effectiveness in detecting viral pathogens and have revolutionized the clinical diagnostic field (Arcenas 2017). With thousands of deaths annually, viral infectious diseases represent an important portion of global public health concerns. Clinical prognosis often relies on early detection of the

infectious agent, from serious pandemics and highly contagious infections to common influenza episodes (Grijalva 2009). This will help in early diagnosis which inturn reduces the mortality rate. Effective prevention and clinical management of infectious diseases are closely linked to early and accurate pathogen screening, not only by detecting infectious particles in the organism, but also by elucidating aspects that confer therapy resistance and profiles of immune escape, including mutations and genotype disparities (Das et al. 2015). Observational information compiled by correct diagnosis plays a significant role in public health by detecting and monitoring outbreaks (Smiline, Vijayashree, and Paramasivam 2018).

Nucleic acid amplification techniques (NAAT):

NAAT is used in biological samples to detect HIV RNA or DNA for diagnosis and/or monitoring of HIV infection. HIV RNA PCR is used to measure the plasma virus charge or extracellular viral RNA. Essays on amplification of nucleic acid**.**NAAT Is a quick screening test for complex Mycobacterium tuberculosis (MTBC). Nucleic acid amplification tests (NAAT) are used in biological samples to detect HIV RNA or DNA for diagnosis and/or monitoring of HIV infection (Marrazzo et al. 2005).HIV RNA PCR is used to measure the plasma virus charge or extracellular viral RNA. NAATs are incredibly sensitive methods of detecting whether the biological sample contains a bacteria or a virus (Black et al. 2002). Nucleic acid research uses a "template" that is a long strand that has a short strand attached to it. The long primer strand has a corresponding (complementary) sequence from the disease organism being identified to a "target" strand (Johnson et al. 2000).

Genomic DNA multiplication is a versatile molecular method not only for fundamental research but also for application-oriented fields such as clinical medicine production, communicable diseases diagnosis, gene cloning and the regulation of industrial efficiency. PCR was the first method for amplification of nucleic acid (Priyadharsini et al. 2018a). Advantages are NAAT is a popular platform for the amplification and detection of low nucleic acid material. Such technique can be used by amplifying different target regions to identify the presence of microorganisms within the human body. While NAATs are sensitive, there have been numerous inconveniences addressed. First is that they are costly and thus may not be available for extensive gene based viral detection methods (Paramasivam, Priyadharsini, and Raghunandhakumar 2020a).

PCR and its application in viral diagnosis:

Many, but not all, nucleic acid detection based molecular diagnostic methods use polymerase chain reaction (PCR) to greatly increase the number of nucleic acid molecules, thus amplifying the target sequence(s) in the patient sample. PCR is the most commonly used DNA sequence detection tool at this time (Girija et al. 2019). PCR is based on three simple steps needed for any DNA synthesis reaction: (1) denaturation of the template into single strands; (2) annealing of the primers for new strand synthesis to each original strand; and (3) extension of the new DNA strands from the primers (Arcenas 2017).

PCR is a method widely used in molecular biology to quickly create millions to billions of copies of a particular DNA sample which allows scientists to take a very small sample of

DNA and amplify it to a sufficiently large amount to study it in detail(Anand and Raghuwanshi 2020). PCR was first discovered by Kary Mullis in 1983. The different components needed for PCR include a DNA sample, DNA primers, ddNTPs known as free nucleotides, and polymerase DNA. The different components needed for PCR include a DNA sample, DNA primers, ddNTPs known as free nucleotides, and polymerase DNA. Broad- range 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) gene is used in clinical specimens to detect and identify bacterial pathogens from patients with high suspicion of infection.PCR is commonly used in the clinical study of infectious agents including HIV, hepatitis, human papillomavirus (the cause of genital warts and cervical cancer), Epstein-Barr virus (glandular fever), malaria and anthrax (Modrow et al. 2013). PCR has many benefits to it. This is relatively easy to understand and use, and yields good results. The technology is especially sensitive, with both the capability to develop millions or even billions of duplicates for sequence analysis, cloning, and analysis of a specific product. Even so, the polymerase chain reaction also has drawbacks. Though highly precise, this functions only under certain conditions. A factor in the degree of precision is the length of the target DNA sequence.

Viral culture based methods & marker based assays:

The conventional approaches used to diagnose CMV infection were viral culture and serological testing but these are gradually being replaced by a confounding variety of alternative approaches. The latter is unusual in that it detects CMV RNA, likely a more accurate active infection predictor than CMV DNA. Four main virus detection methods are in use today: scanning, integrity checking, identification and heuristic detection. Scanning and capture are very typical of these, with the other two common being in less commonly used anti-virus kits. The modern age of diagnostic virology dates from the first reports Weller and Enders made of viral isolation in cell culture in 1948 and Enders et al. in 1949. In addition, the need for cell culture techniques is the for virology labs as separate entities from other general clinical microbiology labs.Although the relative importance of viral isolation as a diagnostic tool is decreasing rapidly, it is still important as it is the only technique capable of providing a viable technique to isolate virus that can be used for further analysis , for example with phenotypic antiviral susceptibility testing (Priyadharsini et al. 2018a).

Virus growth in cell culture is usually detected by visualizing cell morphological changes, called cytopathic effect (CPE). Often, the CPE characteristics are sufficiently distinctive to allow the laboratory to suspect which virus is responsible for. HSV is the virus which culture remains most useful. Often cell culture is also used to detect CMV, VZV, adenovirus, RSV, parainfluenza and influenza viruses, rhinovirus, and enteroviruses (Shahzan et al. 2019). Reliable and practical transfer of genes Transduction levels in non-human primates up to 40 percent of HSCs is the advantage and disadvantage will be Small levels of transcription One or less copies of provirus per cell Chromosomal sensitive effects DNA sensitive repeats, introns.

Fluorescent in situ hybridization (FISH):

Buongiorno-Nardelli as well as Amaldi in Rome, utilizing 3H-labeled rRNA on Chinese hamster tissue sections embedded with paraffin. FISH is a kind of cytogenetic technique that

uses fluorescent probes that bind parts of the chromosome to show a high degree of complementarity in sequence. Fluorescence microscopy can be used to determine where the fluorescent probe is attached to the chromosome (Paramasivam, Priyadharsini, and Raghunandhakumar 2020a). Fluorescent in situ hybridization (FISH) can be used to check different chromosome regions for presence or absence, and is also used to detect small chromosome deletions such as Williams syndrome (Paramasivam, Priyadharsini, and Raghunandhakumar 2020b). This involves the application of a specific DNA probe that recognizes the region to be tested (Mathivadani, Smiline, and Priyadharsini 2020).
Multiplex-FISH is a technique of 24-color karyotyping and is the method of choice to study complex interchromosomal rearrangements.Includes three main steps in the process (Priyadharsini et al. 2018a). Fluorescent in situ hybridisation is a molecular method that can be used to determine the physical status of HPV (episomal or integrated) within the infected cell (Corneli 2005), (Priyadharsini et al. 2018b). A FISH examination can detect genetic variations that are too small for microscope viewing. FISH also doesn't have to be conducted on healthy dividing cells which will be the advantage of FISH and disadvantage is it is quite expensive and requires skilled manpower in making a non isotopic labelling. Multi-labeled fish probes up to 48 samples targeting the same RNA that can be pooled and labelled together. The identification of rearrangements involving more than 5 Mb of DNA is restricted to traditional karyotyping. The resolution of the FISH technique is around 100kb-1Mb in size, using fluorescent probes.

Antibody test (Serology):
This is also known as serological testing is a blood test that can detect if you have been infected with COVID-19 beforehand. The presence of specific antibodies suggests you have been exposed to the disease and pro-inflammatory cytokines in various viral disease including CoV (Girija, Shankar, and Larsson 2020). Antibodies are molecules that are produced by the body's immune system to fight a specific viral infection (Greer and Alexander 1995). The antibodies bind themselves to a cell attacked with the virus and kill the infection. This check searches for antibodies to a particular viral infection (Brown 2012). Usually, it is done on a blood sample. Immunofluorescence or immunoperoxidase assays are widely used to determine whether a tissue sample contains a virus (Goudsmit 1998). Such experiments are based on the idea that if the tissue becomes infected with a virus, it can be binded to by an antibody unique to that virus (Priyadharsini et al. 2018a).
Many of the rapid tests substitute slowly the viral culture for all of the mentioned viruses in the detection of suitable markers (Marquardt 2004). Antigen identification approaches include staining of the fluorescent antibody (FA), staining of the immunoperoxidase, and EIA(29). Of these, the most widely used in diagnostic virology is FA staining (Choi and Chae 2003). Four main virus identification approaches are in use today: screening, validity checking, interception and heuristic identification (Jung, Choi, and Chae 2002). Scanning and capture are very typical of these, with the remaining two typical only in less commonly distributed antivirus kits (Ha, Choi, and Chae 2004), (Calmettes et al. 2011). The antibody test might not be able to display the virus' current existence (like you may experience with a nasal or throat swab test) because it may take 1-3 weeks after symptoms to establish anticorps.

Other molecular diagnostic Methods:

(a)Nested PCR: Nested PCR is a modification of the polymerase chain reaction which aims to reduce non- specific binding in products due to the amplification of unintended binding sites(Mwesigwa n.d.). (b)Strand displacement amplification(SDA): SDA is an exothermic, in vivo immunochemical technique based on HincII 's ability to nick the unmodified hemi phosphorothioate shape of its recognition site and the ability of klenow (exo-klenow) deficient exonuclease to expand the 3'-end at the nickname(Li et al. 2020).

(C) Probe amplification techniques: Sample amplification methods amplify products that contain only the sequence of the probes. It includes Chain Reaction Ligase, Cycling Probe Technology, etc. LCR amplification is based on the sequential rounds of two juxtaposed oligonucleotide probes dependent template ligation(Sanahuja et al. 2019).

(D) Microarrays: A microarray is a laboratory device which is used simultaneously to detect the expression of thousands of genes. DNA microarrays are microscope slides printed at defined positions with thousands of tiny spots, each spot having a known DNA sequence or gene(Ng et al. 2020).

(E) Allele-Specific oligonucleotide: The allele-specific oligonucleotide (ASO) is a short piece of artificial DNA complementing a predetermined target DNA sequence. It is intended (and used) in a way which makes it specific to only one version of the DNA being tested, or an allele(Studencki and Bruce Wallace 1984).

(F) Gel electrophoresis: Gel electrophoresis is a method that is used to separate the Fragments of DNA by their size. At one end of a gel, DNA samples are loaded into wells (indentations), and an electric current is applied to pull them through the gel. Fragments of DNA are negatively charged, so they pass towards the positive electrode(Rabindra and Raju 2012).

2. CONCLUSION:

Molecular diagnostic methods have replaced the conventional protocols on viral culture and further detection of potential viral markers. It has also seemed to be superior to the various serological methods though for some viral identification, serology is still considered as the gold standard method for viral detection. However, the rapidity, sensitivity and specificity in the detection of specific viral antigens could be best achieved by the latest molecular techniques. This review thus had reinforced the applications of various molecular methods used in viral detection with their advantages and disadvantages.

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Author contributions:

M.Dhakshinya:

1) Execution of the work
3) Data collection

4) Drafting of manuscript.

Smiline Girija AS:

1) Concept and design of the study
2) Validation of the data collection
3) Revision and proof-reading of the review

Ezhilarasan D:

1) Validation of the data collection
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*Conflict Of Interest***:** None to declare

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