Advanced viral detection techniques and methods

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DESCRIPTION

Viral infections are a major cause of morbidity and mortality worldwide, especially in the context of emerging and re-emerging pathogens. Rapid and accurate diagnosis of viral infections is essential for timely initiation of appropriate treatment, prevention of transmission, surveillance and outbreak response. However, viral detection poses many challenges due to the diversity, variability and evolution of viral genomes, the low viral load in some clinical specimens, the presence of inhibitors or contaminants in samples, and the limited availability of resources and expertise in some settings. Therefore, there is a need for developing and improving viral detection techniques that are sensitive, specific, fast, cost-effective, user-friendly and adaptable to different scenarios. Viral detection techniques can be broadly classified into two categories: Direct and indirect methods. Direct methods aim to detect the presence of viral components or functions in the sample, such as nucleic acids, antigens, enzymes or infectivity. Indirect methods rely on detecting the host immune response to viral infection, such as antibodies or cytokines. Both categories have advantages and limitations depending on the type of virus, the stage of infection, the quality and quantity of the ample, and the purpose of the test. Among direct methods, nucleic acid-based techniques are widely used for viral detection due to their high sensitivity and specificity. Polymerase Chain Reaction (PCR) is the most common technique that amplifies a specific region of the viral genome using primers and a thermo stable DNA polymerase. PCR can be performed in Real-Time (RT-PCR) to monitor the amplification process and quantify the viral load. PCR can also be multiplexed to detect multiple viruses or variants in a single reaction. However, PCR requires high quality nucleic acid extraction, thermal cycling equipment, trained personnel and biosafety measures. Moreover, PCR may fail to detect novel or divergent viruses that do not match the primers. To overcome some of these limitations, alternative nucleic acid-based techniques have been developed or improved in recent years. For example, isothermal amplification methods such as Loop Mediated isothermal amplification (LAMP) or Recombines Polymerase Amplification (RPA) can amplify nucleic acids at a constant temperature without thermal cycling equipment. These methods are faster, simpler and cheaper than PCR and can be performed at point of care settings or low resource settings. However, they may have lower specificity or higher risk of contamination than PCR. Another emerging nucleic acid-based technique is Next Generation Sequencing (NGS), which can sequence millions of Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) fragments in parallel without prior knowledge of the target sequence. Next Generation Sequencing (NGS) can provide comprehensive information on viral genomes, such as identification, characterization,

phylogeny, diversity and evolution. NGS can also detect novel or unknown viruses by comparing the sequences with databases or using bioinformatics tools. However, NGS is still expensive, time consuming and complex to perform and analyse. Moreover, NGS may generate false positive or false negative results due to sequencing errors or low coverage. Besides nucleic acid based techniques, antigen based techniques are also widely used for viral detection due to their simplicity and rapidity. Antigen based techniques rely on detecting viral proteins or glycoproteins using antibodies that bind specifically to them. The most common antigen based technique is Enzyme Linked Immuno Sorbent Assay (ELISA), which uses colorimetric or fluorescent signals to indicate the presence of antigens. ELISA can be performed in micro plates or Lateral Flow Devices (LFDs), which are commonly used for point of care testing or home testing. However, antigen based techniques have lower sensitivity than nucleic acid-based techniques and may not detect low level or variant viruses. Other direct methods include enzyme activity based techniques that detect viral enzymes such as reverse transcriptase or protease using substrates that produce detectable signals upon cleavage. These methods are fast and simple but have low specificity and may not distinguish between different viruses that share similar enzymes. Another direct method is virus culture that detects viral infectivity by inoculating cell lines or animals with the sample and observing cytopathic effects or disease symptoms. This method is highly specific but slow, labor intensive and ethically questionable. Among indirect methods, antibody based techniques are widely used for viral detection due to their ability to measure past or present exposure to viral infection. Antibody based techniques rely on detecting host antibodies that bind specifically to viral antigens using labelled antigens or antibodies. The most common antibody based technique is also ELISA.