Strengthening Diagnostic Approach for Emerging Pathogens in the Philippines Through TEM-based Ultrastructural Pathology

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ABSTRACT

The emergence of novel pathogens poses significant challenges to public health systems worldwide, demanding rapid and coordinated outbreak response mechanisms. The case of "Pathogen X," a hypothetical novel pathogen, highlights the complexity of such efforts, which require rapid identification, diagnostic readiness, and effective containment strategies. Leveraging recent advances in ultrastructural pathology and genomics is essential to ensure a timely and robust response. Within this diagnostic framework, transmission electron microscopy (TEM) serves as a powerful imaging modality at high magnification that enables direct visualization of pathogen particles and infection-induced ultrastructural cellular changes. Unlike molecular techniques that infer presence through nucleic acids, TEM offers same-day morphological triage, confirmation of pathogen presence, and identification of co-infections. Through techniques such as negative staining and ultrathin sectioning, TEM reveals hallmark structural features that not only support provisional taxonomic classification and biosafety risk assessment but also inform the design and quality control of downstream assays such as metagenomic next-generation sequencing (mNGS) and targeted PCR. As such, TEM significantly accelerates and reinforces the overall outbreak investigation workflow. Its high-resolution imaging capabilities make it particularly valuable in situations where speed, morphological clarity, and pathogen diversity necessitate a direct visualization approach.

Key words: electron microscopy, transmission electron microscopy, ultrastructural pathology, emerging pathogens, Pathogen X

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ELECTRON MICROSCOPY AS REVOLUTIONARY TECHNOLOGY

The first prototype of the electron microscope was developed in 1931, engineered by Ernst Ruska and Max Knoll in Berlin, revolutionizing how scientists visualize life at the smallest scales.[1] This groundbreaking invention laid the foundation for modern electron microscopy. In 1939, the first commercially available electron microscope was introduced by Siemens, with Ernst Ruska playing a key role in its development. Following World War II, numerous companies developed commercial electron microscopes, notably JEOL in Japan and Philips in Europe, leading to widespread adoption in biological and materials research.[2]

Today, modern transmission electron microscopes (Figure 1), with resolutions reaching as fine as 0.23 nm and magnification exceeding 2 million times, can reveal structures far beyond the reach of conventional light microscopy.[3,4] From viruses and bacterial ultrastructure to molecular-level entities such as prions, TEM provides an unparalleled window into the architecture of disease. For researchers at the forefront of pathogen detection, this translates to faster diagnostics, deeper understanding of novel threats, and a critical advantage in pandemic prevention.

Recent advancements in TEM protocols have significantly expanded its diagnostic applications across infectious agents. Ranjan et al. demonstrated that negative staining enables sample preparation for foot-and-mouth disease virus detection in under 10 minutes, highlighting TEM's

Figure 1. ***JEOL JEM-F200 Transmission Electron Microscope*** installed at the Research Institute for Tropical Medicine.

Photo taken by J.G. Vinarao.

efficiency in urgent diagnostic settings.[5] Laue further emphasized the evolution of diagnostic electron microscopy from a routine laboratory method to a specialized tool increasingly used in outbreak investigations and pathogen characterization.[6] Notably, SARS-CoV-2 was initially identified using TEM, which provided rapid morphological insights into viral particles before genomic data were available, thereby guiding subsequent metagenomic sequencing efforts.[6,7]

In addition to structural visualization, TEM techniques such as immunogold labeling have proven valuable for biochemical and functional characterization. This approach enhances the detection of specific antigens and extracellular vesicles associated with pathogens, offering insights into mechanisms of disease transmission and immune evasion.[8] Such capabilities position TEM not only as an imaging modality but also as a functional diagnostic tool. Further illustrating its versatility, Jiao et al. employed TEM to trace virion assembly and intracellular movement in plant virology, demonstrating its applicability across diverse biological systems.[9]

CELLULAR, TISSUE, AND BACTERIAL CROSS-SECTION ULTRASTRUCTURAL ANALYSIS

Transmission electron microscopy remains indispensable for nanoscale visualization of pathogens and host cellular architecture. High-quality ultrastructural imaging requires advanced instrumentation, meticulous specimen preparation, and specialized technical expertise. At the Research Institute for Tropical Medicine (RITM), personnel

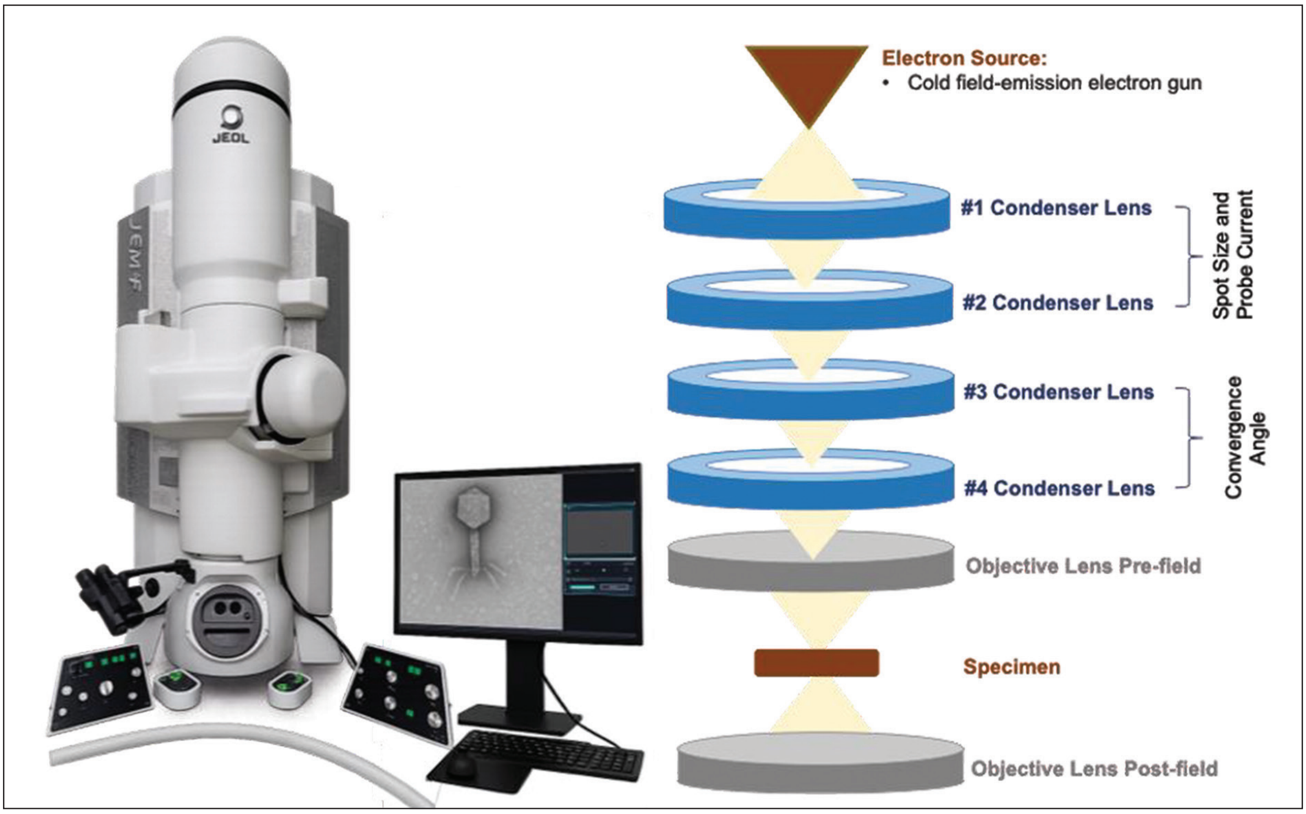

Figure 2. ***JEOL JEM-F200 transmission electron microscope and its lens system.*** The JEM-F200 is equipped with a cold field emission gun (CFEG), producing a highly coherent electron beam with low energy spread, ideal for resolving nanoscale viral structures. The condenser lens system (CL1 and CL2) modulates beam intensity and spot size, enabling optimization for imaging and analytical techniques such as energy-dispersive X-ray spectroscopy and electron energy loss spectroscopy. The specimen stage is positioned at the center of the column, where the objective lens—critical for high-resolution imaging—focuses transmitted electrons. Downstream intermediate and projector lenses determine final magnification and imaging mode, supporting applications ranging from low-magnification surveys to high-resolution diagnostics.[10]

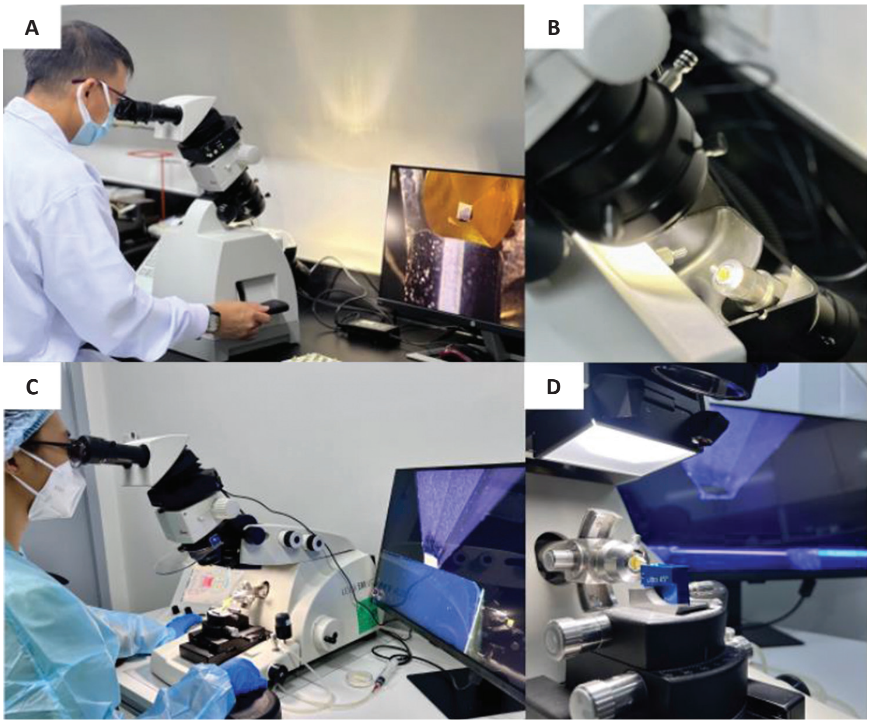

Figure 3. ***Transmission electron microscopy sample preparation procedures:*** **(A and B)** semi-thin and **(C and D)** ultra-thin sectioning using a diamond knife.

utilize modern TEM (Figure 2) platforms alongside the Leica UC7 ultramicrotome, enabling the production of ultrathin sections essential for ultrastructural analysis (Figure 3). Preparation workflows include negative and positive staining as well as ultrathin sectioning at sub-micron thicknesses, procedures that demand exceptional technical proficiency.

Beyond imaging, ultrastructural analysis plays a key role in elucidating pathogen-induced cellular disruption. Yang et al. demonstrated that duck Tembusu virus compromises the blood–brain barrier, revealing novel cytopathological changes through TEM that shed light on neuropathogenesis.[11] In bacterial infections, Liss and Hensel identified previously unrecognized ultrastructural features in *Salmonella enterica*-infected cells, challenging existing models of intracellular bacterial environments.[12] Similarly, Schulte et al. showed that ultrastructural heterogeneity among bacterial morphotypes correlates with physiological states, offering insight into bacterial viability and stress responses.[13]

Collectively, these studies underscore the ability of ultrastructural methods to reveal biological features that may be overlooked by molecular or immunological assays. Integrating TEM into pathogen detection workflows enhances characterization of emerging agents, refines disease definitions, and may facilitate discovery of novel diagnostic biomarkers.

TEM IN VIRAL DIAGNOSTICS AND INTEGRATION WITH GENOMICS

Transmission electron microscopy continues to serve as a critical diagnostic modality, particularly for rapid detection of viral and atypical pathogens. Negative staining (Figure 4) allows rapid preparation and high-contrast visualization of viral particles (Figure 5) using electron-dense stains such as uranyl acetate or phosphotungstic acid. Jiang et al. demonstrated that TEM with negative staining can identify multiple pathogens from bronchoalveolar lavage fluid within 48 hours, outperforming many molecular diagnostics in turnaround time.[14] Importantly, TEM's reagent-independent nature makes it especially valuable for detecting unknown or emerging pathogens.

While metagenomic next-generation sequencing has revolutionized infectious disease diagnostics through hypothesis-free detection of known and novel pathogens, TEM remains a vital complementary tool. Unlike sequencing methods that infer pathogen presence from genetic material, TEM provides direct visual confirmation and enables assessment of infection-induced cellular pathology. Although mNGS excels at detecting unexpected or co-infecting organisms,[15] its sensitivity can be limited by high host DNA background unless extensive enrichment strategies are applied.[16]

Advances such as cryo-electron tomography and immunogold labeling have further expanded TEM's capabilities, enabling molecular-level visualization of pathogen–host interactions.[17] Integration of TEM into public health and reference laboratories strengthens surveillance and outbreak response capacity, particularly for morphologically distinctive or highly transmissible agents. A synergistic diagnostic framework combining TEM's specificity with the broad detection range of NGS offers a more robust and accurate approach to infectious disease diagnosis.

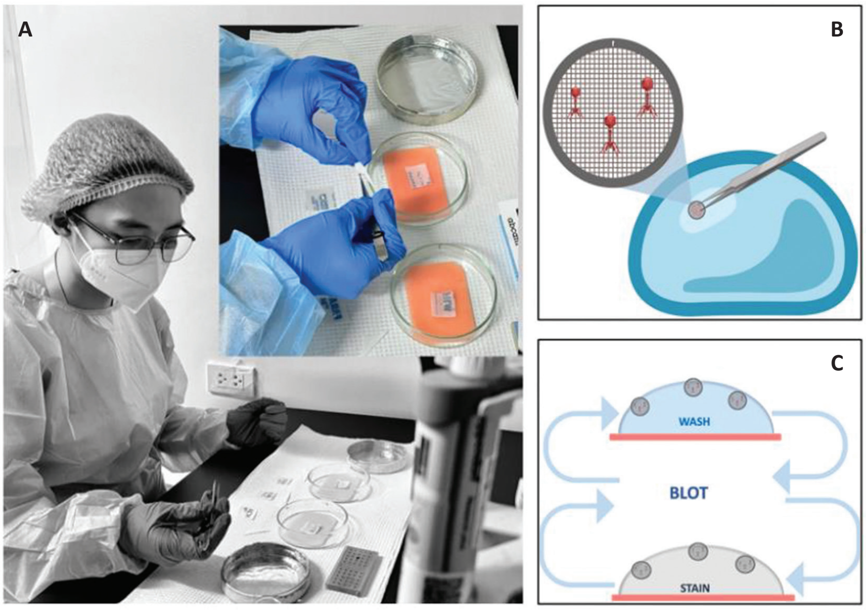

Figure 4. ***Negative staining workflow for enhanced ultrastructural visualization of viral particles.*** **(A)** Setup of the negative staining station, including essential tools and reagents; **(B)** Schematic representation of the staining procedure showing sample adherence to grids, followed by **(C)** sequential washing, blotting, and drying steps.

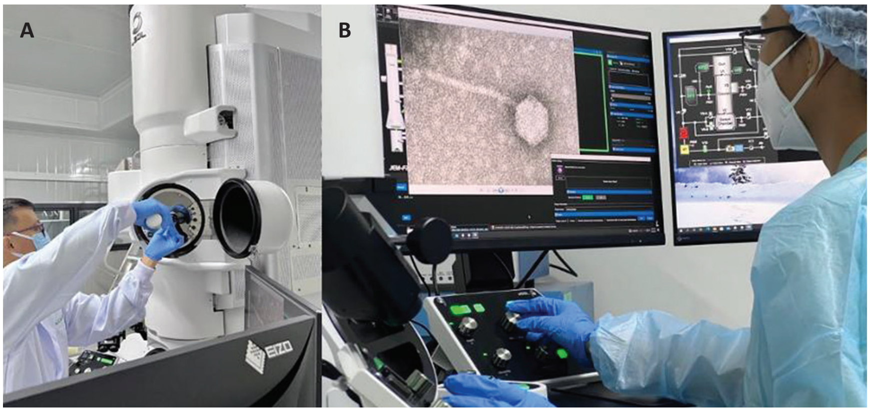

Figure 5. ***Generating electron micrographs using the transmission electron microscope (TEM).*** **(A)** Insertion of the sample holder into the TEM column; **(B)** Operation of the TEM user interface for image acquisition and parameter adjustment.

CONCLUSION

Seeing the unseen and future-facing vision

As the world confronts emerging and re-emerging pathogens—including Ebola, Zika, COVID-19, and avian influenza—early detection has become a cornerstone of public health preparedness. The upgraded TEM laboratory at RITM was established to strengthen national capacity for infectious disease surveillance, diagnostics, and outbreak response. In this context, TEM serves not merely as an instrument but as a sentinel technology, bridging classical microscopy and modern molecular diagnostics.

The commissioning of this advanced TEM system marks a significant step forward in the Philippines' preparedness for biological threats. Beyond diagnostics, it enables cutting-edge research in structural virology, vaccine development, nanomedicine, and materials science, reinforcing its role as a cornerstone of future-facing infectious disease research.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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