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From the Publisher's Desk

Welcome to Biotechnology Kiosk!

The February'2022 issue of BK is now live for our readers with the regular features. This issue includes cutting edge articles on rapid diagnostics for infectious diseases and electron microscopy for the analysis of viruses and the regular section on editor picks along with a popular article.

We hope our readers will enjoy reading these news and views on the current cutting-edge topics that include latest research breakthroughs in different areas of medicine and biotechnology. We look forward to receiving your feedback. We do hope that you will enjoy reading this issue of Biotechnology Kiosk. Please do write to us with your comments. Your suggestions are always appreciated.

Dr. Megha Agrawal & Dr. Shyamasri Biswas.

Editors-in-Chief, Biotechnology Kiosk

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Recent Advances in Rapid Diagnosis and Analysis of Infectious Agents in Human Blood Stream

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Abstract

The ability to rapidly diagnose and analyze infectious agents such as pathogens in human blood stream is considered pivotal and an important step for patient treatment and effective monitoring. This is also essential to ensure the safety of the donor blood supply. This has driven research in medical biotechnology and the focus has been on developing rapid diagnostic ability that enables detect and identify pathogens in blood borne infections. This research seeks to mitigate the challenges involving infections such as pathogens induced sepsis. These infections are believed to be a major cause of mortality in hospitalized patients worldwide. It is of general consensus that establishing a rapid diagnosis would not only enable an early and adequate antimicrobial therapy but it would also result in positive clinical outcomes for patients. In this perspective, we have presented a brief overview of some of the notable recent advances in diagnostics to detect infectious agents in human blood.

Keywords: Diagnostics, infectious diseases, pathogens, viruses, lasers, blood borne pathogens, spectrometry

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Introduction

In battling against complex diseases, a significant public and medical concern is the quality and safety of blood products that are used in transfusions. It is believed that blood transfusion-transmitted infections or infectious agents can result in serious medical conditions such as sepsis. This can lead to fatal consequences especially in highly vulnerable populations including neonates, the elderly, or immunocompromised persons. Studies conducted over the last decades have shown emerging pathogens that can severely affect blood safety. To battle against such infectious pathogens, the focus has been to employ rapid molecular tests for detection of blood-borne pathogens such as West Nile virus, dengue virus (DENV), and Babesia microti etc. [1, 2].

There are several existing processes of blood culture that are employed for the detection of an infection and the identification of the responsible organism for diagnosis of human blood stream infections. However, most of these processes involve a fairly long time period to complete the tests. Hence, it is not rapid at times that usually influences vital treatment decisions. The time-consuming process of blood culture is due to the fact that it involves steps starting from micro-organisms that are present in a blood sample and are enriched in cultivation medium in blood culture bottles under continuous monitoring. In the next step, Gram staining is done that allows the first adjustment of antibiotic therapy. Additionally, sub cultivation may also be required for identification and antimicrobial susceptibility testing. This entire process of blood culture therefore becomes quite complex that could essentially involve up to total 72 hours depending on the pathogen. This also includes the use of highly skilled personnel and complex sample preparation. Furthermore, the process becomes even more complex and time consuming in the event of transporting the blood sample to a microbiology laboratory for advanced analysis. All these complexities have triggered research in medical biotechnology to develop next generation diagnostics that ensures prevention of fatal diseases that are caused by pathogens in blood born infections [3, 4].

To this end, medical technologists and biotechnologists have been involved in cutting edge research to develop diagnostic technology mostly based on lasers and spectrometry that gives the ability to rapidly (within minutes) diagnose blood borne infections on-site. It is believed that on-site diagnostic technology is transformative as it requires simple sample preparation and no requirement for highly skilled personnel. This significantly enhances the ability to identify, contain, and treat blood borne infections. This is expected to greatly reduce the time needed to screen donated blood for infections. Researchers envision that the development of laser-based

blood diagnostic technology with these capabilities could pave the way to future breakthroughs that would take the laboratory diagnosis to the next level, where patients could be screened for infections and receive prescribed treatments immediately [5-9].

Here, we have described some of the recent significant advances in rapid diagnostic technologies for quick analysis of human blood.

1. Ability to Detect Parasites, Bacteria and Viruses in Human Blood: Laser-Based Point-of-Care Diagnostic Technology

In a recent study of major significance, researchers showed the potential of a laser-based diagnostic method to detect the presence of parasites, bacteria and viruses in human blood. The technique was based on Laser-Induced Breakdown Spectroscopy (LIBS).

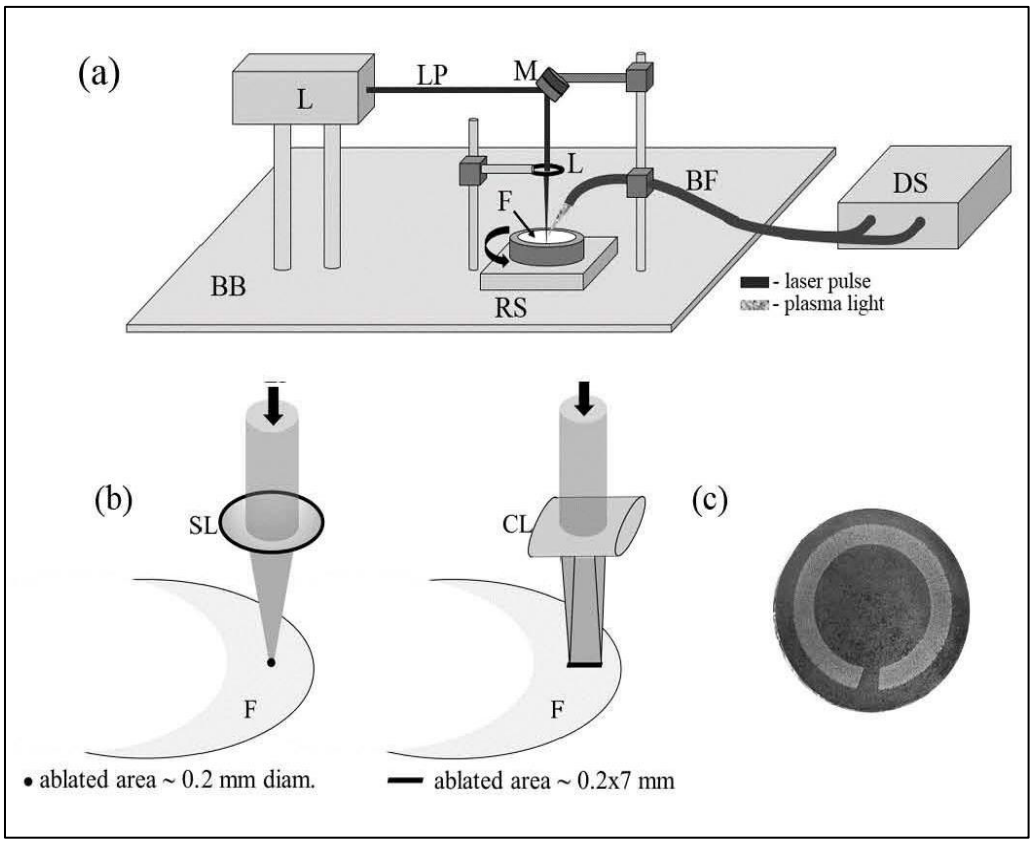

Figure 1: *(a) A schematic diagram showing the apparatus used to record spectra (BB: breadboard; L: laser; LP: laser pulse; M: mirror; L: lens; F: filter; BF: bifurcated fiber optic; DS: dual channel spectrometer; RS: rotating stage). (b) A geometry is shown for focusing the laser pulses using a spherical and cylindrical lens (SL: spherical lens; CL: cylindrical lens). (c) A display photo is shown for a filter following interrogation by 620 laser pulses that are focused using the cylindrical lens [Source: J Appl Microbiol. (2019)].*

It showed the capability of point-of-care diagnostic based on the analysis of spectra collected from a series of laser sparks that were formed on a blood sample. In this method, a laser pulse is used to simultaneously vaporize a small sample mass that subsequently excites the resulting atoms. This results in emitting light formation of a hot plasma on the sample surface. Finally, light from the plasma is collected and spectrally resolved and subsequently recorded for spectra (Figure 1) [8].

The technique was shown to be capable of generating analysis results within minutes. Researchers showed the presence of pathogens in the blood using a novel analysis approach and successfully demonstrated to clinically relevant levels of 10 cells, copies, or parasites per ml. It showed the differentiation of blood spiked with viruses, bacteria, or protozoan parasites to clinically relevant levels in six blood types (O+, O, AB+, A+, A-, B+). These results pave the way to simultaneously detect multiple pathogens in blood that could be employed for more rapid blood analysis in the future [8].

2. Rapid Diagnostic Technology for Human Visceral Leishmaniasis (Kala-Azar)

Leishmaniasis is considered a deadly disease. This disease is believed to be endemic in 98 countries, with the highest burden of disease in India, Brazil and other developing nations. Studies on Leishmaniasis have suggested that the disease is caused by protozoan parasites of the genus Leishmania and the most severe form of this disease is Visceral Leishmaniasis (VL), which is also known as Kala-Azar. It has been suggested that Kala-Azar is caused by L. chagasi/L. infantum in the Americas and L. donovani and L. infantum in Afro-Eurasia. Rapid and precise diagnosis of human VL is the suggested treatment pathway to battle against human VL, which is otherwise believed to be a fatal disease. To this end, researchers believe that for VL eradication, it is essential to develop novel, effective and affordable assays for rapid diagnosis of VL [10-13].

In a recent study, researchers demonstrated a novel diagnostic technique based on Laser Direct-Write (LDW) technology coupled with a Lateral Flow Device (LFD) with double-channel geometry. The advantage of this diagnostic technology lies in the fact that it can work on a low-cost paper platform that can be leveraged for a rapid and accurate serodiagnostic assay for human VL. Researchers employed the Duplex VL-LFD that was based on a laser-patterned microfluidic device using two recombinant Leishmania proteins, β-tubulin and LiHyp1, as novel diagnostic antigens (Figure 2) [14].

They further tested the VL-LFD assay with blood/serum samples from patients diagnosed with VL, Tegumentary Leishmaniasis and

Leishmaniasis of unknown identity. The study also involved other parasitic diseases with similar clinical symptoms. Using LBW-LFD technique, out of the 22 positive samples from patients that were diagnosed clinically with VL infection, 20 tested positive with the Duplex VL-LFD, compared to 17 patients that were tested positive with the available commercial kit [14].

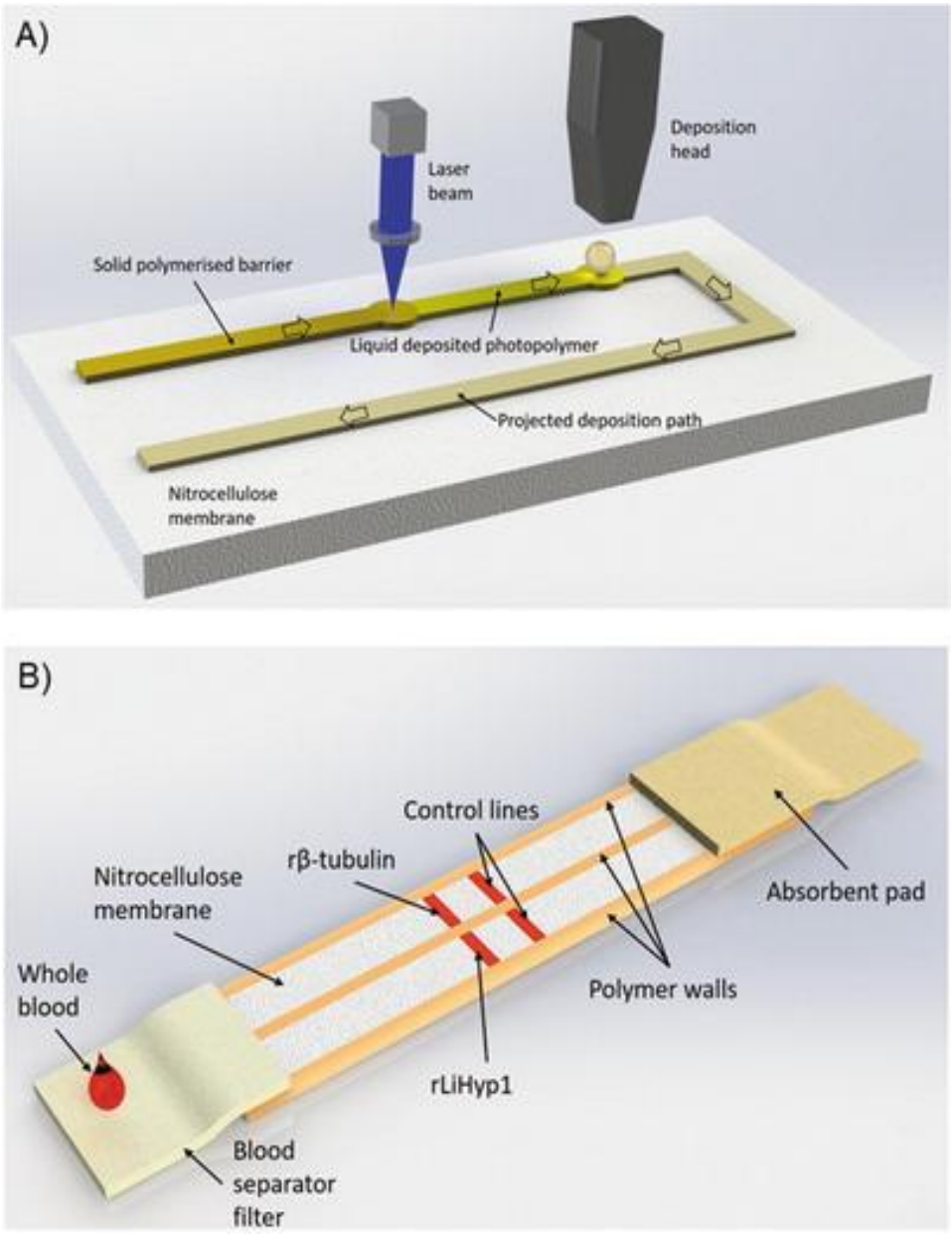

Figure 2: *(A) Schematic depiction of a Laser Direct Write (LDW) setup that shows the deposition head printing the liquid photopolymer onto the nitrocellulose membrane and its subsequent photo-polymerization by the laser beam. (B) A schematic diagram showing the Laser Direct Write Lateral Flow Device (LDW LFD) that consists of a double-channel geometry as a rapid serodiagnostic assay for human VL [Source: Journal Emerging Microbes & Infections (2019)].*

Further, researchers showed the better performance of LBW-LFD technique compared to the standard methods to diagnose and screen patients with VL. It is envisioned that the applications of this new VL-LFD diagnostic technique could be extended to employ to other comparative testing in larger patient groups focusing in areas with endemic VL. This could be a significant step forward to improve diagnosis and disease management of Kala-Azar [14].

3. Identification of Bacteria in Human Bloodstream by Immunoaffinity Mass Spectrometry

As we described in the beginning, blood stream infections (BSI) associated sepsis can lead to serious medical conditions. BSI is caused by the presence of bacteria or fungi in the blood stream. According to one estimate, about 600,000 BSI episodes occur in North America every year, and about 1, 200,000 BSI episodes affect Europe. This results in roughly 86,000 and 157,000 deaths, respectively. To mitigate the challenges posed by BSI, a rapid blood test and diagnosis is recommended to effectively battle such bacterial infections in the blood stream [15-18]. To this end, researchers recently demonstrated a sensitive method to rapidly and accurately identify bacteria in human blood samples. They employed a combined optimized matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and efficient immunoaffinity enrichment/separation method for the rapid diagnosis (Figure 3). A proof-of-concept level demonstration was reported with whole blood spiked with a low initial concentration (102 or 103 cells per mL) of bacteria that was cultured in commercial blood culture bottles and analyzed by the developed method after different blood culture times [19].

The employed method showed a distinct advantage of high sensitivity. It was shown that the blood culture time required for diagnosis could be significantly reduced. The bacteria was successfully identified after 4 hours of blood culture (Figure 3) [19]. This method was further optimized for the development of an entirely new diagnostic process that could be accurately accomplished within half a day from start of the tests to the completion of the process. This paves the way for employing immunoaffinity mass spectrometry method in all future studies that could facilitate anti-bacterial therapy in a timely fashion to mitigate the risk of mortality from bloodstream infections [19].

4. Case Study on Rapid Diagnosis of Bloodstream Infections in Critical Patients

Previous studies have shown that prompt treatment with targeted antibiotics can be leveraged to not only mitigate the challenges of the financial impact but also positively impact the

clinical outcome of bloodstream infection and associated sepsis that represent a major source of mortality in industrialized countries [20-27]. Researchers conducted a case study to assess the usefulness of the IRIDICA BAC BSI Assay, a PCR/ESI-MS-based technology for the early diagnosis of bloodstream infections from primary blood samples in critical patients [28]. This evaluation was performed by comparison with the traditional culture-based methods. In this study, researchers investigated a total of 300 prospective whole blood specimens obtained from patients suspected of sepsis (Figure 4). They found that the overall concordance between the two techniques was of 86%, with a calculated sensitivity of 76% and an assay specificity of 90%. Further, the clinical significance of discrepant results was evaluated reviewing the patients' clinical records and the results of additional relevant microbiological tests [28].

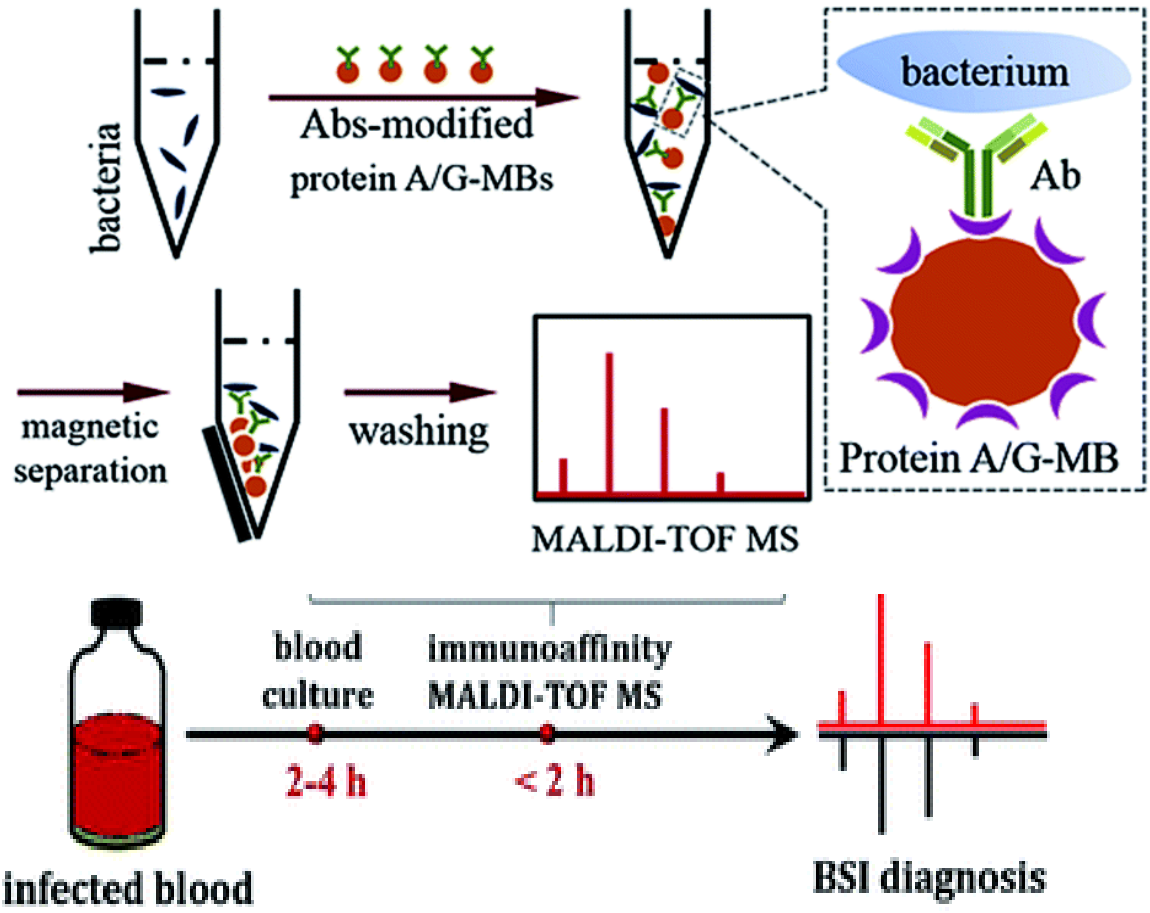

Figure 3: *Schematic illustrations showing the immunoaffinity MALDI-TOF MS procedure and the rapid diagnosis of bloodstream infections (BSI) [Source: Chem. Sci. (2016)].*

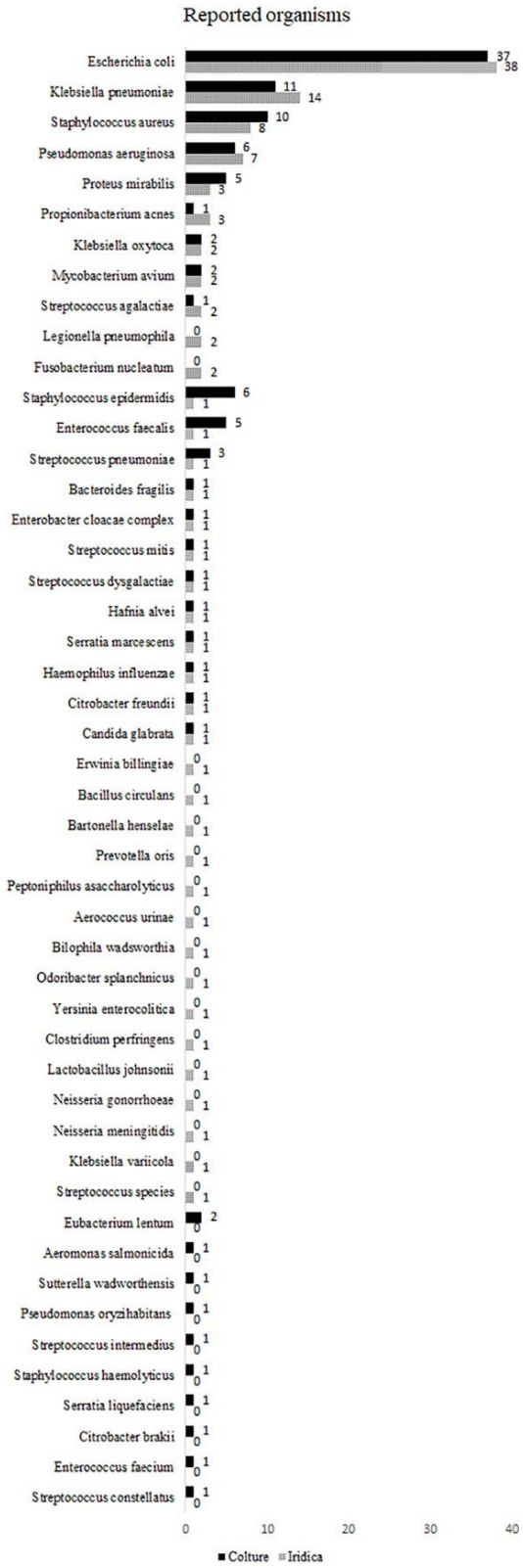

Figure 4: *Distribution of the organisms is shown. The organisms reported by culture (solid bar) and PCR/ESI-MS (patterned bar) are sorted by decreasing order of PCR/ESI-MS reported organisms [Source: PLoS One (2018)].*

This case study was significant as the data supported the ability of the IRIDICA BAC BSI Assay to identify a broad range of bacteria directly from primary whole blood samples, within eight hours, which implied a timely administration of a suitable treatment [28].

Concluding Remarks

In conclusion, we have described the requirements for new clinical diagnostics for rapid detection of pathogens in human blood. Such rapid identification has been shown to be useful in combating complex diseases that are caused by blood pathogens. This can also be leveraged to adapt to newly emerging infectious agents and to screen simultaneously for multiple infectious agents in bloodstream. To this end, several studies have shown promise of laser-assisted diagnostics and the unique capability of laser technology for rapid identification of infections in bloodstream. This new diagnostic technology platform is expected to transform the existing blood culture process to a far more superior and rapid process of identification of infections within a few hours and also on-site. The laser-based approaches can be leveraged to achieve sensitive, multiplex detection that requires minimal sample preparation while at the same time providing rapid results that are generated within minutes. Thus, this versatile point-of-care diagnostic offers a disruptive technology platform that could potentially be a game changer in next generation diagnostics and therapeutics industry. Recent studies have suggested that these application-relevant properties can be leveraged to boost the diagnostic abilities. This can be further equipped with the additional features such as flexibility in the technology that allows to add new agent detection by simply adjusting the detection programming. These steps are considered critically important to achieve the ultimate goal of superior clinical diagnosis of bloodstream infections along with prevention of associated medical fatalities in the future.

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Electron Microscopy for Structural Determination and Analysis of Viruses

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Abstract

Viruses are known to be associated with large-scale, dynamic conformational changes that take place to facilitate cell entry and genome delivery. It is also known that a replication machinery is involved in the advanced stage of the infectious cycle that enables to read and synthesize nucleic acid strands. This process results in the generation of new copies of genetic material. In this process, the function of structural proteins helps to assemble and package the appropriate contents to produce new infectious particles. Lately, there has been a great deal of research interest on structural elucidation of these events. This interest is primarily driven by the significance of virus structural identification, which helps understand these processes and also their inhibition by antiviral agents such as neutralizing antibodies and drugs. To this end, the development of electron microscopy (EM) techniques for studies in virology has played a major role for structural determination and analysis of viruses. In this mini-review, we have highlighted some of the latest developments in this field. We have briefly described important role of EM in virology. We have also discussed notable application examples of EM in elucidating various virus structures to gain insights into identification of deadly pathogens and other infectious agents and outbreaks along with anti-viral developmental strategies.

Keywords: Electron microscopy, cryo-electron microscopy, virus, structure, antiviral therapy, genome delivery, cells, replication.

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Introduction

The Role of Electron Microscopy in Virology

Viruses are known to be extremely diverse in shape and size. Over the years, numerous studies on viruses have shed light on their evolution that has led to a limited number of viral classes or lineages [1-6]. In addition, virologists have used the term virosphere to describe general features of viruses that not only include the space where viruses are found but also the space, they influence that can extend their impact on the environment. This scenario explains the complexity of the interactions involved in the viral assembly mechanisms that are restricted to two general pathways. The co-assembly of capsid proteins and single-stranded nucleic acids is one such pathway. The other pathway involves a sequential mechanism in which scaffolding-mediated capsid precursor assembly is followed by genome packaging [7-12].

In order to prevent viral disease along with virus control and establishing efficient and reliable virus diagnosis, it is critical that studies on the biology of viruses and the etiology of virus disease are conducted. To address these issues, researchers have shown that knowledge of the atomic resolution structures of viruses can be a powerful tool for vaccine discovery and design to mitigate the challenges of global pandemics caused by deadly viruses [13-14]. To this end, the role of electron microscopy (EM) in virology is believed to be pivotal that can be leveraged to identify the causative agents of infectious diseases. EM has been shown to be an essential tool in the detection and analysis of virus replication. For example, the technique has been proven to be important to diagnose pathogens and in testing to identify microorganisms. New EM methods and ongoing technical improvements have been shown to cover a broad range of applications that have allowed in-depth investigation of viral impact on not only the host but also the environment. It is now possible to conduct such investigations close to atomic resolution with the latest cryo-EM based methods. Further, it has been shown that in combination with bioinformatics, the transition from two-dimensional '2D' imaging to three-dimensional '3D' remodeling can be accomplished. This allows structural and functional analyses that advance our fundamental understanding of the diversity in virus structure and life cycle. Especially, EM in combination with advanced techniques such as confocal laser scanning microscopy can be leveraged for live imaging of cells and tissues with high-resolution analysis [7, 15-17].

EM is advantageous over conventional light microscopy for the reason of the technique's capability to analyze small size of virus particles that cannot be visualized by light microscopy-based techniques [18-21]. Researchers have

shown the versatility of EM to resolve 3D structures of individual viral proteins and whole virions in multiple functional states that include cells at different stages of infection. This makes EM as a very popular technique that can be employed to identify and analyze unknown emerging viruses even when there are no primers, antibodies or probes available. Thus, EM is considered extremely advantageous because of a generic approach that it follows that offers the potential to detect all viral particles present in a sample [22, 23].

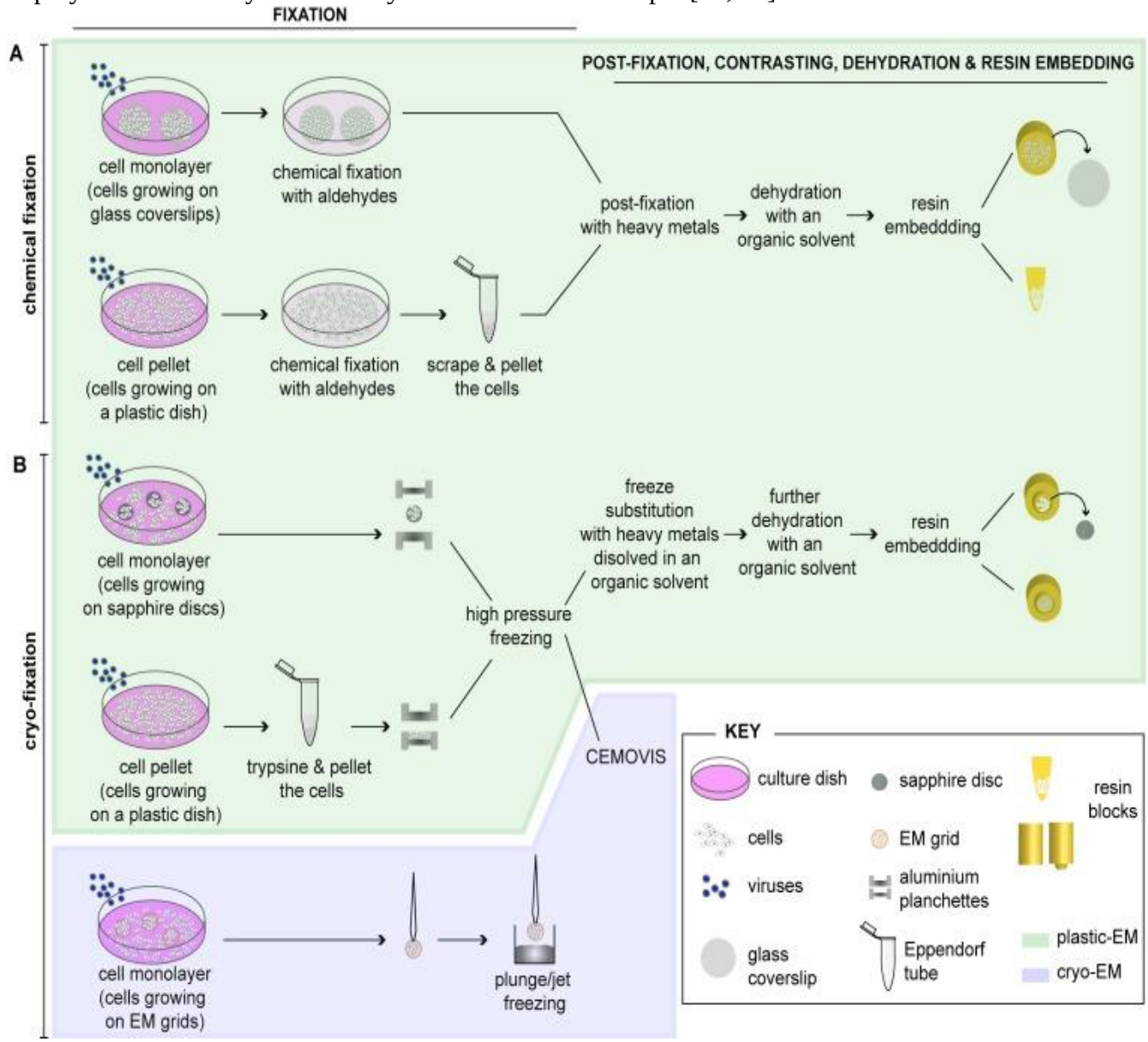

Figure 1: *[A-B] Schematic depiction highlighting different methods for preparing virus-infected cells for electron microscopy [Source: Viruses (2015)].*

Preparation and Analysis of Virus-Infected Cells for Electron Microscopy

It is very important that the ultrastructure is preserved in a state that is as close as possible to a snapshot of the living state. Accordingly, it is suggested that the preparation of cells for EM should follow the major goal of preserving the cell structure that is visualized at the resolution limit of the electron microscope. To this end, researchers have shown several methods and standard recipes. These methods can be frequently modified when addressing particular biological questions [22, 23].

Figure 1 shows the standard method to prepare cells for routine EM that involves fixation, embedding and sectioning [23]. As Figure 1 shows, adherent cells can be either chemically fixed with aldehydes or fast frozen by high-pressure freezing (HPF) or plunge/jet freezing. Subsequently, infected cells can be further processed as a cell monolayer (e.g. grown on glass coverslips) or be pelleted prior to further processing for EM after chemical fixation. Post-fixation processing can be done with heavy metals using osmium tetroxide (OsO4) and uranyl acetate (UA). This results in sample dehydration with increasing concentrations of an organic solvent using ethanol or acetone. This is followed by embedding into a plastic resin (plastic-EM; highlighted in green) (Figure 1). It is also suggested that coverslips are removed from the polymerized resin block which is done by successive immersions in liquid nitrogen and hot water. Further, cella must be grown as monolayers on sapphire discs for rapid immobilization by HPF. This can be achieved by clamping in-between two aluminum planchettes and subsequently loading into a HPF machine for rapid freezing [23]. Alternatively, this can be done by directly placing cell pellets into the aluminum planchettes or into capillary tubes for freezing. Frozen cells can then be subjected to freeze substitution (FS), dehydration with an organic solvent and resin embedding (plastic-EM; highlighted in green in Figure 1). Further, as an alternative approach, high-pressure frozen cell pellets can be analyzed by CEMOVIS (cryo-electron microscopy of vitreous sections) (cryo-EM, highlighted in violet in Figure 1). Cells growing on EM grids can then be plunge/jet frozen and analyzed directly by cryo-EM. Both these cryo-EM approaches do not require further processing of the cells and allow visualizing cells in their closest-to-native status [23].

With respect to analyzing virus-infected cells, the main research focus has been on visualization comprising different steps of the viral life cycle that include attachment, entry, replication, assembly and egress along with the study of the ultrastructure of the infected cell. To this end, an important goal is to understand virus-induced modifications of a targeted subcellular

organelle. This is to elucidate not only the function of different viral proteins, but also aimed at designing novel antiviral drugs and eventually vaccines [24].

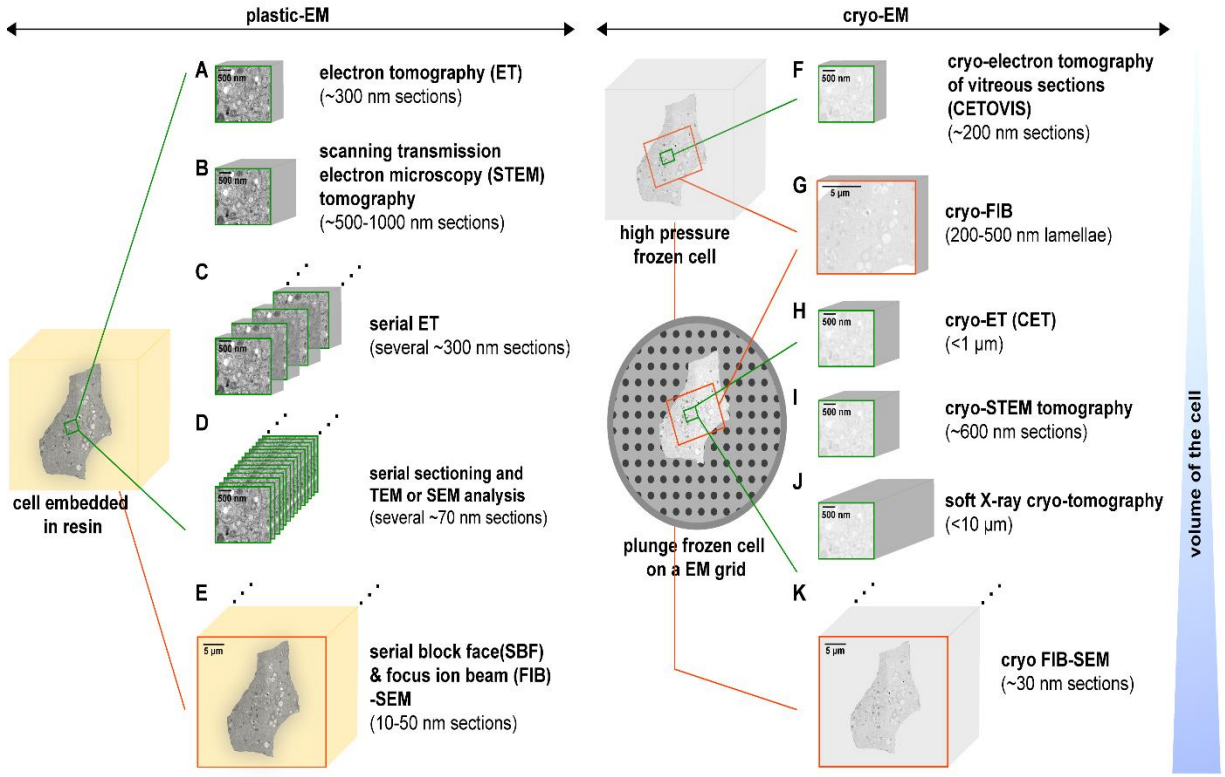

Figure 2: *An overview of the 3D-EM methods that show various approaches to analyze the architecture of virus-infected cells. This is achieved by employing plastic-EM (A-E) or cryo-EM (F-K) [Source: Viruses (2015)].*

Several analytical approaches have been developed to gain 3-D information of cellular structures by EM. Researchers have shown the possibility to unravel the 3-D architecture of cellular organelles and even complete virus-infected cells with the implementation of ever advancing novel EM techniques (Figure 2) [23]. For example, negative stain transmission electron microscopy (TEM) is frequently considered for imaging microbial samples especially in diagnostic virology. However, there is a challenge in negative-stain TEM that requires an adequate concentration of bacterial cells or virus particles. To address this issue, researchers have grown microbes to a high tire and/or concentrated by centrifugation. However, such a process is somewhat difficult to do with patient specimens or agents that are not easy to culture. There has been some progress in filtration techniques that have shown that both TEM and scanning electron microscope (SEM) identification of viruses can be carried out with as little as 5000 total particles per

sample. Studies have also shown that SEM can be employed to study morphological features of isolated organisms for diagnosis purposes. In such cases, biological specimens are required to be coated with thin film evaporation or sputtering of carbon or metal in a vacuum coater to produce an electrically conductive surface for SEM [24].

Lately, cryo-EM has emerged as a popular technique for virus detection and analysis of specimens that have not been stained or fixed in any way. Cryo-EM and cryo-electron tomography (cryo-ET) (Figure 2) are the latest EM technologies that are revolutionizing the field of structural biology. Recent research has shown the huge promise of cryo-EM to determine high-resolution structures of many viral assemblies as well as those of assembly intermediates. This paves the way for further development and redesign of virus-based platforms for advanced biomedical and biotechnological applications [25, 26]. In the following sections, we have described some of the important applications of electron microscopy in detection, identification and analysis of complex viruses.

Investigating Novel Viruses from Mud Crab Scylla paramamosain with Multiple Infections by Cryo-Electron Microscopy

Identification of deadly pathogen is considered very important for early intervention in unknown infectious outbreaks. Such early identifications are especially recommended for pathogens that can cause dual or multiple infections. One example is the mud crab Scylla paramamosain, which is an economically important aquaculture species that are found in China, India, Australia, and many other countries. The problem is however, the prevalence of sleeping disease (SD) that is found in this species that results in significant economic losses. Previous studies involving molecular investigations were conducted that showed two major viral pathogens including mud crab reovirus (MCRV) and mud crab dicistrovirus (MCDV) with diameters of 72 and 30 nm, respectively. MCDV has been reported to belong to the family Dicistroviridae, which contains three genera involving Cripavirus, Triatovirus, and Aparavirus [27-31].

SD in crabs with multiple infections is known to affect aquaculture worldwide in addition to potential outbreaks in human population. Researchers addressed these issues in a recent study and reported discovery and identification of a novel virus in mud crabs with multiple infections by employing cryo-EM. Such virus structural analysis was not previously done by molecular, immune, or traditional electron microscopy methods. In this study, researchers showed high-resolution structures of pathogenic viruses that provided new ways for a molecular understanding and developing new disease prevention methods. They showed that the 3D structure of the mud crab

tombus-like virus (MCTV) and mud crab dicistrovirus (MCDV) could assist the development of antiviral inhibitors. The identification of a novel virus in multiple infections demonstrated the applicability of this strategy for investigating multiple infectious outbreaks including humans and other animals [32].

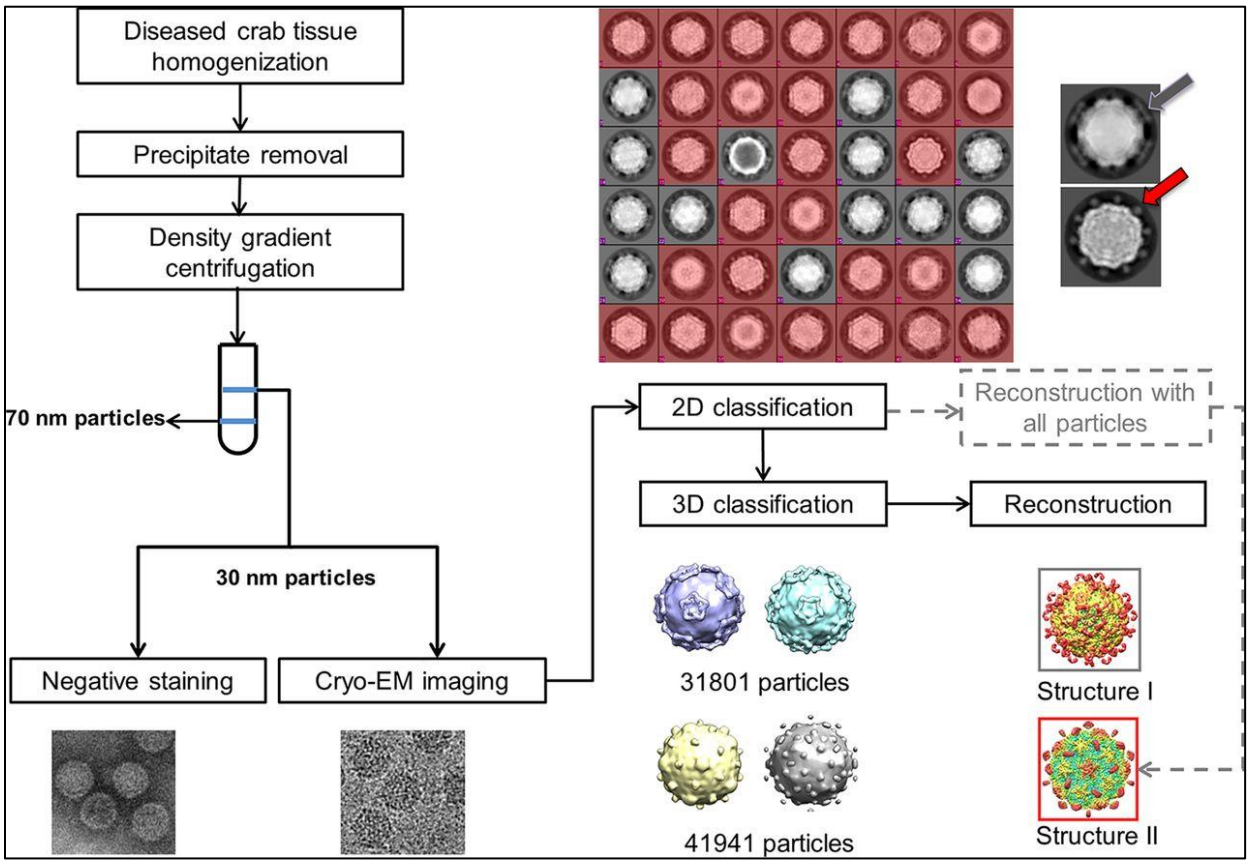

Figure 3: *Flow chart is shown for sample isolation and data processing that revealed two kinds of virus based on the shape of protrusions including one (colored red) that showed dot-like protrusions (red arrow), and the other (gray) showed lamina-shaped protrusions (gray arrow). These results confirmed the presence of two kinds of virus with different protrusions [Source: Journal of Virology (2019)].*

Single-particle cryo-EM was employed to investigate viruses associated with SD following novel antiviral developmental strategies (Figure 3) [32]. The results revealed the structure of mud crab dicistrovirus (MCDV) along with identifying a novel mud crab tombus-like virus (MCTV) that was not previously detected using molecular biology methods. Moreover, the structure of MCDV at a 3.5-Å resolution identified three major capsid proteins (VP1 to VP3) that were found organized into a pseudo-T3 icosahedral capsid, which confirmed the existence of VP4. The researchers also made an unusual observation on MCDV VP3 that contained a long C-terminal

region, which formed a novel protrusion that was not observed in other prior studies on dicistrovirus. These results revealed that MCDV can release its genome via conformation changes of the protrusions when viral mixtures were heated. Also, the structure of MCTV at a 3.3-Å resolution revealed a T 3 icosahedral capsid with common features of both tombusviruses and nodaviruses [32].

Identification and Analysis of Viral Infection of Tissues in COVID-19 by Transmission Electron Microscopy

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, causing coronavirus disease 2019 (COVID-19) has devastated the global population that resulted in millions of deaths worldwide. To gain insights into the novel virus, there have been intense investigations into the pathogenesis of this disease. With the emergence of SARS-CoV-2, one of the research areas that has received great attention is the use of EM to help identify virally infected cells and uncover the pathogenesis of this disease. To this end, several articles have used EM that have proposed direct evidence of infection of the kidney and other tissues by SARS-CoV-2. These reports have triggered speculation that the morbidity and mortality of COVID-19 have resulted from direct infection of tissues throughout the body. These studies have indicated that the identification of pathogens/viruses by electron microscopy help to establish critical information that is needed to better understand the biology of the disease and achieve effective treatments for this and future pandemics [33-39].

In a recent relevant study, researchers employed TEM for the identification of novel viruses including SARS. They showed effectiveness of TEM for the identification of COVID-19 in tissues and morphologic features of the viral particles that were consistent with the prior knowledge of the virus. This study focused on understanding the size and uniformity, formation of higher order structures including aggregates/arrays/inclusions, the absence or presence of a clearly discernible membrane, and the qualities of nucleocapsid and peplomers/spikes electron densities [40].

By employing TEM, researchers investigated the SARS-CoV2 viral particles that were shown to be with an average diameter of 64 nm (range 56-75 nm) (Figure 4) [40]. It was also shown that tissue preservation was critical because poor preservation for autopsy material could often compromise objective interpretation of electron micrographs and the ability to conclusively identify viral particles. Location of viral particles was also studied by TEM that conformed to the known biology of viral replication. This provided strong supporting evidence that was required when attempting to identify viral particles in

tissues with suboptimal preservation, necrosis and autolysis in order to differentiate these particles from normal cellular structures. As shown in Figure 4, coronavirus particles were found inside the cisternae of the ER-Golgi and secretory compartment, as well as outside of cells [40].

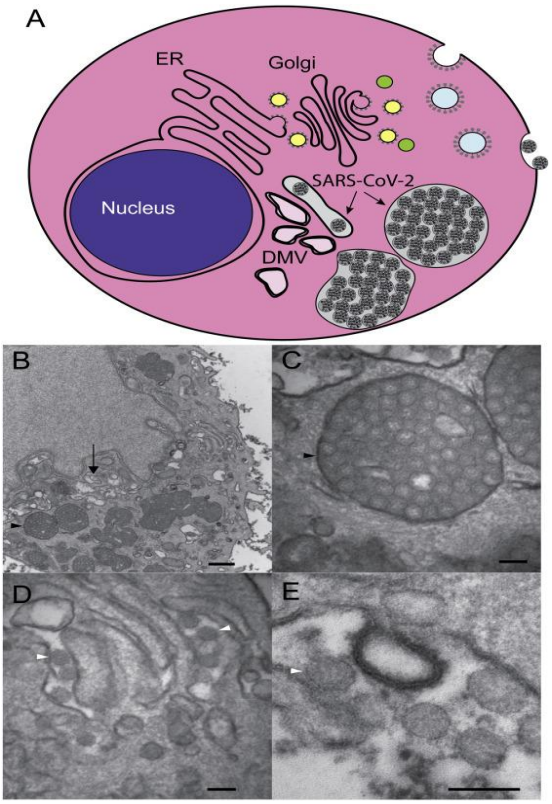

Figure 4: *[A-E] Schematic depiction of cell demonstrating structures that are associated with the investigations of coronavirus infection in cells by transmission electron microscope (TEM). This shows double-membrane vesicles (DMV) that are found near the nucleus and represent the site of viral genome replication. Coronavirus particles bud into the cisternae of the ER/Golgi and they accumulate in cytoplasmic vesicles that fuse with the plasma membrane and release virus particles into the extracellular space. Typical TEM images are shown for a SARS-CoV-2 infected HBEC3-KT cell with perinuclear DMV (arrow) and enlarged vesicles (black arrowhead) filled with viral particles (bar = 500 nm) [Source: The American Journal of Pathology (2020)].*

Scanning Transmission Electron Microscopy (STEM) Detector for Virus Quantitation

Previous studies have shown that virus quantitation is an important factor when studying the environmental impact of viruses, or virus impact on a specific host. To this end, an accurate method for the quantitation of virus particles is considered vital and therefore, the research in virology has been focused on developing a universally accepted method that can be adopted by the scientific community. It is believed that an accurate method for the quantitation of virus particles is very useful. However, a universally accepted method has not been adopted by the

scientific community so far. To address the issue, researchers have employed direct EM quantitation, which is considered a valuable technique due to the reason that it provides enumeration of all virus particles including infectious non-infectious [41-44].

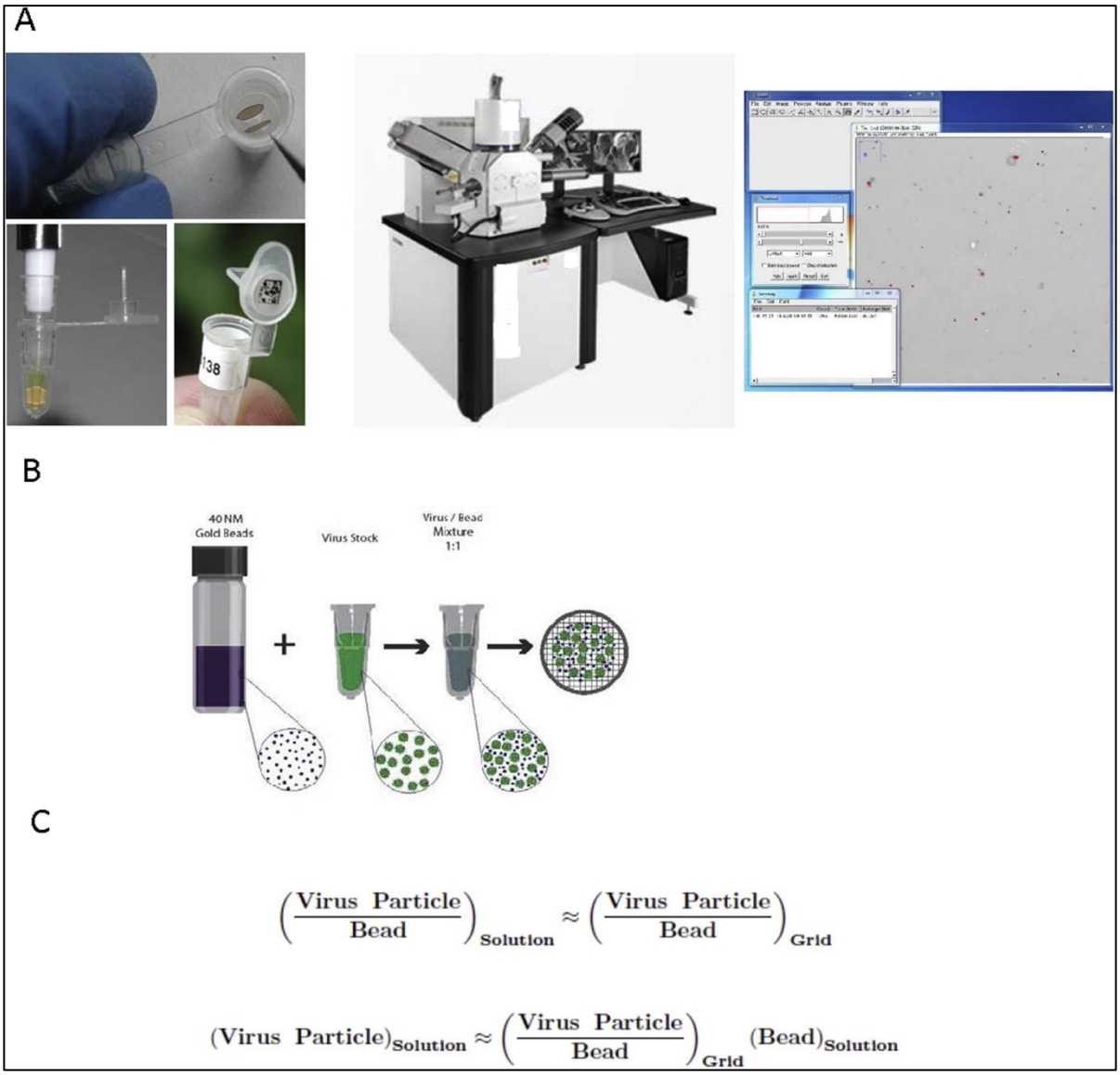

Figure 5: *[A-C] An overview of the employed STEM-VQ method is shown with the three major phases that are needed for determining particle concentration (left) sample preparation using mPrep/g system, (middle) STEM imaging in the SEM and (right) particle counting using image. A mixture of a known concentration of gold beads is also shown with an unknown concentration of virus stock and a formula is shown that is used to calculate the number of unknown viral particles based on the known concentration of gold beads and the virus-gold ratio [Source: Journal of Virological Methods (2017)].*

In a recent study, researchers have demonstrated a method for accurate quantitation of virus particles based on electron microscopy quantitation that provides direct morphology information and

counts of all viral particles, whether or not they are infectious [45]. In the past, EM negative stain quantitation methods were cited as inaccurate, non-reproducible, and that were too high to be useful. In this study, researchers improved accuracy and reproducibility with detection limits by employing a method termed Scanning Transmission Electron Microscopy – Virus Quantitation (STEM-VQ), which simplified sample preparation and that used a high throughput STEM detector in a SEM coupled with commercially available software. They demonstrated STEM-VQ with an alphavirus stock preparation to present the method's accuracy and reproducibility, including a comparison of STEM-VQ to viral plaque assay and the ViroCyt Virus Counter (Figure 5) [45].

Conclusion

In this mini-review, we have highlighted advanced electron microscopy (EM) based methods that have proven to be immensely promising in elucidating and deeper understanding of the structures of complex and deadly viruses. We have presented some notable examples of EM based methods that have been shown to be capable of detecting and analyzing viruses such as SARS-CoV-2. It is believed that these investigations are of huge importance because a deeper understanding of virus structural morphology could help pave the way to major breakthroughs in future in the areas of new therapeutics, such as antibodies and drugs and eventually highly effective and safe vaccines. It is a multidisciplinary research field that involves thin film technologists and electron microcopy experts along with biotechnologists and virologists, to name a few. It is anticipated that future studies will include a combination of different EM techniques to elucidate ultra-small structural features of viruses that will help understand different stages of infectious cycle along with new insights into virosphere. This will eventually help prevent future outbreaks and pandemics.

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Popular Article

Safe Space: An Introduction to Cybersecurity

Janet Campbell

Elderspark.com

Cybersecurity is a complicated field but one that is increasingly pertinent to businesses operating in the digital space. Today, Biotechnology Kiosk explores precisely how cybersecurity works and what can be done to protect sensitive information online.

What Is Cybersecurity?

In a nutshell, cybersecurity is the protection and maintenance of computer systems/networks against theft, damage, or disruption. The need to secure our personal information, intellectual property, and critical data runs parallel to our increased dependence on technology. As politics and business grow increasingly entwined with digital applications, so do threat actors multiply with the aim to infiltrate and disrupt our flow of data and money.

For all these reasons, it's good to have someone on your staff (perhaps you yourself) who is well-acquainted with cybersecurity and how to implement it. If you've ever thought about going back to school, this may help protect your business and be well-worth it in the long run – especially if you take advantage of online learning platforms that allow you to work remotely and learn at your own pace.

Identifying Risk

Larger businesses tend to have specialized personnel (such as Chief Information Security Officers) to oversee and strategize against cybersecurity risks. In the absence of an expert, it's up to the business owners themselves to read up, identify risks and prepare for a potential attack. This begins with:

- Documentation of systems, applications, and information in company use with an aim to ensure confidentiality, integrity, and availability at all times.
- Pre-established risk management frameworks that are applicable to your existing systems.
- A framework aimed to identify, document, and manage attacks (written in expectation of and prior) in relation to existing systems and applications.

To help with this, it makes sense to familiarize yourself with the various risks posed to your business in relation to your digital practices, systems, and applications.

Preparing Systems

Implementing the protocols that can protect against an aggressor is essential to help avoid or defend against a potential breach.

- Systems and applications should be delivered (and maintained) by trusted suppliers only.
- Personnel should be available to understand and secure any vulnerabilities in systems/applications.
- There should be pre-existing rules that ensure only trusted, supported operating systems, applications, and computer code are used on company hardware and databases.
- Information should be encrypted at all times, including during transit between systems - this information should also be inspectable, controlled, and auditable.
- Everything should be backed up.
- Only trusted, qualified personnel should be granted access to systems, applications, and sensitive data repositories - this exposure should be limited whenever possible, and authentication should be used.
- Team members should be familiar with basic cybersecurity practices.
- Physical access to systems should be restricted to authorized personnel only.

With comprehensive preparatory controls, you can reduce the possibility of an attack and limit any damage should a breach occur.

Detecting Threats

You can't react to an attack if you don't know when one is occurring. In order to detect and analyze threats, it's important to use the relevant tools and applications.

- Firewalls are designed to block unauthorized access to your computer system/network
- An Intrusion Prevention System (IPS) regularly monitors your network for any malicious activity and can be programmed to prevent it by reporting, blocking, or destroying threats.
- An Endpoint Detection and Response System (EDR) monitors and collects activity data that might indicate a threat, automatically responds to identified threats and, using advanced technology, provides a forensic analysis for use by security personnel.

Responding to Attacks

A security breach is certain to negatively impact a business. This could mean damage to the brand image, lost revenue, reduced productivity or even legal implications.

- Ensure that any incidents are identified and reported both internally and externally to any relevant bodies. Speed is of the essence.

- Incidents should be contained, eradicated and recovered from as quickly as possible. Hesitancy in the event of an attack can prove damaging.
- Recovery/continuity plans should be enacted to help mitigate damage.

Companies or business entities that fail to protect themselves from cyber threats are the ones most likely to be targeted by them. By taking the necessary steps today, it's possible to reduce the likelihood of a breach and mitigate the damage should one occur.

Image by Pexels

***Biotechnology Kiosk** was founded in May, 2019 by Dr. Megha Agrawal and Dr. Shyamasri (Shya) Biswas to serve the global biotechnology and medical science community. If you have any questions, please email publisher@biotechkiosk.com.*

Biotechnology Advances around the World

Editor's Picks

Every issue of Biotechnology Kiosk presents select latest research news picked by the editors-in-chief on significant research breakthroughs in different areas of biotechnology around the world. The aim is to promote further R&D in all of these cutting-edge areas of biotechnology. The editors have compiled and included the following innovations and breakthroughs to highlight the latest biotechnology advances.

Dr. Megha Agrawal
Co Editor-in-Chief

Dr. Shyamasri Biswas
Co Editor-in-Chief

Pluripotent Stem Cells

New gene replacement therapies for arrhythmogenic cardiomyopathy

Studies have shown that arrhythmogenic cardiomyopathy, which is a heart disease, occurs as a result of mutations in genes involved in desmosomes. This forms welds between cells that help them communicate and move in a coordinated way. One of these genes, *PKP2* is known to encode a protein. This protein is known as plakophilin-2 that is crucial to maintaining heart cell structure.

In a study published in January in Stem Cell Reports (Modeling reduced contractility and impaired desmosome assembly due to plakophilin-2 deficiency using isogenic iPS cell-derived cardiomyocytes. *Stem Cell Reports*, 2022; DOI: 10.1016/j.stemcr.2021.12.016), researchers from Osaka University, Japan reported that heart cells from a patient with an inherited heart disease called arrhythmogenic cardiomyopathy do not contract correctly when grown in the laboratory, and that replacing the mutated gene responsible for this effect fixes this defect.

In their study, researchers found that induced pluripotent stem cell--derived cardiomyocytes from a patient with arrhythmogenic cardiomyopathy could recapitulate the reduced contractility and impaired desmosome assembly associated with this disease. This provided a rapid and convenient platform for developing new treatments such as gene replacement therapy.

Researchers replaced the mutated *PKP2* with an intact copy of the gene repaired the defects in both cell contraction and desmosome assembly. They were able to observe using a time-lapse approach and fluorescently labeled desmosomes. These findings suggest that cardiomyocyte cell lines can recapitulate the pathology of arrhythmogenic cardiomyopathy and provide a rapid and convenient platform for developing gene-based therapies for this disease,

Furthermore, since *PKP2* is the most common gene associated with arrhythmogenic cardiomyopathy, and that *PKP2* mutations can lead to severe disease, it is believed that new therapeutic approaches as shown in this study could help halt disease progression. The findings from this study could pave the way for the development of new gene replacement therapy that could be a valuable way of treating patients with this condition. The cell lines created in this study could be considered a viable model to test new therapies for arrhythmogenic cardiomyopathy.

Compiled and Edited by Dr. Megha Agrawal and Dr. Shyamasri Biswas.


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