

Negative Stain Transmission Electron Microscopy (TEM) for Viral Particle Characterization

Methodology, Applications, and GMP-Compliant Services at Minaris Advanced Testing

Introduction

Negative stain transmission electron microscopy (TEM) is a critical analytical tool for viral particle detection, morphological characterization, and contamination assessment in biologics development and manufacturing. Unlike molecular or immunological assays that rely on virus-specific reagents, negative stain TEM provides unbiased, direct visualization of virus-like particles based on morphology and size.

Minaris Advanced Testing, formerly WuXi AppTec Philadelphia, has developed a standardized and regulatory-aligned negative stain TEM methodology that enhances quantitative accuracy using a latex bead reference system.

This approach improves reproducibility over traditional pellet-based estimations and supports compliance with FDA guidance for biologics manufacturing.

Operating one of the few GMP-compliant TEM facilities in the United States, Minaris Advanced Testing delivers high-resolution imaging, reliable viral load quantification, and rapid turnaround times to support viral clearance studies, seed stock qualification, and contamination investigations.

This white paper outlines the scientific foundation of negative stain TEM, describes the Minaris quantification methodology, highlights key applications, and details the instrumentation and GMP infrastructure supporting our services.



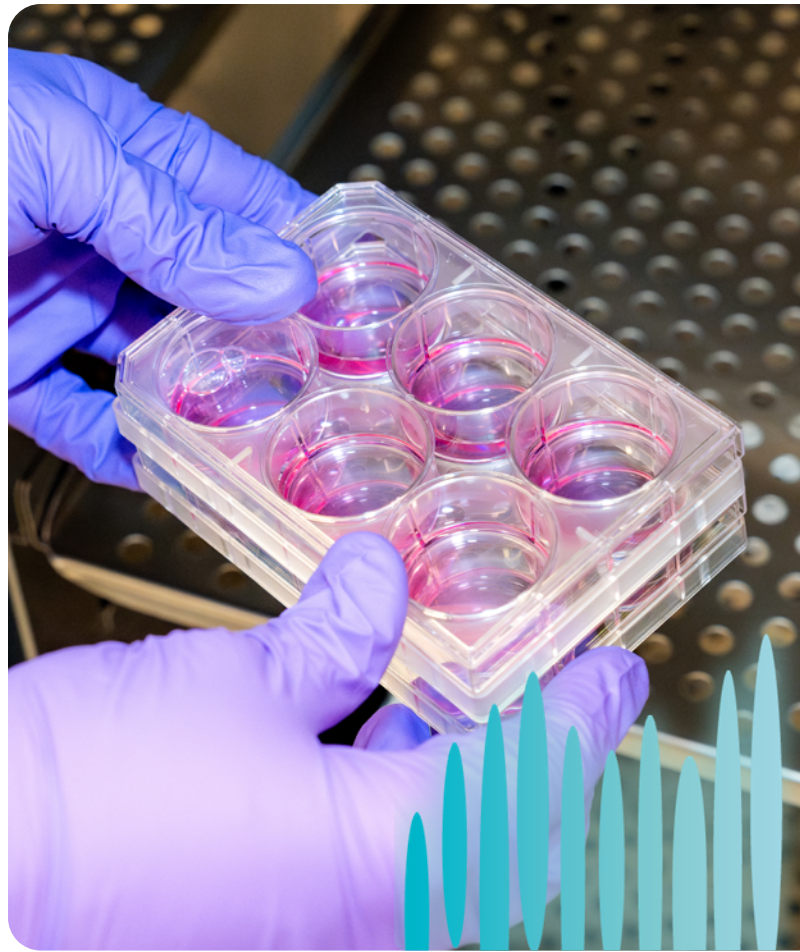
The Role of TEM

Viral safety and characterization remain central priorities in the development and manufacture of biologics, monoclonal antibodies, viral vectors, and cell-based therapies. Regulatory agencies require robust testing strategies to ensure product safety, purity, and quality.

Negative stain transmission electron microscopy (TEM) plays a vital role within this framework by enabling:

- Direct visualization of virus-like particles
- Morphological characterization independent of virus-specific reagents
- Detection of unknown or unexpected contaminants
- Quantitative particle estimation

When used alongside orthogonal assays such as PCR, ELISA, and infectivity studies, TEM strengthens overall viral safety strategies by providing structural confirmation and unbiased contaminant detection.



Scientific Basis of Negative Stain TEM

Negative stain TEM is a high-resolution imaging technique that enhances contrast by surrounding biological particles with electron-dense stain. The resulting images allow clear visualization of viral morphology and size.

Because all samples are processed identically, negative stain TEM allows for consistent comparative analysis and identification of virus-like particles based on structural features rather than molecular markers.

This makes TEM particularly valuable for detecting unknown, unexpected, or adventitious viral contaminants in mAb unprocessed bulk for example.

Key attributes of negative stain TEM include:



Uniform sample preparation across all materials



Elimination of virus-specific detection bias



High-resolution morphological analysis



Visualization of particles ranging from ~20 nm to several hundred nanometers

The Minaris Advanced Testing Quantification Methodology

Limitations of Traditional Quantification

Conventional negative stain quantification methods frequently rely on crude virus pellet volume or area estimations. These approaches can introduce variability and reduce reproducibility.

Standardized Latex Bead Referencing

Minaris Advanced Testing has developed an improved quantification methodology that enhances accuracy and consistency.



Our approach includes:

- 1 Mixing the sample material with a standardized latex bead reagent of known concentration.
- 2 Preparing and imaging the mixture using negative stain TEM.
- 3 Counting a minimum of 500 total particles (latex beads and/or virus particles).
- 4 Calculating viral load in terms of virus particles per milliliter (particles/mL) based on comparative ratios.

This approach provides:

- Reduced estimation variability
- Improved quantitative precision
- Standardized internal referencing
- Reproducible viral load determination

Importantly, this method does not require virus-specific reagents or specialized processing, ensuring unbiased analysis of all sample types.

Regulatory Alignment

The Minaris negative stain TEM methodology was developed in accordance with FDA guidance, including:

- Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)
- Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997)

By aligning with these regulatory frameworks, Minaris ensures that testing results support quality assurance, regulatory submissions, and biologics manufacturing compliance.

Applications of Negative Stain TEM

Negative stain TEM supports multiple stages of product development and manufacturing.

Viral Clearance Studies

Evaluation of process intermediates and cellular debris for virus-like particles to support viral safety validation.

Virus Bank and Seed Stock Screening

Confirmation of virus bank morphology, structural integrity, and titer accuracy for quality assurance.

Contamination Investigations

Investigation of suspected contamination in:

- Cell cultures
- Reagents
- mAb bulk harvest
- In-process samples

Because TEM does not rely on predefined targets, it is particularly effective when the source or identity of contamination is unknown

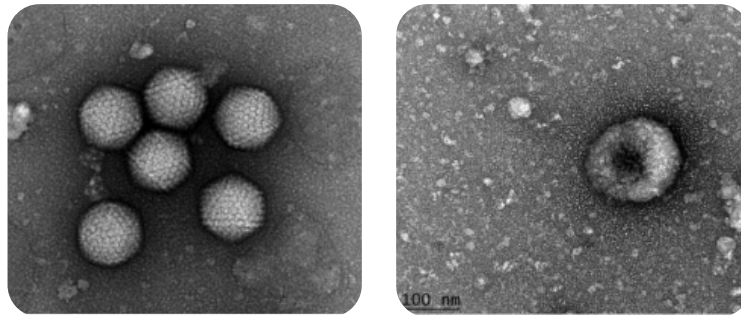


Figure 1: Picture on left is adenovirus type 5 and picture on right is an example of a 120 nm retrovirus-like particle (MuLV) among cell debris at 30,000x magnification.

GMP Infrastructure and Operational Advantages

GMP-Compliant Facility

Minaris Advanced Testing operates one of the few GMP TEM testing facilities in the United States. All sample preparation and testing are conducted under stringent GMP conditions to ensure:

- Data integrity
- Regulatory defensibility
- Consistent documentation
- Audit readiness

Improved Turnaround Time

Our in-house, U.S.-based equipment and experienced microscopists eliminate the need for third-party outsourcing. This reduces logistical complexity and supports consistent turnaround times.

Superior Quantification Approach

The standardized latex bead methodology minimizes estimation variability and improves confidence in reported viral load values.

Instrumentation and Technical Capabilities

Minaris Advanced Testing utilizes a JEOL 1400 Flash 120kV transmission electron microscope, equipped with:

- LaB6 filament
- High-contrast pole piece
- Gatan Orius 832 digital camera

The instrument achieves a maximum resolution of 0.38 nm, exceeding the resolution requirements for viral particle analysis, given that viruses typically range from ~20 nm to several hundred nanometers in diameter.

Digital Imaging Workflow

Gatan Digital Micrograph software enables:

- Real-time image screening
- Accurate digital acquisition
- Enhanced visualization of key sample features
- Elimination of film development

This digital system improves efficiency, supports data integrity, and reduces the need for re-examination.



The Role of TEM in Modern Viral Safety Strategies

As advanced biologics, viral vectors, and cell and gene therapies continue to expand; orthogonal analytical methods are increasingly essential. Negative stain TEM contributes uniquely by providing:

- Direct morphological evidence
- Detection of unknown viral contaminants
- Quantitative particle assessment
- Complementary confirmation to molecular assays

Its unbiased nature makes it a critical component of comprehensive viral safety and characterization programs.

Conclusion

Negative stain transmission electron microscopy remains a cornerstone technology for viral particle characterization and contamination assessment in biologics development and manufacturing e.g. of mAb bulk harvest. By enabling direct visualization and morphology-based identification without reliance on virus-specific reagents, TEM provides unbiased and complementary insight to molecular assays.

Minaris Advanced Testing enhances the traditional negative stain approach through a standardized latex

bead quantification method, regulatory-aligned procedures, GMP-compliant operations, and optimized high-contrast instrumentation. This combination ensures reliable, reproducible, and defensible results that support viral safety programs and regulatory expectations.

As biologics and advanced therapies continue to evolve, robust analytical tools such as negative stain TEM will remain essential to ensuring product safety, quality, and compliance.

