
The Royal Melbourne Hospital
MISERIS SUCCURRERE DISCO
VIDRL
Victorian Infectious Diseases Reference Laboratory
CELEBRATING
50 YEARS
1975 - 2025
Electron Microscopy & Structural Virology
50th Anniversary Annual Report

Front cover image: False coloured electron micrograph of Reovirales-like virions and bacteriophage taken by Margit Homola in June 1975. This image represents Fairfield Hospital's first known clinical electron micrograph using the Philips 301 electron microscope commissioned in 1975. Magnification 45,000x.
Rear cover image: Original version of the electron micrograph taken by Margit Homola in June 1975. Scale bar = 100nm. Magnification 45,000x.
Headers and Footers: Original resin embedded HIV culture material from 1985 was recut and imaged using the current TEM. Images were false coloured to highlight HIV virions.

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FEI Tecnai T12 Spirit 120kV Transmission Electron Microscope housed at the Peter Doherty Institute for Infection and Immunity.

Half a Century of Advances in Electron Microscopy

For five decades, the Electron Microscopy and Structural Virology Laboratory (EMSV) has served as a critical sentinel within Victoria's public health system. Its history spans multiple electron microscopy technical milestones and reflects the profound shifts in human infectious disease investigation over the modern era. This brief synopsis documents the EM facility's evolution through three distinct institutional phases: the Fairfield Infectious Diseases Hospital (1975–1998), VIDRL at Jane Bell House (1998–2014), and the Royal Melbourne Hospital and University of Melbourne collaborative venture of the Peter Doherty Institute for Infection and Immunity, established in 2014.

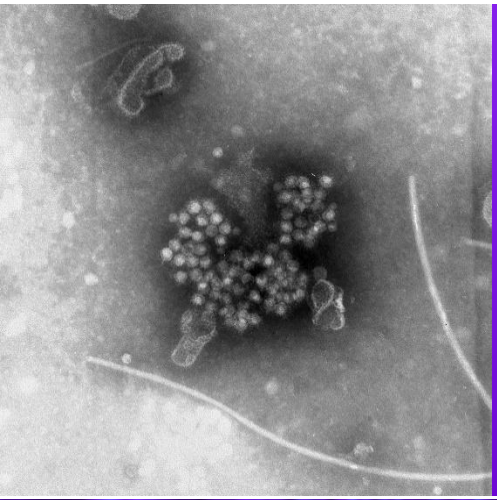
Before transmission electron microscopy (TEM) became the mainstay of 20th century clinical virology, medical scientists relied on inference via cytopathic effects, serology, or animal models. The 1975 introduction of TEM at Fairfield revolutionised this paradigm, enabling the direct visualisation of pathogens. Clinicians could have gold standard visual confirmation, not just a probability, for undiagnosed illnesses. Through the apertures of three primary instruments—the Philips 301, Philips CM12, and FEI Tecnai Spirit T12—this laboratory has witnessed the decline of the "golden age" of bacterial control, the HIV/AIDS crisis, the rise of zoonotic spillovers, and the SARS-CoV-2 pandemic.

The Fairfield Era, 1975–1998 (The Phillips 301)

In the mid-1960s to early 1970s, Fairfield Hospital faced a hepatitis A epidemic, admitting 700–800 patients annually. In 1953, Dr. Alan Ferris (Virus Laboratory Director, 1948–1970), who had adapted the virology lab for tissue culture to investigate poliovirus vaccine safety, led efforts to identify the elusive agent of hepatitis A. In 1974 his team included a young PhD student, Stephen Locarnini, who used a serological technique known as Immune Electron Microscopy (IEM), was able to identify Hepatitis A (HAV) particles in patient faeces. Lacking the necessary on-site equipment, he had used the Alfred Hospital's Zeeman microscope.

This work, conducted in parallel with the National Institutes of Health in the USA (though the American group published slightly earlier), demonstrated global-level expertise. Crucially, it gave additional justification to install the laboratory's first dedicated TEM: the Philips 301 in July 1975. Dr Ian Gust, who by 1970 had succeeded Ferris as Director, secured a Victorian Ministerial Grant to operate the laboratory as a Victorian Public Health Reference facility, which enabled the construction of a modern microbiology facility.

The Philips 301 instrument, a 60 kV analogue, film-based microscope, gave the unit greater independence. In 1976, Locarnini and Gust confirmed HAV in stools and specific IgM in sera. The HM175 strain isolated at Fairfield was shared with the NIH, adapted to cell culture, and became the basis for Havrix, the first effective hepatitis A vaccine, confirming EM's unique morphological confirmation capabilities as a powerful global health tool.



1975 image of Hepatitis A particles taken using the Philips 301 by Stephen Locarnini, using negative contrast stain IEM.

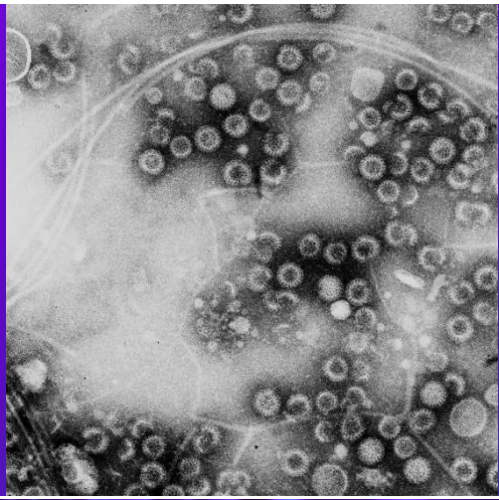


Image of negative contrast stained rotavirus particles, representing the first image taken by John Marshall in 1977.

The 1970s was a decade of profound discovery in virology, driven almost entirely by the application of EM to clinical samples. The 1973 discovery of rotavirus by Ruth Bishop at the Royal Children's Hospital Melbourne highlighted a growing global need for enteric virus characterisation expertise. Fairfield's EM unit immediately became a critical hub for characterising the deluge of "small round viruses" that followed. In 1977, a pivotal study by Chris Birch, Ian Gust, and colleagues utilised the new microscope to survey infants admitted to Fairfield. The findings were significant: rotavirus was identified in approximately 42% of cases, with a marked prevalence during the winter months. Crucially, the study demonstrated that direct electron microscopy was more sensitive than the counter-immunoelectrophoresis methods then in use. 1977 was also the year Dr John Marshall joined Fairfield Hospital to lead the electron microscopy team.

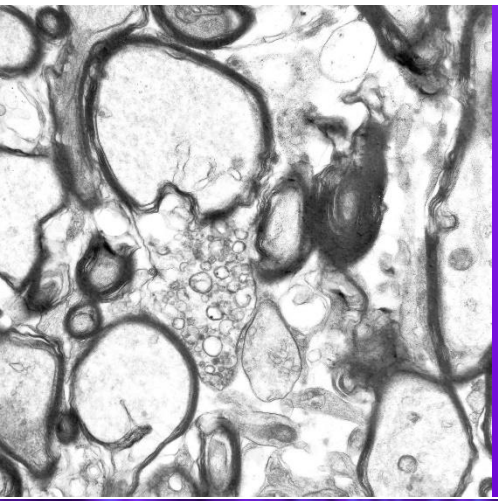
By the end of the 1970s, the laboratory staff had developed an expert eye for viral morphology. They routinely differentiated the distinct, 75nm wheel-like structure of rotavirus from the smaller, 28nm star-shaped astroviruses and the amorphous, rugged surfaces of the roughly 35nm caliciviruses (later classified as noroviruses). A 1979 comparative study by Birch et al. reinforced the value of the microscope, noting that while emerging molecular methods like Enzyme-Linked Immunosorbent Assay (ELISA) offered higher throughput, EM provided an "open view." Unlike an assay designed to find a specific agent, the electron microscope could reveal novel or unanticipated agents.

The clinical utility of the microscope was tested during the major echovirus 11 epidemic of 1979–1980. Over an 11-month period, the virus was isolated from 174 patients. The outbreak presented with a spectrum of severe illness, including viral meningitis (66% of cases) and respiratory infections. The EM unit provided rapid identification of isolates, supporting the clinical teams dealing with a surge of critically ill neonates and young children.

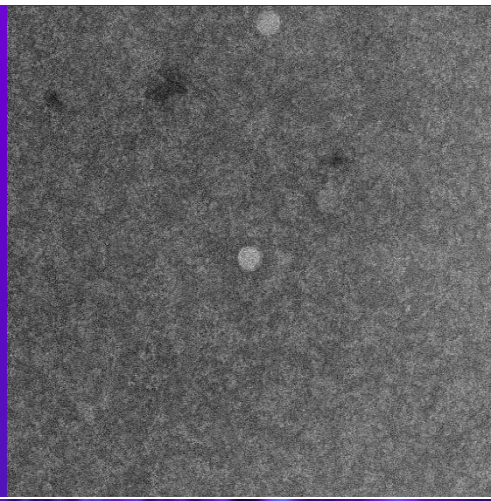
A 1976 Lassa Fever scare in Melbourne (involving a patient exposed in London) revealed inadequate high-consequence pathogen facilities. This spurred the construction of the Perrin Norris Building (Ward 18), opened in 1982. Despite design flaws in its pressurisation systems, it served as a training facility and handled a suspected Lassa case in 1985. The experience proved crucial to informing future high-containment design.

The 1980s saw the emergence of HIV. By 1984, Fairfield had confirmed its first two Australian AIDS cases and became the National HIV Reference Laboratory and a WHO Collaborating Centre. The Philips 301, though ageing, was proved to be a critical resource: in September 1985, Dr. John Marshall captured the first Australian EM images of HIV from cell culture, providing definitive confirmation when serology was ambiguous.

In 1992, Ms Jenny Doultree working with Dr John Marshall and Dr Scott Bowden, authored a paper describing new staining methods for viruses in suspension, this work was done with Professor Jia-Yee Lee, a PhD student at the time working on rubella virus and employing the 'Tokuyasu' method for IEM-based tissue examinations.



Last image taken using the Phillips 301 of a brain biopsy in August 1992.



First image taken using the Phillips CM12 of a possible parvovirus in October 1992.

Around the same time in 1992, the Pathology and Virology laboratories at Fairfield formally merged to form VIDRL, governed by Melbourne Health. Professor Stephen Locarnini, whose research proved pivotal in hepatitis A discovery, was now the Director and led the amalgamation.

The Philips 301 was retired in August 1992 after 17 years. A new Philips CM12 (120 kV, microprocessor-controlled) was commissioned in September 1992, installed at Fairfield, representing a major technological leap for high-throughput demands.

A 1992 international flight gastroenteritis outbreak was traced to Norovirus contamination in orange juice, highlighting EM's unique value: when the cause is unknown, the microscope may reveal unanticipated agents. The 1994 emergence of Hendra virus intensified the need for investigating disease with an unknown aetiology, where EM retained a key niche. The lab continued characterising novel caliciviruses (e.g., Camberwell virus in 1996, Carlton in 1997).

VIDRL at Jane Bell House, 1998–2014 (The Phillips CM12)

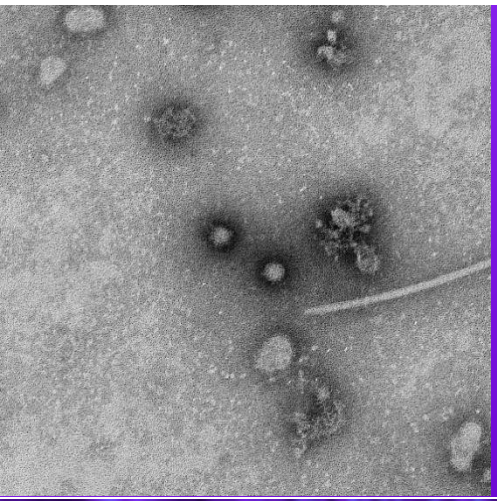
Following the 1996 closure of Fairfield Hospital VIDRL and the EM facility relocated in 1998 to a new brick facility, Jane Bell House, in North Melbourne. The Wreckyn Street building, designed for high containment, housed the National High Security Quarantine Laboratory. The Philips CM12 became the EM workhorse for 16 years. Dr. Mike Catton (Director from 2001), an RCPA Fellow, integrated lab science with public health intelligence, a synergy critical during crises. Dr Catton also oversaw the 2006–2008 relocation of the WHO Collaborating Centre for Influenza to the Jane Bell building.

In April 2000, the Melbourne Aquarium Legionella outbreak (125 confirmed cases) tested VIDRL's resilience. While not the primary tool, the EM unit supported environmental characterisation and excluded viral pathogens.

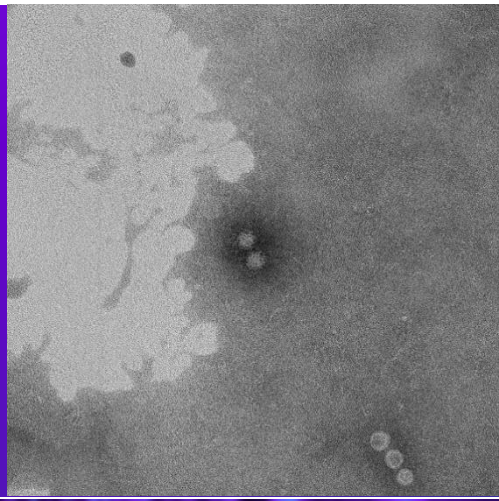
Following the 2001 anthrax letter attacks in the US, hundreds of "white powder" samples arrived at VIDRL; light microscopy and EM could rapidly identify Bacillus anthracis spores or rule out threats like poxviruses within minutes, critical for public order and emergency service response.

The 2003 SARS outbreak, with unknown aetiology, demanded rapid action. EM was globally instrumental in identifying the causative agent, a coronavirus. At VIDRL, the CM12 was once again used to confirm virus morphology in order to aid molecular test development.

As the scientific landscape evolved, with the rise of molecular techniques like the polymerase chain reaction (PCR) from the late 1990's to early 2000's, EM adapted to find a new and vital niche. In an acute outbreak, where the causative agent is unknown, PCR is of limited use. The CM12, with its "open view," became the laboratory's frontline tool for rapid, unbiased investigation. It could provide a morphological diagnosis, identifying a virus to its family, such as a calicivirus, within hours of receiving a sample.



Last image taken on the Philips CM12 at Fairfield Hospital in April 1998 by Jenny Daultree.



First image taken on the Philips CM12 after relocation to Jane Bell House. May 1998 by John Marshall.

This initial finding would then guide the specific and sensitive molecular tests that followed. Dr Marshall expertly oversaw this synergistic workflow, a critical process that forms the basis of EM testing to this day. He was supported by a dedicated team, including his deputy from 2008, Dr Leesa Bruggink, who was instrumental in the laboratory's norovirus surveillance programs.

As the Wreckyn Street era neared its end, the CM12 would play a final role. In 2013, Dr. Marshall and Dr. Jason Roberts, a polio-virologist working with the World Health Organization's Regional Poliomyelitis Reference Laboratory at VIDRL, published a finding with implications for global polio surveillance. The team identified that L20B cells, a murine cell line used worldwide for poliovirus isolation, chronically expressed an endogenous retrovirus, later identified as a murine leukaemia virus. While not necessarily unexpected, this discovery, made possible only by the visual acuity of the electron microscope, served as a warning against complacency regarding cell culture materials and the presence of adventitious agents.

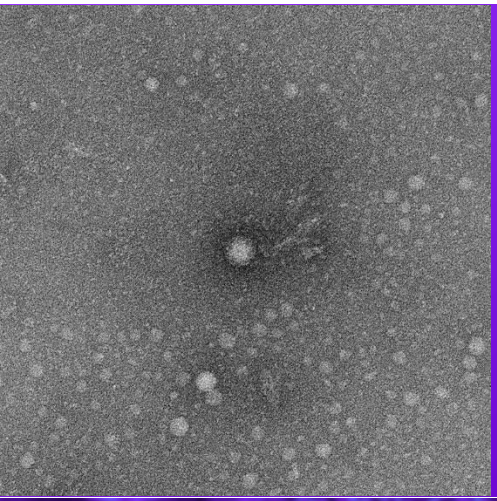
VIDRL at The Doherty Institute, 2014–2025 (The FEI Tecnai T12)

In 2014, VIDRL underwent its most significant transformation since the closure of Fairfield relocated to the newly constructed Peter Doherty Institute for Infection and Immunity in Parkville. This joint venture between the University of Melbourne and the Royal Melbourne Hospital realised the long-held vision of a comprehensive public health campus, integrating diagnostics, research, and education under one roof.

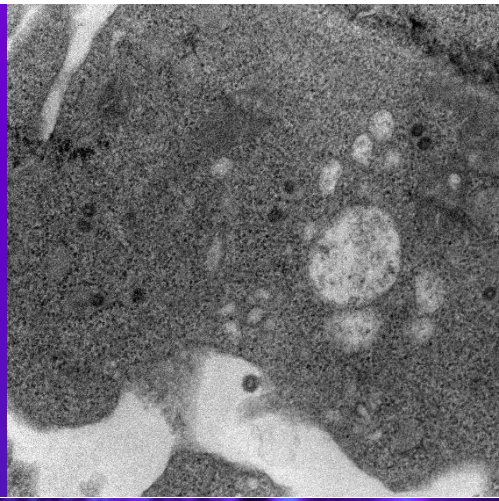
In September 2014, the laboratory commissioned its third instrument, the FEI Tecnai Spirit T12. This microscope represented a transition to a fully digital workflow. Equipped with a LaB6 (Lanthanum Hexaboride) filament and a high-resolution 4k CCD camera, it eliminated the need for darkroom processing. Images appeared instantly on high-definition monitors, allowing for immediate analysis and dissemination. The instrument also introduced Cryogenic Electron Microscopy (Cryo-EM) capabilities, allowing samples to be imaged in a near-native frozen-hydrated state.

After a 41-year career, the long-serving Dr. John Marshall passed away in February 2018, leaving a legacy of success and reinvention in his wake. The electron microscopy laboratory underwent a strategic review and was subsequently renamed the Electron Microscopy and Structural Virology (EMSV) Laboratory in 2019 in order to best capture the new global direction that electron microscopy was headed, termed the “Resolution Revolution”. A term referring to the quantum leap in camera technology and computational capabilities, combined with emerging cryogenic electron microscopy techniques. Associate Professor Jason Roberts was appointed as Head of the lab in 2019, due to his background in the traditional and molecular diagnostic virology, and the application of computational biology to public health surveillance.

In January 2020, reports of a "pneumonia of unknown aetiology" in Wuhan, China, sent a familiar shiver through the laboratory. Virologists at VIDRL had already been monitoring concerning reports in 2019, via well-established



Last image taken on the CM12 at Jane Bell House, showing a negative stained Norovirus particle. Image taken by John Marshall March 2014.



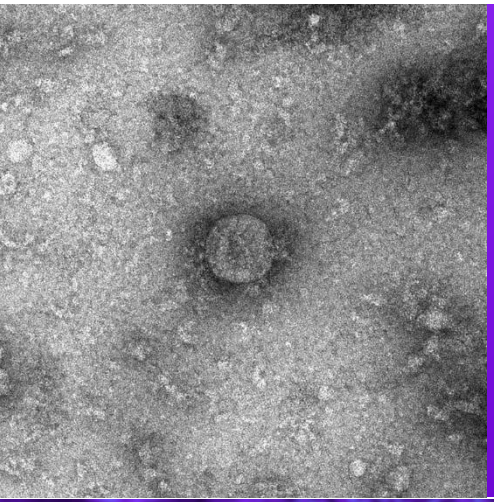
First image taken on the FEI T12 at the Doherty Institute. Showing endogenous murine retrovirus like particles in a L20B cell. Taken by John Marshal in November 2015.

global networks formed over the preceding decades, and began preparing for the worst. On 25 January 2020, VIDRL received a clinical specimen from Australia's first confirmed COVID-19 case, a traveller returning from China. The isolation team, led by Dr. Mike Catton and Dr. Julian Druce, worked around the clock in the Biosafety Level 3 (BSL3) laboratory. They achieved the first SARS-CoV-2 culture outside China; sharing this isolate was a critical step in the global development of diagnostics and therapeutics.

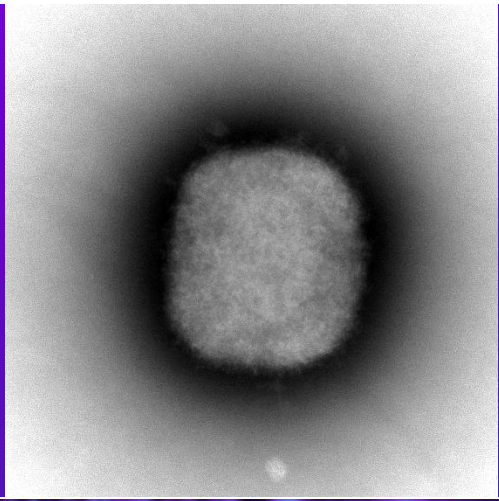
On 31 January 2020, A/Prof Jason Roberts, with the assistance of A/Prof Andrew Lees of WEHI, captured the first Australian images of SARS-CoV-2. These images were taken using an FEI Talos L120C instrument housed at the Ian Holmes Imaging Centre at the Bio21 Institute. This collaborative effort highlighted the generous nature of the Australian national electron microscopy community, with the facility head Prof Eric Hansen prioritising access to the 120kV instrument, after complications with VIDRL's FEI T12 cooling system just the week prior.

Lessons learned during the SARS-CoV-2 pandemic highlighted that the sample preparation methods from the late 20th century were no longer sufficient for the requisite rapid response to emerging global pandemic threats. The unit implemented new protocols to increase throughput. Ms. Jamie Mumford, who joined the team in 2020, introduced microwave-assisted sample processing. This innovation reduced the turnaround time for thin-section preparation (embedding tissues in resin) from five days to approximately six hours, a critical improvement for visualising pathogens infected cellular material. The laboratory also adopted electron tomography methods for 3D reconstruction of viral and cellular ultrastructure, moving from simple 2D imaging to volumetric analysis.

This proved timely in May 2022, when the laboratory responded to Australia's first confirmed pandemic mpox case. The new methods enabled rapid, high-fidelity results with enhanced ultrastructural detail. Coupled with advanced 3D data analysis, they marked the integration of artificial intelligence into pathogen characterisation, a pivotal step in transforming VIDRL's public health electron microscopy. This shift signified a new era, where structural insights could be delivered by electron microscopy more rapidly and with greater precision, supporting real-time outbreak response and global surveillance.



First Image of SARS-CoV-2 in culture taken by Jason Roberts, in collaboration with Andrew Lees at Bio21 in January 2020



First image of pandemic Monkeypox virus from the first Australian case in May 2022. Taken by Jason Roberts

Electron Microscopy Activities

The Electron Microscopy and Structural Virology Laboratory is a critical national resource, serving as one of only two Australian facilities equipped to examine high consequence pathogens of public health significance, under Biocontainment Class 2 (BC2) conditions. The laboratory maintains an 'approved arrangement' with the Department of Agriculture, Fisheries and Forestry for the examination and secure storage of samples originating from Biocontainment Class 2 (BC2), and inactivated samples from Biocontainment Class 3 (BC3), and Class 4 (BC4) environments (Figure 3). This capability enables the rapid generation of gold-standard morphological and morphogenic data for high-consequence pathogens, providing essential evidence during public health emergencies, as demonstrated during the COVID-19 and mpox pandemics.

Since 2019, the laboratory's service portfolio has expanded beyond traditional negative contrast staining (including immuno-gold electron microscopy) and thin sectioning, to include transmission electron tomography (TET), and rudimentary cryo-electron microscopy (CryoEM). These advancements reflect a growing demand for high-resolution structural insights into viral and microbial pathogens.

The number of samples received for electron microscopy examination is a key indicator of laboratory engagement and workload. While raw sample numbers received provides a useful benchmark, it does not fully capture the complexity of EM services, as turnaround times vary significantly depending on the procedure. Simple techniques such as negative contrast staining may take as little as 30 minutes to one hour per sample, whereas more advanced analyses, such as thin sectioning or transmission electron tomography (TET), can require days or even weeks of processing. For the 2024–2025 fiscal year, the laboratory received a total of 117 samples, requiring 160 examinations (Figure 1).

Historically, thin section examinations were limited by the labour-intensive nature of sample preparation. However, the implementation of rapid microwave-based preparation methods and optimised staining protocols between 2021 and 2022 significantly improved sample quality, enhancing ultrastructural preservation and contrast, while reducing preparation time from an average of five business days to one business day for prioritised samples. This advancement resulted in a statistically significant increase in throughput, rising from an average of 40 thin sections per year over preceding 42 years (1975 and 2017), to 51 samples per year between 2019 and 2025 (Figure 2). This increase coincided with heightened demand during the COVID-19 and mpox pandemics, where the rapid techniques facilitated rapid response and mitigation efforts.

Transmission Electron Tomography (TET) was not consistently available as a service prior to 2015 due to the procedure's technical complexity, specialised sample preparation requirements, and the need for advanced computational resources for 3D reconstruction. Significant progress was made following dedicated support from the instrument provider in 2023, who identified and addressed critical hardware and software issues with the instrument. Combined with optimisation of data acquisition protocols and the implementation of state-of-the-art reconstruction algorithms and AI-based analysis methods, tomography project timelines have been reduced from weeks to days (Figure 4).

The annual number of images produced by the laboratory directly correlates with electron microscope usage. Prior to a period of reduced activity in 2018, the laboratory averaged 169 images per year. Since 2019, image output has increased significantly to an average of 2,332 images annually (Figure 5), representing a 15-fold rise. While the introduction of advanced imaging techniques like image montaging and tomography represent significant methodological progress, these do not contribute directly to this total image count. Therefore, fluctuations in image output may occur as new techniques are adopted.

Electron Microscopy Examinations

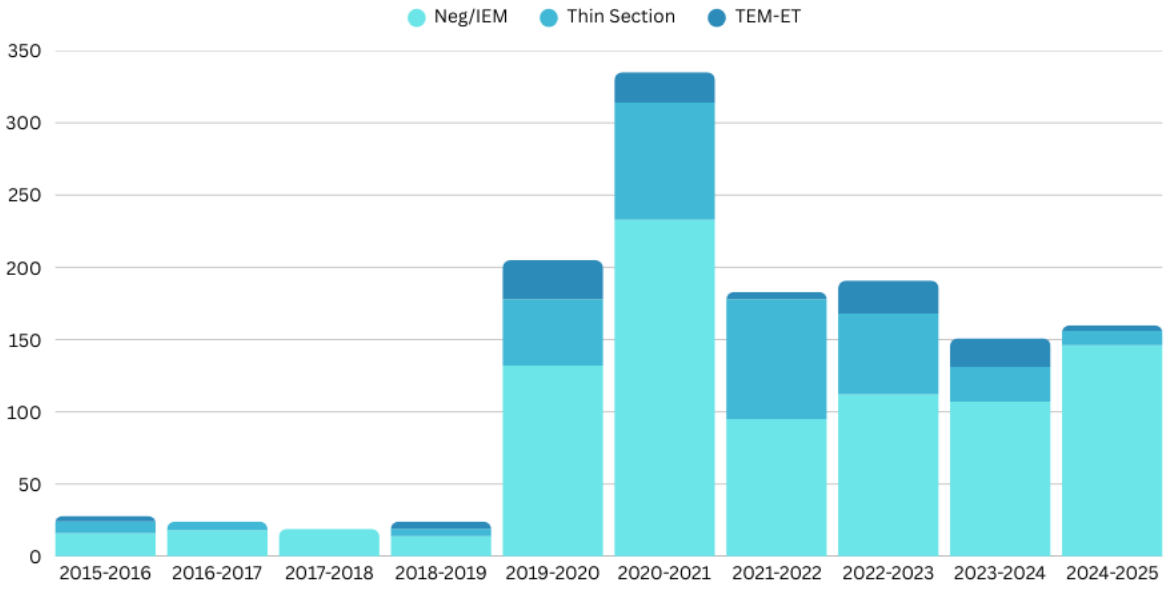


Figure 1: Breakdown of Samples by examination type, fiscal years 2015-2025.

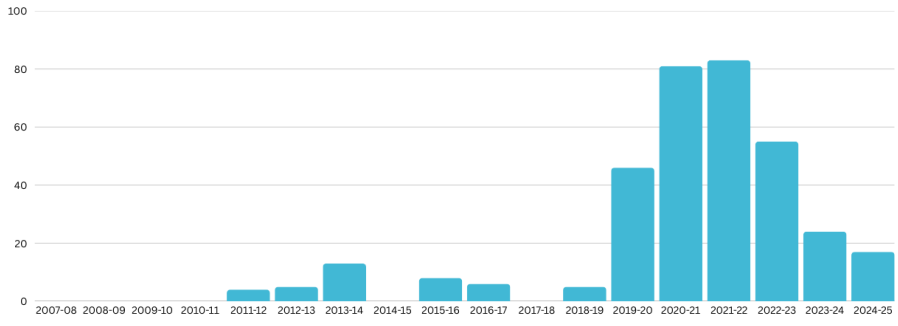


Figure 2: Samples received for thin-section preparation for the fiscal years 2007 to 2025.

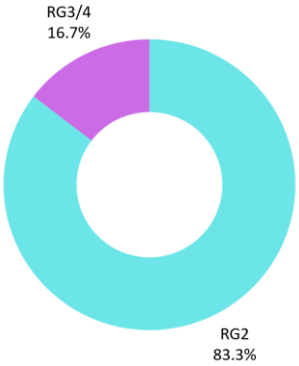


Figure 3: Breakdown of samples by risk-group 2019-2025.

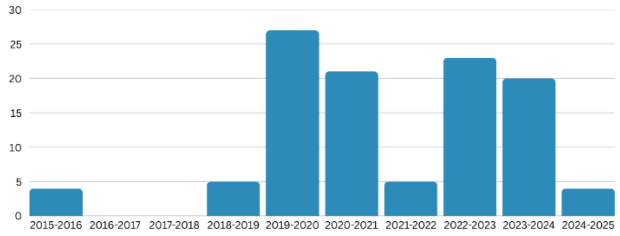


Figure 4: Total number of TEM tomography reconstructions performed during the fiscal years 2015-2025.

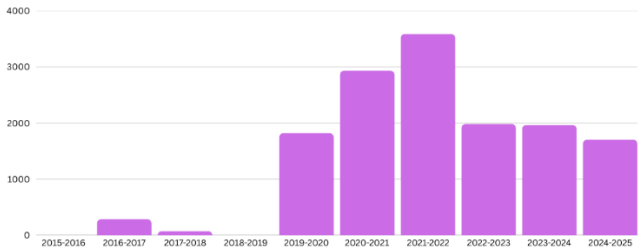


Figure 5: Annual image output for fiscal years 2015-2025.

Scientific Advisory, Public Engagement, and Training

The Electron Microscopy and Structural Virology Laboratory (EMSV) maintains a framework for knowledge sharing across multiple audiences through expert advisory services, strategic public engagement, and structured training programs. These programs ensure that scientific insights are translated into practice and awareness across the relevant scientific community, policy-making bodies, and the broader public.

Expert Advisory Services

Operating as part of VIDRL, the EMSV provides critical scientific advice on technical, scientific, and public health matters through multiple channels including acting as the Virologist Specialist for the Australian National Certification Committee for the Eradication of Polio, and the Chair of the VIDRL Artificial Intelligence Advisory Committee.

Public Engagement and Outreach

Public engagement is essential for promoting understanding of electron microscopy's critical role in global health security. The EMSV leverages innovative engagement strategies including scientific outreach, media engagement, and visual storytelling through imagery. The provision of high-quality electron micrographs to media relations at the Doherty Institute, VIDRL, and external stakeholders has generated significant public engagement and international recognition, demonstrating the value of investing in scientific visualisation.

Training and Mentorship

Recognising the need for specialised skills development, the EMSV has implemented structured training initiatives across multiple areas. We are in the process of establishing a mentorship program for postgraduate students, as well as early-career and late-career scientists, this program is designed to provide hands-on training and guidance from experienced EMSV staff members, enabling young professionals to gain practical experience and build skills in electron microscopy.

In 2024 we welcomed our first structural virology PhD student, Mr Wilson Hu, a University of Melbourne undergraduate student. Additional collaborative training has been untaken with the Australian Centre for Disease Preparedness; this training and mentorship relates specifically to rapid EM diagnostics and AI integration. Our training approach has evolved to incorporate computational biology and artificial intelligence applications, reflecting a shift toward integrated diagnostics. Recent advancements in AI-powered image segmentation and analysis have been incorporated into our training curriculum, enhancing the capabilities of staff to handle complex analytical workflows.

WHO 30th Meeting of the Regional Commission for the Certification of Poliomyelitis Eradication WPRO, Laos 2024, attended by A/Prof Jason Roberts.

Current Capabilities and Standards

In alignment with the key strategic priorities outlined in the VIDRL 2022–2025 Strategic Plan, the Electron Microscopy and Structural Virology (EMSV) Laboratory has systematically categorised its operational functions into three distinct tiers: Primary (Core) Functions, Secondary Functions, and Tertiary Functions.

Operational Framework

Primary (Core) Functions

The EMSV Laboratory's primary functions are central to VIDRL's mission as a trusted public health reference and diagnostic facility. These core activities underpin pandemic preparedness, outbreak response, and global health security, conducted within a certified microbiological containment environment.

- **Public Health Reference**
  - Pathogen characterisation - structure determination and morphometry.
  - Pandemic response and preparedness, agent identification and characterisation.
  - Novel pathogen morphologic and morphogenic characterisation.
  - Confirmation of provenance and quality for reference cell culture collections.
  - Morphologic authentication for reference pathogen culture collections.
  - Protocol establishment and dissemination for High-Containment and Polio Essential Facilities.
  - Clinical microbiology and public health policy advisory, State, Federal, International (WHO).

- **Public Health Diagnostics**
  - Clinical diagnostics – agent identification.
  - Diagnostic reagent development – assay design and validation.

Secondary Functions

The EMSV Laboratory's secondary functions provide in-kind support to public health initiatives at the national, regional, and global levels. These activities specifically involve:

- Application of gold-standard methodologies in pathogen morphology, morphogenesis, and ultrastructural pathology.
- Provision of technical and scientific advisory services to national and international partners, supporting collaborative research.
- Participation in inter-laboratory validation studies and outbreak investigations to strengthen surveillance and response frameworks.

Tertiary Functions

Tertiary functions encompass cost-recovered electron microscopy services provided to external entities for defined research projects. These are carefully triaged by the Principal Scientist to ensure alignment with public health priorities and optimal utilisation of VIDRL's resources. Projects are evaluated based on scientific merit, relevance to emerging infectious diseases, and capacity to contribute to the broader mission of pandemic preparedness and response.

Testing Repertoire

Prior to 2020, the Electron Microscopy (EM) Laboratory performed basic electron microscopy examinations of clinical samples and derivatives using either negative staining of suspensions or thin-section examination of cells and tissues. As of 2022, the laboratory has significantly expanded its testing repertoire to include a range of advanced protocols:

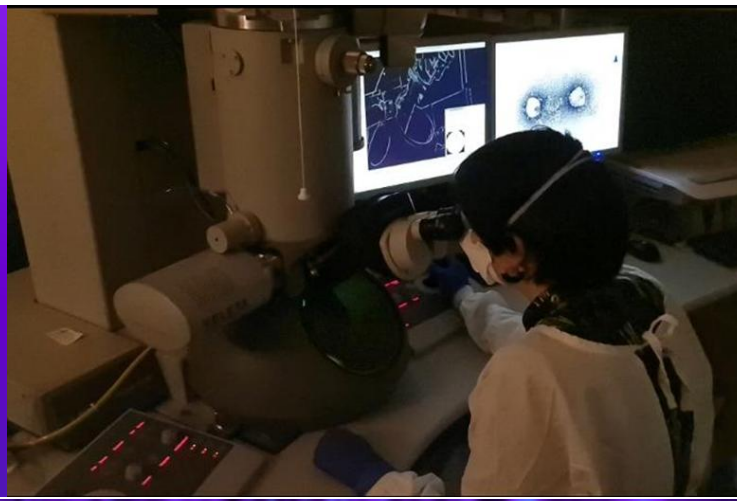
Core Testing Capabilities

- **Negative Contrast Staining** (Suspensions): Routine use of uranyl acetate, phosphotungstic acid, and ammonium molybdate for rapid visualisation and characterisation of pathogens.
- **Thin Section** (Cells, Tissues): Comprehensive workflows including rapid sample preparation, embedding, ultramicrotomy, and 2D imaging.
- **Immuno-Gold Electron Microscopy** (IEM): Both negative stain IEM and thin-section IEM for targeted antigen localisation, enhancing specificity in complex samples.
- **Electron Tomography** (Plastic-Embedded Sections): High-resolution 3D reconstruction of viral ultrastructure using serial sectioning and computational reconstruction. This capability is now enhanced by AI-powered image filtering and segmentation, significantly improving data quality and turnaround time.

Advanced and Emerging Protocols

The laboratory is actively developing next-generation techniques to meet the demands of modern virology:

- Artificial Intelligence-based image filtering and object segmentation.
- Single Particle Analysis (SPA) – Targeted for high-throughput structural determination of viral components.
- Cryo-Electron Tomography (Cryo-ET) – Being implemented to study macromolecular complexes in their native state.



Medical Scientist – Ms Jamie Mumford – examining SARS-CoV-2 positive culture material on the FEI Tecnai T12, 120kV transmission electron microscope.

Quality Assurance and Regulatory Compliance

Patient-related reporting can be performed via exemption under the TGA Special Access Scheme and the laboratory continues to conform to the NATA/TGA based VIDRL quality management requirements.

The Electron Microscopy and Structural Virology Laboratory is designed to handle risk group 2 pathogens under containment level 2 (PC2+) conditions. The EMSV serves as one of two EM facilities operating under an 'approved arrangement' with the Department of Agriculture Forestry's and Fisheries for the examination and storage of Biocontainment Class 2 (BC2), inactivated BC3, and inactivated BC4 agents, facilitating access to gold-standard morphological and morphogenic analysis methods during high-impact public health events, such as the COVID-19 and mpox pandemics.

The laboratory's certification is ensured through rigorous audits by:

- The Office of the Gene Technology Regulator (OTGR) as a Physical Containment level 2 (PC2) facility.
- The Department of Agriculture, Fisheries and Forestry (DAFF) as a Biocontainment Class 2 facility (BC2), operating under a quarantine "Approved-Arrangement" framework.

Entry to the VIDRL PC2+/BC2 EM suite, housed within the Peter Doherty Institute for Infection and Immunity.

The Future: Innovation and Collaboration

As outlined in the VIDRL Strategic Plan 2022-2025, the laboratory has been working towards several key objectives that will drive our growth and success, as follows:

Strategic Priorities	Electron Microscopy and Structural Virology Laboratory Alignment
Dedicate effort in key focus areas that best respond to public health and external stakeholder needs	• Strengthen collaboration with international and national network contacts. • Develop innovative approaches for examining high-containment samples. • Establish collaborative One Health initiatives across human and animal sectors.
Define, integrate, and grow research and innovation across all our services	• Exploit the silicon-based "resolution revolution" in EM methods. • Develop artificial intelligence-powered analysis tools. • Integrate bioinformatics and computational biophysics expertise.
Harness our expertise to consolidate presence and profile as a trusted leader in public health	• Develop quality systems implementation guidelines. • Enhance stakeholder engagement through education and outreach. • Provide expert scientific input and advice across sectors.
Structure and focus VIDRL's services to maximise value and sustainability	• Develop and incorporate emerging hardware and software advances. • Optimise sample processing capacity and efficiency. • Continually review and refine TEM protocols.
Invest in our people and systems to create a collaborative, high performance workplace	• Provide ongoing education and training opportunities, including rapid EM preparation techniques and AI segmentation for tomography. • Foster partnerships with academic institutions. • Develop formal agreements for access to external partners.

Technological Advancements for Enhanced Pandemic Preparedness

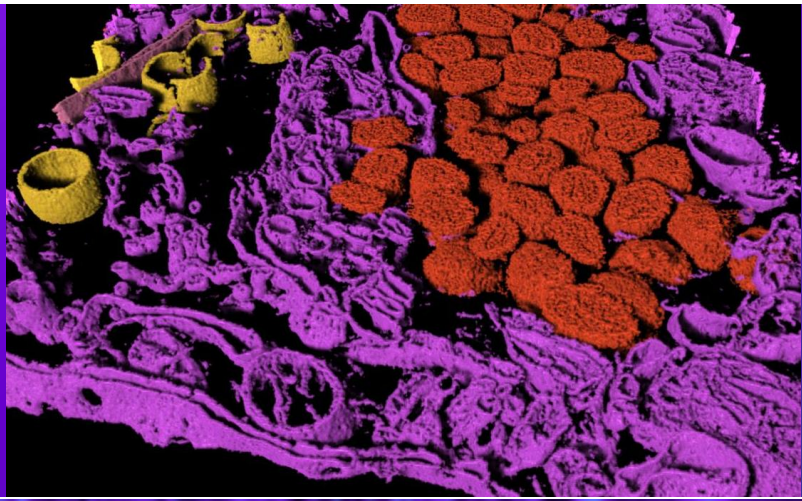
The COVID-19 pandemic demonstrated the critical need for rapid, high-fidelity structural characterisation of emerging high-consequence pathogens. Traditional sample preparation methods, which took up to five days from sample receipt to image provision, proved inadequate for a timely response. The development of microwave-assisted rapid thin-sectioning protocols has reduced this preparation time to under eight hours while significantly improving ultrastructural preservation. These protocols use reduced osmium and en-bloc staining techniques that minimise exposure to aromatic compounds, preventing the extraction of subcellular components and maintaining structural integrity.

These technological advancements, combined with resin-embedded transmission electron tomography (TET) and AI-assisted 3D segmentation, now enable us to deliver high-quality 3D structural data in as little as 16 hours from sample receipt—an 85% reduction in turnaround time compared to traditional methods. This integrated approach has become the gold standard for high-resolution ultrastructural analysis of pathogens in our facility, offering a safer alternative to cryo-ET methods that require vitrified high-titre samples. Our optimised 3D reconstruction workflow now uses 150-200nm sections for TET with a 3°-4° Saxton tilt scheme, typically capturing 40-60 images for reconstruction rather than the traditional 60-132 images, significantly reducing beam exposure damage and data acquisition times. For rapid preliminary examinations, a U-net 2D algorithm is used, while more complex structures require 2.5D U-net or 3D Sensor algorithms.

These technological and IT advancements are positioning the EMSV at the forefront of pandemic preparedness and will enable us to provide critical support for public health responses to emerging infectious diseases while continually improving our diagnostic capabilities and operational efficiency. Future work will focus on adapting cryo-ET-derived deep learning workflows, such as the CryoCARE and DeepDeWedge algorithms found in Relion5, to our TET data, potentially addressing current limitations like low signal-to-noise ratios and the “missing wedge artefact”. Integrating this platform with genomic and proteomic data could provide a more comprehensive view of viral pathogenesis, enhancing both pandemic preparedness and fundamental research into viral assembly and host-pathogen interactions.

AI Integration: Governance, Challenges, and Lessons Learned

In order to address resource limitations and an increasing workload, in particular relating to methodological complexity and an evolving quality and compliance landscape, we looked to the augmentation of processes and procedures via Artificial Intelligence technology. Our facility had already implemented deep learning algorithms for image analysis, using pre-existing GPU-accelerated computational hardware granted to A/Prof Jason Roberts for atomistic molecular dynamics simulation. The existing hardware by its nature is ideal for hosting local AI solutions, specifically methods involving deep learning and secure, local Large Language Models (LLMs).



Initial AI training of MPXV TEM tomography segmentation data, initial 3D object recognition after additional 30 min training.

Conclusion: A Legacy and a Vision for the Next Fifty Years

The Electron Microscopy and Structural Virology Laboratory's 50-year journey represents a foundational contribution to public health microbiology in Victoria and Australia, marked not by theoretical promises but by concrete achievements in identifying and understanding viral pathogens. From the critical visualisation of hepatitis A virus in 1975 to the rapid characterisation of SARS-CoV-2 in 2020, and the subsequent response to the mpox pandemic, this laboratory has consistently provided gold-standard morphological data that has directly informed outbreak management and national health responses.

Our work has not merely been about the advancement of technology; it has been about the application of these tools in service of public health. We have demonstrated this through historical events such as:

- Critical roles in the national outbreak responses, such as the seminal work on hepatitis viruses, the 1979–80 echovirus 11 epidemic, ongoing norovirus surveillance expertise and general viral gastroenteritis agent identification, supporting clinical and public health teams with real-time pathogen identification.
- The first Australian morphological confirmation of HIV in culture in 1985, contributing invaluable assistance to Australia's early implementation of blood supply screening.
- The first published growth and subsequent sharing of SARS-CoV-2 cell culture isolates in January 2020, underpinning rapid global response efforts.
- The development of rapid TEM examination protocols that reduce processing times improving turnaround for high-consequence pathogen characterisation, including critical work on the 3D reconstruction of high consequence pathogens.

This laboratory's legacy is preserved in a physical archive of over 40,000 original electron micrographs and 2,000+ resin embedded clinical samples, as well as a dozen peer-reviewed collaborative co-authored publications in the last five years, contributing to the multitude of manuscripts published over the preceding 50 years. The International Society for Ultrastructural Pathology recognised the EMSV's accomplishments in 2024, via Ms Jamie Mumford's award-winning work on rapid thin-section protocols. These contributions are significant, the EMSV is one of a handful of facilities in the Southern Hemisphere with the ability to examine risk-group 3 and risk-group 4 human pathogens, and our ability to safely examine and store these samples has been indispensable during public health emergencies.

While we focus on providing high-quality EM services, leveraging expertise for public health reference and research, building collaborative networks, and informing evidence-based policy, our current capacity remains a limiting factor. The laboratory's 50-year record of service to Victorian public health, national reference laboratories, and international partners demonstrate the value of this dedicated public health resource.

False coloured image of a 20-year old, historical sample of Astrovirus retrieved and imaged using negative staining TEM

Appendix 1. Society for Ultrastructural Pathology Conference Poster

Development of Microwave-Assisted Thin Section Protocols for the Examination of Pathogens of Public Health Significance

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Introduction

Traditional methods for preparing thin sections for examination using transmission electron microscopy (TEM) can be time-consuming and labour-intensive, and may result in fixation or dehydration artefacts, and poor contrast of structures of interest. Prior to 2020 in the Victorian Infectious Diseases Reference Laboratory (VIDRL) Electron Microscopy and Structural Virology Laboratory, standard osmium tetroxide protocols and uranyl acetate en-bloc staining with lead citrate post-staining, were the most commonly used rapid approaches to sample preparation, resulting in a total preparation time of approximately 3 days. More complex reduced osmium tetroxide-thiocarbohydrazide-osmium tetroxide (rOTO) protocols that incorporated en-bloc staining with uranium and lead salts were used more for three-dimensional ultrastructural reconstruction procedures and took up to 5 days to complete.

The initial SARS-CoV-2 pandemic response in January 2020, demonstrated that the development of faster TEM sample preparation protocols, that were fine-tuned for viral infections, was necessary. Microwave systems allow traditional protocols to be accelerated, while also increasing the preservation of ultrastructural integrity due to the decrease in permeation time of fixatives and reduced exposure time of the cells to aromatic compounds responsible for the extraction of some sub-cellular components. The VIDRL EM laboratory has modified and implemented two previously described, rapid, tissue preparation protocols, using a Pelco BioWave Pro+ microwave processing system[1,2].

Aim

To develop and implement two microwave-assisted rapid thin-section protocols to replace current in-house protocols, in order to optimise sample preservation and reduce processing time.

Results

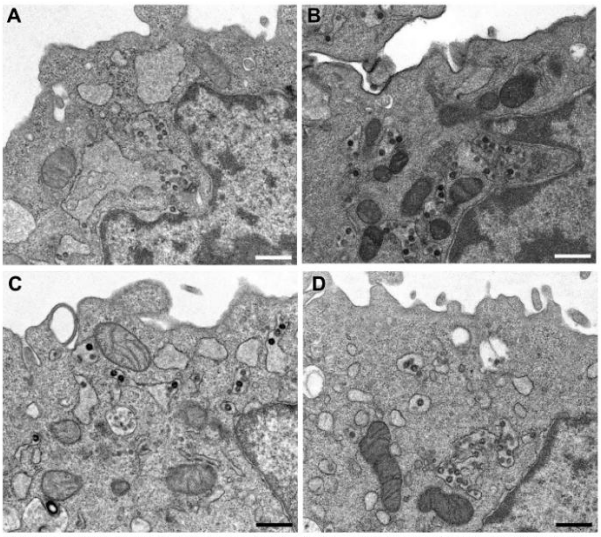

Figure 1. L20B control cells expressing an endogenous retrovirus (**A**) 3-day standard osmium en-bloc protocol with lead citrate post stain (**B**) 4-day standard rOTO en-bloc protocol, (**C**) Microwave 8-hour rapid rOTan en-bloc protocol with lead citrate post stain and (**D**) Microwave 24-hour rapid rOTO en-bloc protocol.

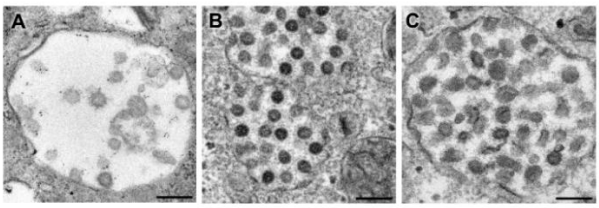

Figure 2. Vero/hSLAM cells infected with SARS-CoV-2, showing vacuoles packed with coronavirus virions (**A**) 3-day standard osmium protocol with lead citrate post stain (**B**) 8-hour rapid rOTan en-bloc protocol with lead citrate post stain and (**C**) 24-hour rapid rOTO en-bloc protocol.

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Methods

The VIDRL EM laboratory previously used two thin-section staining protocols: a standard osmication protocol, comprising a single osmium tetroxide incubation with uranyl acetate en-bloc staining, followed by post-staining with lead citrate, and a standard (rOTO) protocol with uranyl acetate and lead aspartate en-bloc staining.

The rapid rOTO en-bloc staining protocol was adapted for use with the microwave from its direct ancestor, the standard rOTO en-bloc protocol, with previous work by Tapia et al used for guidance[1]. The microwave based rapid reduced osmium tetroxide-tannic acid (rOTan) en-bloc protocol was adapted from a single step reduced osmium tetroxide en-bloc staining protocol described by Laue[2]. The protocol comprises an altered osmium tetroxide staining process, using tannic acid and sodium sulphate, followed by uranyl acetate en-bloc staining. Both protocols utilise an optional 3% lead citrate post-stain to increase contrast prior to observation, this is routinely performed for the rOTan protocol but is not generally necessary for the rOTO protocol.

Development and implementation of the new two rapid protocols involved the use of an internationally standardised WHO control cell line, L20B, obtained from the WPRO WHO Regional Poliomyelitis Reference Laboratory cell repository, curated by the National Enterovirus Reference Laboratory at VIDRL. In 2012 it was noted that the L20B cell line expressed endogenous retrovirus-like particles[3]. Given the consistent observation of both intracellular and extracellular particles in L20B cells, they were selected as a laboratory quality control (LQC) for tissue preparation and thin sectioning.

Initial experiments using L20B LQC material was processed using the following four protocols: standard osmication en-bloc, standard rOTO en-bloc, rapid rOTan en-bloc, and rapid rOTO en-bloc.

Subsequent comparative assessment of protocol suitability was performed using Vero/hSLAM cells infected with SARS-CoV-2 processed with the following protocols: standard osmication en-bloc, rapid rOTan en-bloc and rapid rOTO en-bloc. All sections were examined using an FEI T12 Spirit TEM operating at an acceleration voltage of 80keV. Electron micrographs were collected using an FEI Eagle 4K CCD camera.

Process	Standard Osmium	Standard rOTO	Rapid rOTan (microwave)	Rapid rOTO (microwave)
Osmication	1 hour	2 hours	10 minutes	18 minutes
En bloc stain	Overnight	Overnight	14 minutes	11 minutes
Dehydration	1 hour	1 hour	6 minutes	8 minutes
Infiltration	Overnight	Overnight	21 minutes	18 minutes
Polymerisation	36 hours (60°C)	36 hours (60°C)	2 - 4 hours (90°C) 12 hours (75°C)	2 - 4 hours(90°C) 12 hours (75°C)
Total	**3 days**	**4 days**	**6 - 24 hours**	**8 – 24 hours**

Table 1: Comparison of processing times for standard versus microwave thin-section protocols. Times denote incubations only and do not include reagent preparation and specimen preparation handling time.

Discussion and Conclusions

The L20B LQC material processed by the standard osmication en-bloc protocol (Fig. 1A) resulted in a relatively low contrast representation with poorly defined, but somewhat distinguishable, organelle and plasma membranes, with clearly defined retrovirus-like particles. The standard rOTO en-bloc protocol (Fig. 1B) indicated strong, preferential staining of membranes and carbohydrates (including glycoproteins), with overstaining of the mitochondrial matrix, and retrovirus-like particles were also clearly defined.

The rapid rOTan en-bloc protocol (Fig.1C) resulted in a suitably balanced staining outcome with exceptionally high contrast of viral nucleic acid complexes. The high signal to noise ratio for virus particles afforded by the rOTan protocol allowed very easily definable virus particles in relation to cellular matrix, a property that is well suited to diagnostic virology.

The rapid rOTO en-bloc protocol (fig. 1D) gave exceptional contrast and ultrastructural resolution and yielded a comparatively balanced staining profile relative to the standard osmium and rOTO protocols. There was a notable decrease in membrane staining allowing lower contrast structures, such as microfilaments, to be easily visualised and the decreased mitochondrial matrix staining resulted in more defined cristae.

The microwave-based protocols permitted a reduction in turnaround time from 3-4 days using standard methods, to as little as 6 hours from sample receipt to sectioning. An advantage of the use of microwave protocols is the dramatic decrease in incubation times (Table 1) required for sample preparation; for example stopping points in the standard protocol such as the overnight aqueous uranyl acetate incubation is replaced by a 3-minute microwave step in the rapid rOTan and rapid rOTO protocols. This reduction in incubation time contributes to a notable reduction in sample damage due to excessive fixation and/or dehydration.

The most dramatic representation of these phenomena can be seen in figure 2, where almost pleomorphic morphology of the SARS-CoV-2 virion aggregates shown in figures 2A and 2C are more accurately resolved using the rapid rOTan en-bloc protocol (Fig. 2B). Here the virions are represented as spherical or ovoid, which is the correct morphology according to previous work performed using CryoEM methods on native SARS-CoV-2 particles. Interestingly, for SARS-CoV-2 the staining profile was dramatically different for the rapid rOTan protocol (Fig. 2B) which gave superior staining of N protein in the core of virions compared to rapid rOTO protocol (Fig. 2C) that was more diffuse in its presentation. In the examination of Monkeypox infected Vero cells, the opposite is observed, with the rapid rOTO protocol providing superior ultrastructural detail compared to the rOTan protocol (data not shown).

Acknowledgements

We thank the staff of the WHO Regional Poliomyelitis Reference Laboratory for the provision of L20B control cells, and the VIDRL Virus Identification Laboratory for their assistance during the pandemic response period, with preparation of SARS-CoV-2 infected cellular material.

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2. Laue, Michael. "Electron microscopy of viruses." *Methods in cell biology*. Vol. 96. Academic Press, 2010. 1-20.
3. Roberts, Jason A., et al. "Electron microscope detection of an endogenous infection of retrovirus-like particles in L20B cells." *Microscopy* 62.4 (2013): 485-486.

Appendix 2. Publications and Awards

Published, (preceding five years):

2025 Dodla, A., Giergiel, M.; McLean, A., Earnest, L., Edeling, M., McAuley, J.L.; Godfrey, D., Purcell, D., Yap, A., Carrera-Montoya, J., Roberts, J., et. al., Spectroscopic characterization and differentiation of SARS-CoV-2 Virus like Particles. *Analytical Chemistry,* 97(32), pp.17405-17414.

2025 Edeling M. A., Earnest, L, Montoya, J.C., Yap, A.H.Y., Mumford, J. E., Roberts, J., et. al., Development of methods to purify SARS CoV-2 Virus-like particles at scale. *Biotechnology and Bioengineering, 122: 1118-1129*.

2024 Mumford, J.E., Druce, J.D., Thorley, B.R. and Roberts, J.A., Development of Microwave-Assisted Thin Section Protocols for the Examination of Pathogens of Public Health Significance. *The Society for Ultrastructural Pathology: UltraPath XXI.*

2024 Carrera Montoya, J., Collett, S., Fernandez Ruiz, D., Earnest, L., Edeling, M.A., Yap, A.H.Y., Wong, C.Y., Cooney, J.P., Davidson, K.C., Roberts, J. and Rockman, S., 2024. Human Nasal Epithelium Organoids for Assessing Neutralizing Antibodies to a Protective SARS-CoV-2 Virus-like Particle Vaccine. Organoids, 3(1), pp.18-31.

2023 Collett, S., Earnest, L., Montoya, J.C., Edeling, M.A., Yap, A., Wong, C.Y., Christiansen, D., Roberts, J., Mumford, J., et. al., Development of virus-like particles with inbuilt immunostimulatory properties as vaccine candidates. *Frontiers in Microbiology,* (14), p.1065609.

2023 Speck, P. et. al. Statement in Support of: "Virology under the Microscope - a Call for Rational Discourse", *Journal of Virology.*, *mSphere.* and *mBio.*

2022 Lim, C. K., Roberts, J., Moso, M., Liew K.C., Taouk, M., Williams, E., Tran, T., Steinig, E., Caly, L., Williamson, D.A., Monkeypox Diagnostics: Review of Current and Emerging Technologies, *Journal of Medical Virology,* 95(1).

2022 Hammerschlag, Y., MacLeod, G., Papadakis, G., Sanchez, A.A., Druce, J., Taiaroa, G., Mumford, J., Roberts, J., Caly, L, Williamson, D.A, Cheng, A.C, McMahon, J.H., Monkeypox infection presenting as genital rash, Australia, May 2022, *Eurosurveillance*, 27(22)

2021 Wood B.R., Kochan K., Bedolla D.E., Salazar-Quiroz N., Grimley., Perez-Guaita D., Baker M.J., Vongsvivut J., Tobin M., Bambery K., Christensen D., Pasricha S., Eden A.K., Mclean A., Roy S., Roberts J.A., Druce J., Williamson D.A., McAuley J., Catton M., Purcell D., Godfrey D., Heraud P., Infrared based saliva screening test for COVID-19. *Angewandte Chemie*, 133(31): 17239-17244.]

2020 May, M. L. A., Durrheim D. N., Roberts J. A., Owen, R., The risks of medical complacency towards poliomyelitis. *Medical Journal of Australia*, 213(2):61-3.

2020 Caly, L., Druce, J., Roberts, J., Bond, K., Tran, T., Kostecki, R., Yoga, Y., Naughton, W., Taiaroa, G., Seemann, T., Schultz, M., Howden, B., Korman, T., Lewin, S., Williamson, D., Catton, M., Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first diagnosis of COVID-19 in Australia. *Medical Journal of Australia*, 212 (10): 457-458.

2020 Taiaroa G, Rawlinson D, Featherstone L, Pitt M, Caly L, Druce J, Purcell D, Harty L, Tran T, Roberts J, Scott N. Direct RNA sequencing and early evolution of SARS-CoV-2. BioRxiv,

Awards, (preceding five years):

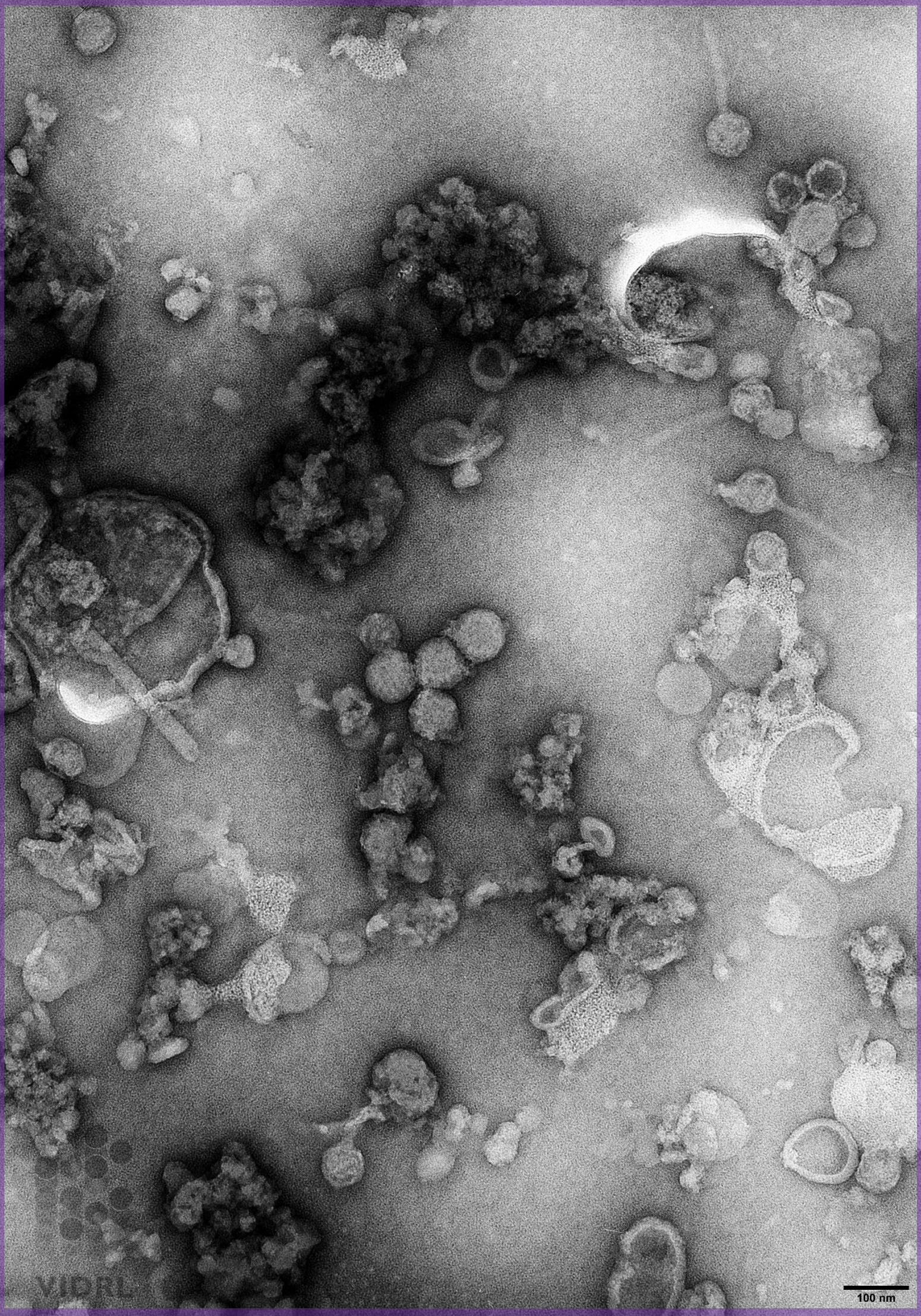
2024 **The Society for Ultrastructural Pathology International Conference XXI Poster Award:**

Ms Jamie Mumford - Rapid Thin-section Protocols for the Examination of High-Consequence Viral Pathogens Using Transmission Electron Microscopy. (Appendix 1).

2024 **Journal of Medical Virology**, top 10 most-cited papers published by the journal in 2023 – Lim et al., Monkeypox Diagnostics: Review of Current and Emerging Technologies, *Journal of Medical Virology,* 95(1).

2022 **The Medical Journal of Australia**, top cited article in 2021 – Caly et al., Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first diagnosis of COVID-19 in Australia. *Medical Journal of Australia*, 212 (10): 457-458.

2020 **Doherty Institute Innovation and Initiative Award,** Dr Jason Roberts – Management of the Electron Microscopy. Awarded in recognition of activities during the COVID-19 pandemic response.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