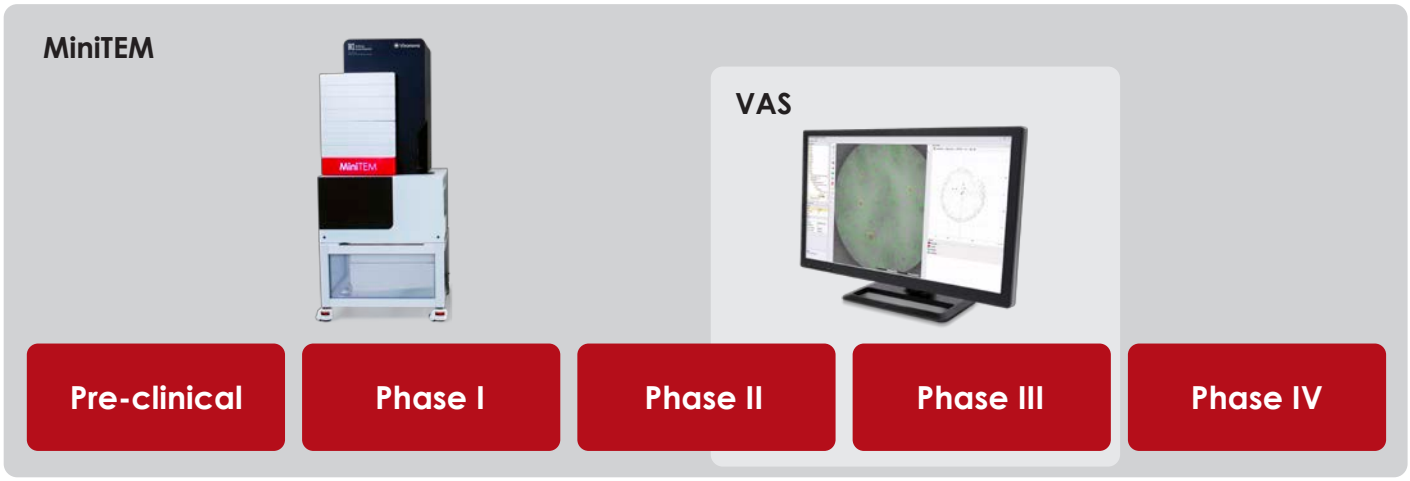
Vironova

Characterization of AAV samples using TEM

Purity analysis from development to commercial process

Transmission electron microscopy (TEM) can provide unique insights when characterizing viral gene delivery platforms such as those based on adeno-associated viruses (AAVs). Morphological characterization can support process development by confirming purity and status of the viral capsids.



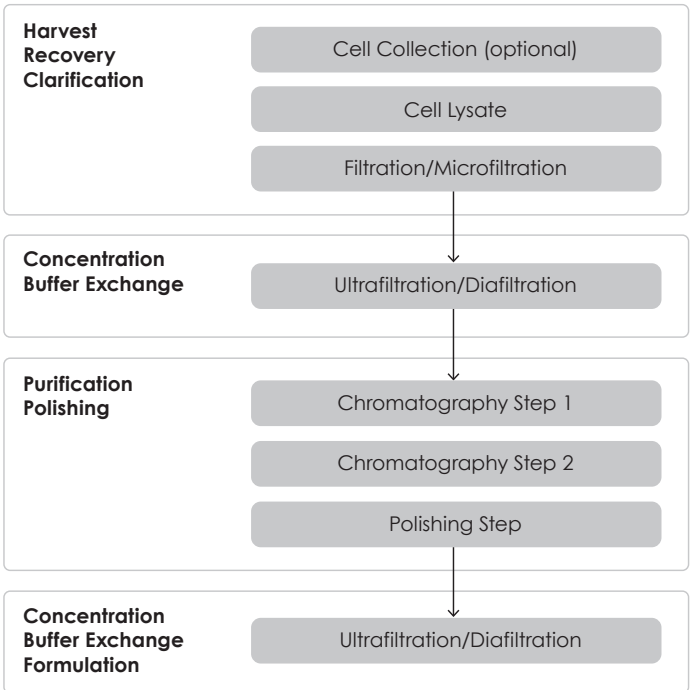
Process development	QC and Manufacturing	Life cycle
Confirming critical quality attributes = overall AAV particle morphology and capsid status, aggregates, process and host cell related impurities	QC = full/empty analysis	Stability testing

Example of a downstream process for AAV

As more and more gene therapy projects move into later clinical phases, manufacturers face new challenges. When developing the commercial manufacturing process scaling up may have impact on the purity profile at different steps. Access to reliable analytical data to base process change decisions on is key to success for a smooth process development.

Impurities or morphology features in AAV samples that can be detected by TEM:

- Broken particles
- Proteasomes
- Protein or membrane-based cell debris
- Residual DNA
- Capsid content (full/empty)
- Aggregates
- Remnants of helper virus



MiniTEM in process development

Experience from providing an EM service for gene therapy vectors, and of the type of features that need to be characterized frequently and in high volume, has inspired Vironova to develop MiniTEM. MiniTEM is a low voltage system that can be placed in any standard lab, close to your process. MiniTEM provides bioprocess workers with their own in-house solution that enables non-experts in electron microscopy to generate quantitative data and images in a few hours - data that can support decision making in process development.

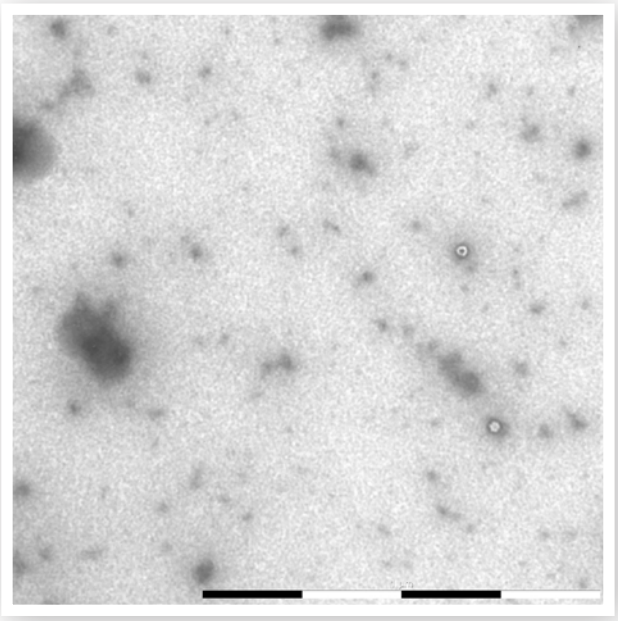
MiniTEM:

- Automates imaging, particle detection and classification
- Visualizes your product and confirms critical quality attributes
- Turns visual evidence into quantitative data
- Generates instant results that allow you to adjust the process within hours

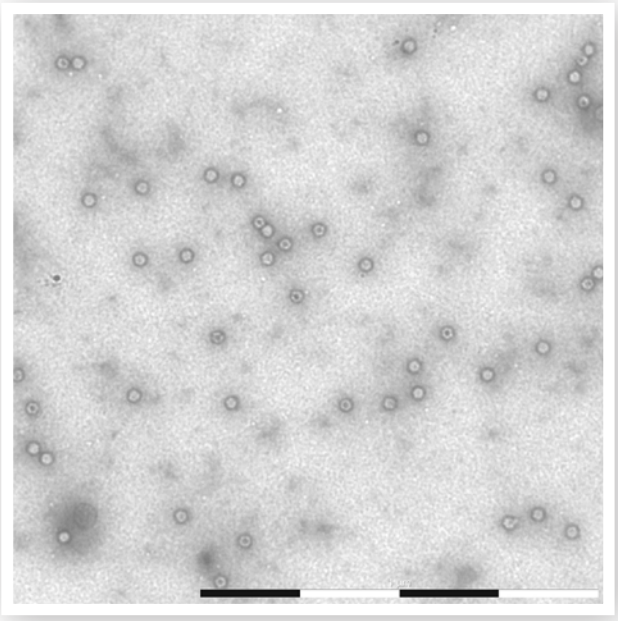
Monitoring each step in the purification of AAV

Negative staining (nsTEM) is a powerful method for assessing process- and product-related impurities. It is a quick and easy preparation technique, supported by MiniTEM, in which the specimen is embedded in a layer of heavy metal salt solution. The use of heavy metal stain significantly increases the contrast in the images.

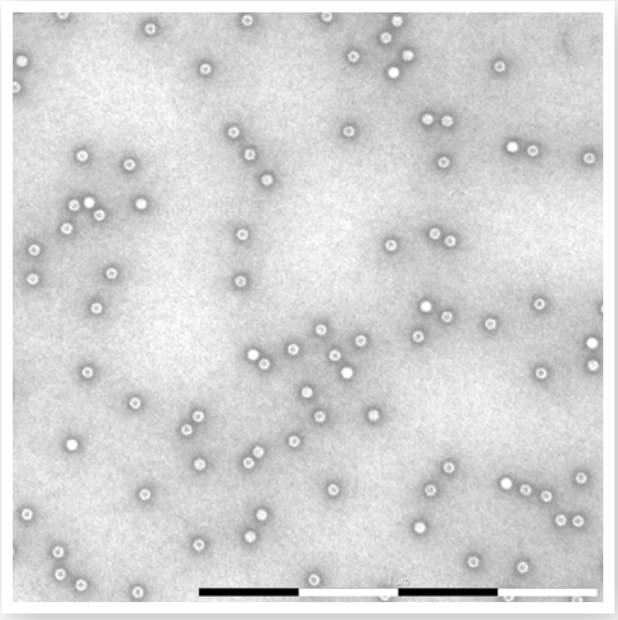
Sample after first purification step



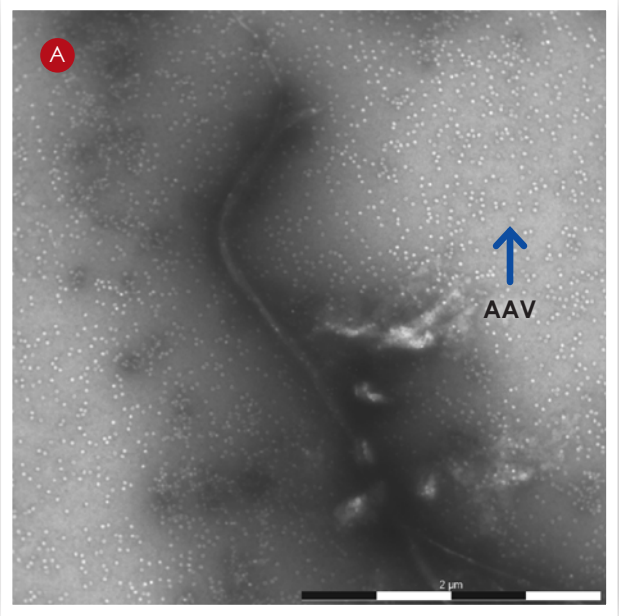
Sample after 2nd purification step



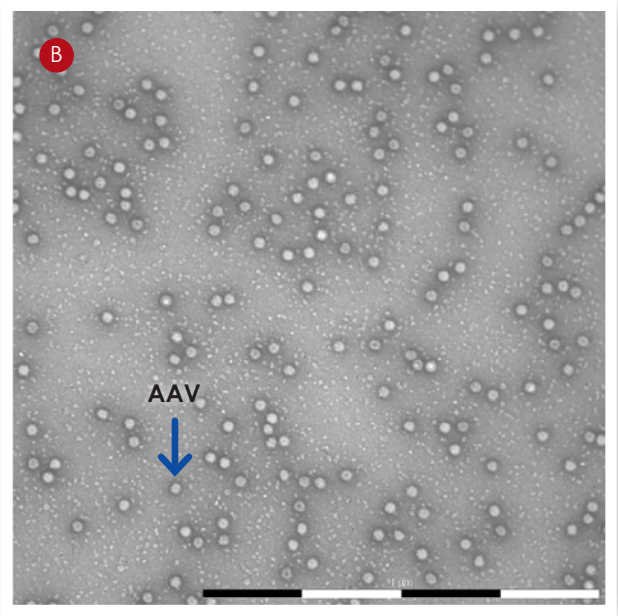
Sample after final purification step



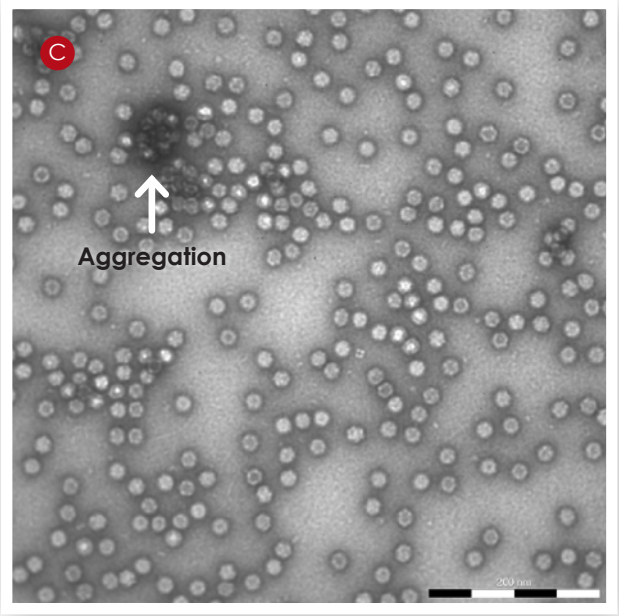
The types of contaminants that may appear



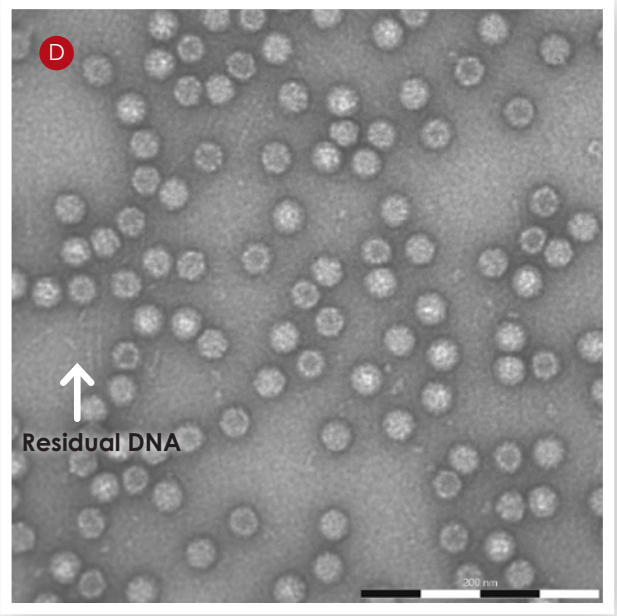
(A) Large size impurities, 4 µm



(B) Small size impurities, 2–15 nm.



(C) Aggregates



(D) Residual DNA impurities, 2 nm thickness

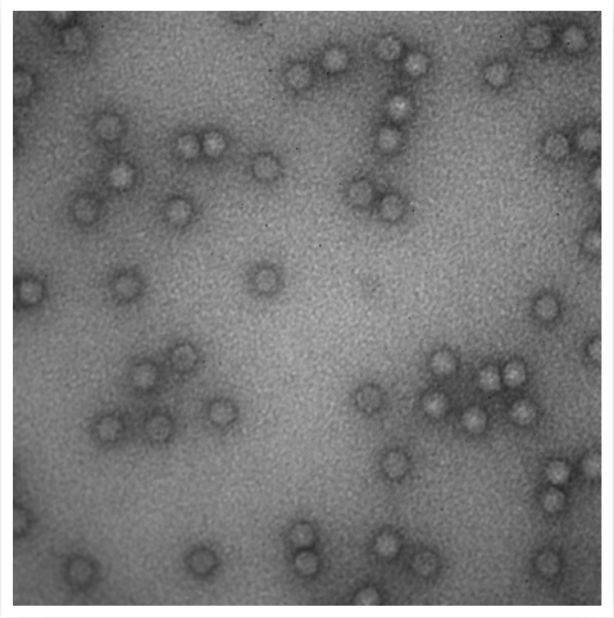
Undesired sample characteristics to consider when optimizing a process

- Aggregation
- Failure to remove host-cell debris or residual DNA
- Loss of particle morphology or integrity

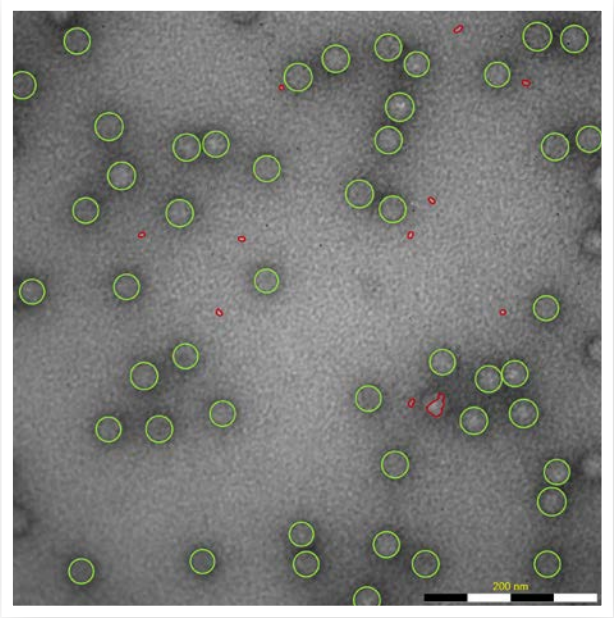
All these types of contaminants can easily be identified and quantified by using the automated image analysis software tools in MiniTEM.

Automated analysis of purity and level of debris

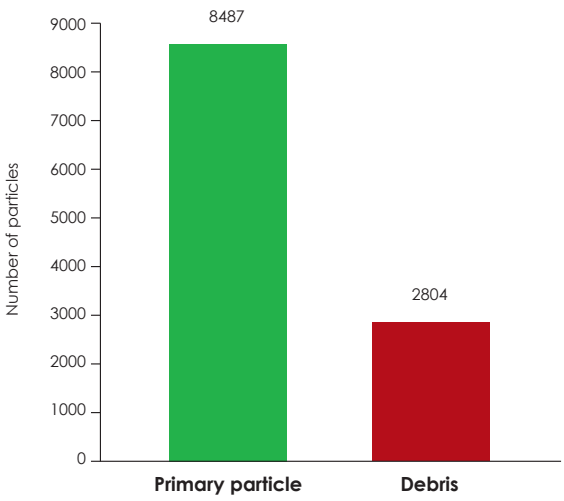
MiniTEM automatically images, detects and classifies particles and debris. Since automation enables detection and analysis of a far larger number of particles than is feasible with manual operation, the results are more consistent, statistically significant and reproducible.



Undetected particles



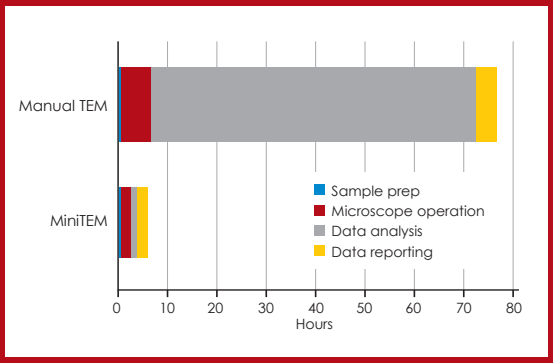
Detected primary particles and debris



Particles detected, classified and quantified using MiniTEM.

Automation saves time

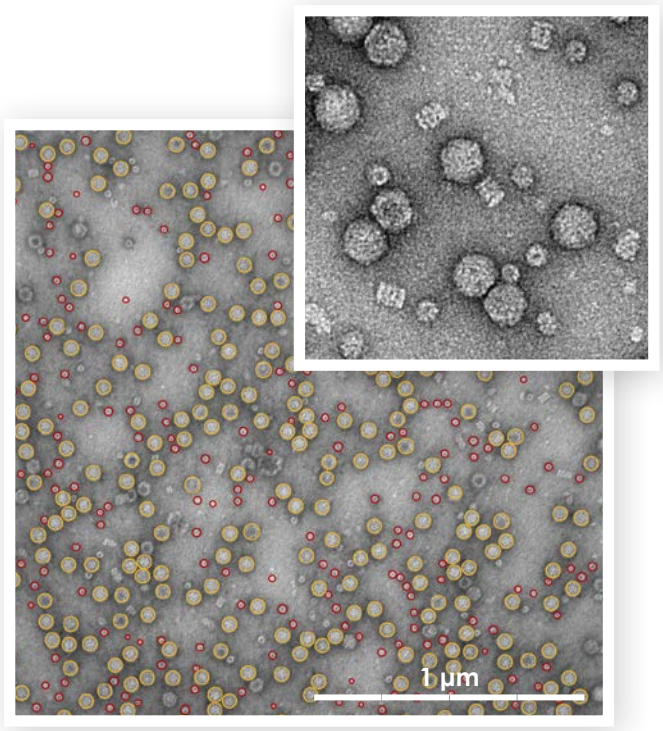
MiniTEM achieves an accurate and reliable result in two hours compared to approximately 65 hours for manual TEM.

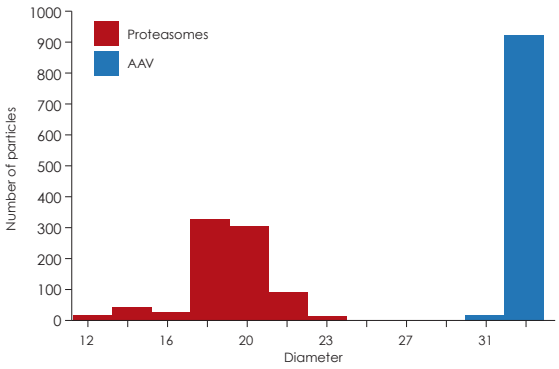


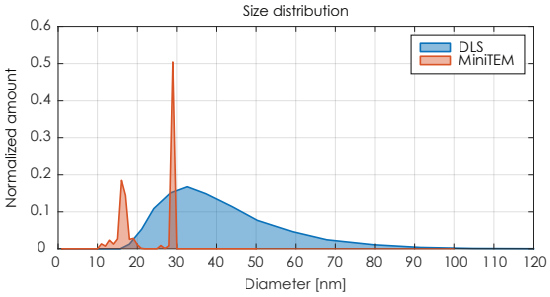
Manual imaging on conventional EM was not performed in its entirety, for practical reasons. In order to compare the time required to obtain data of similar accuracy using manual handling, the time for data analysis of 20 images was extrapolated to estimate the time that would be needed for 504 images.

Proteasomes could be hiding in your AAV sample

Comparing MiniTEM analysis with DLS size distribution analysis on the same sample demonstrates that the quanititative data based on image analysis by MiniTEM can reveal the presence of proteasomes where DLS gives only one broad peak.

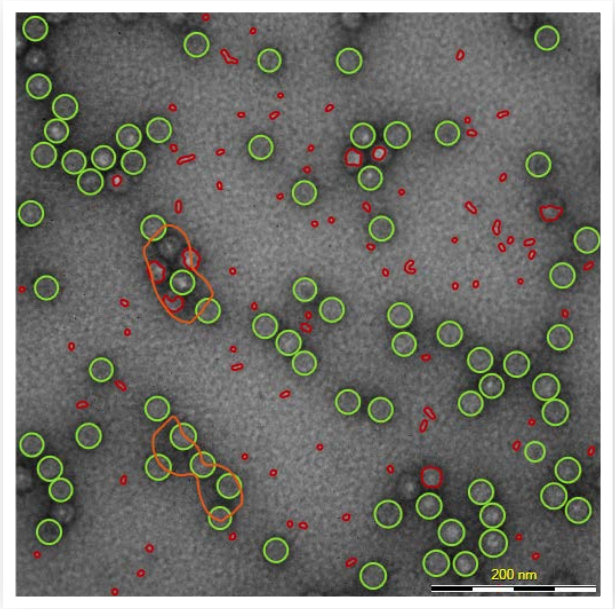


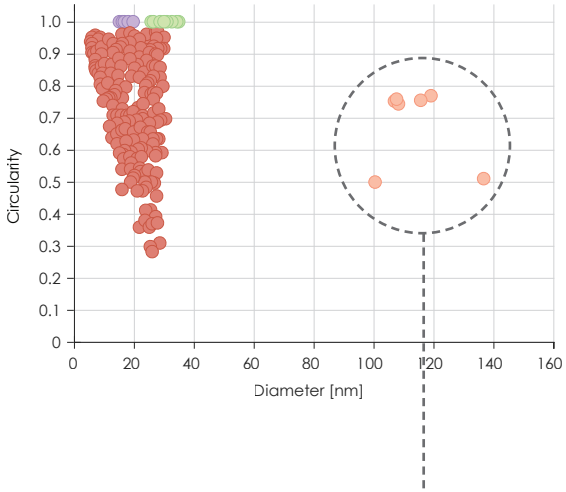




Size distribution analysis to reveal aggregates and debris

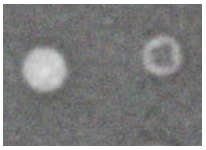
Particles present in the sample that deviate from the expected size and shape can be the undesired outcome of an un-optimized process step. In this example a large aggregate as well as smaller size debris are detected and automatically measured.



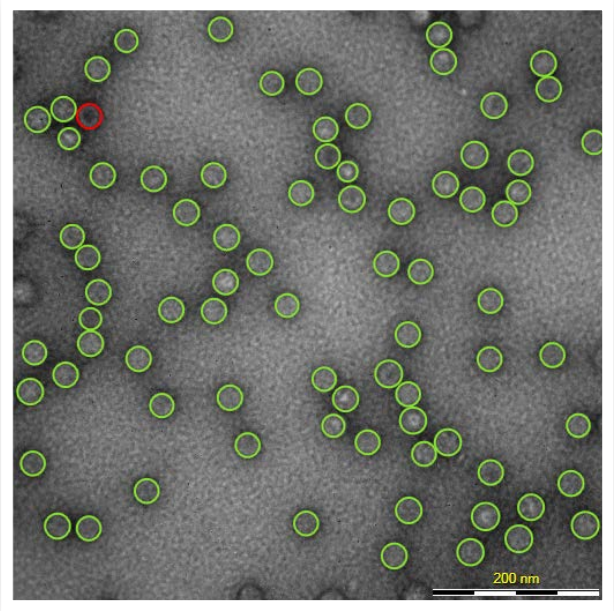


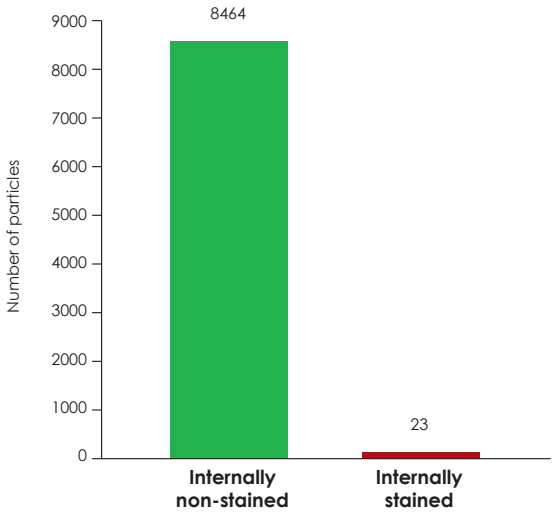
Outliers represent potential aggregates. By clicking on the outlier MiniTEM takes you to the image and you can determine whether it's a true aggregate or not.

AAV capsid integrity variations observed with MiniTEM



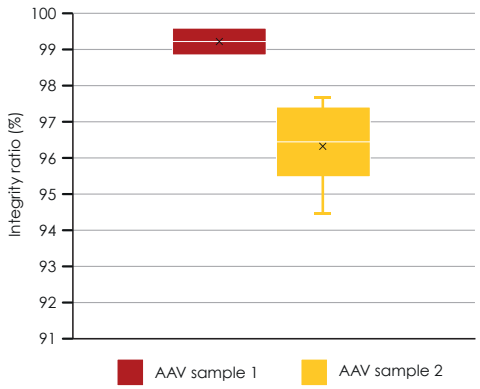
Negative-stained samples of AAV particles display variations in staining patterns that provide a clear indication of capsid integrity status. Intact AAV particles appear as lacking internal staining as opposed to broken AAV particles that exhibit internal staining because of presumed disrupted virus capsid structure. Particle doublets and triplets that can occur under certain conditions can also be detected.



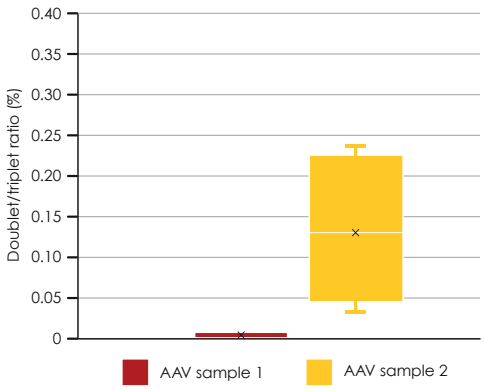


Using MiniTEM to compare the capsid status in two different samples

A **Particle integrity ratio**



B **Ratio of doublet/triplet particles**



In this MiniTEM study, the integrity of two different samples of purified AAV particles was automatically quantified and compared.

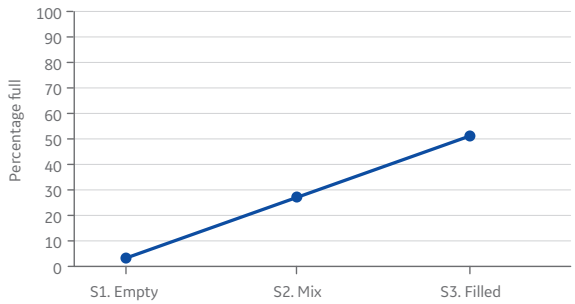
The study showed that AAV Sample 1 had a larger portion of intact particles (A) compared with Sample 2 (99% intact particles compared with 97% in Sample 2. (B) No doublets or triplets were detected in Sample 1, whereas 42 doublets or triplets (0.125%) were detected in Sample 2.

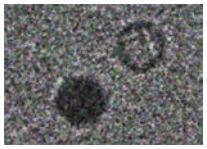
Linearity proves accuracy of cryoTEM full/empty analysis

CryoTEM is a method by which the biological specimens are vitrified by rapid freezing in liquid ethane, embedding the molecules in a layer of amorphous ice. CryoTEM does not involve using stains or other chemicals, so therefore samples prepared in this way preserve their native structure. The particles observed with cryoTEM with internal dark staining represent particles containing DNA. CryoTEM in combination with Vironova Analyzer Software (VAS) has been validated for quantification of percentage full AAV analysis. In this example three different samples are analyzed: empty (S1), filled (S3) and a third sample that is a mix 1:1 of sample S1 and S3 (S2). The cryoTEM shows the expected linear relationship between particle content and staining.

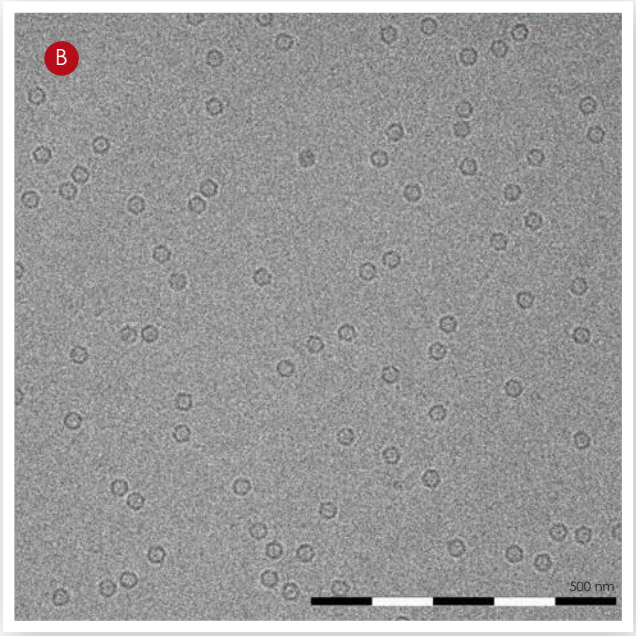
A

CryoTEM linearity assessment of AAV packaging

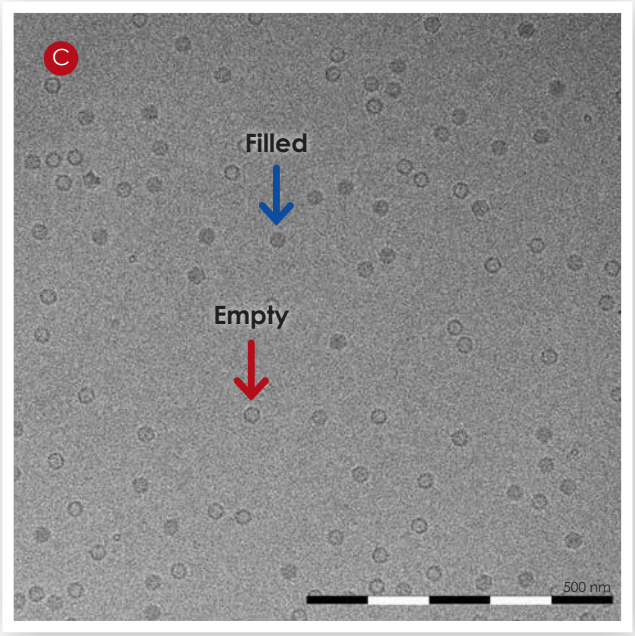




The particles observed with cryoTEM with dark internal staining represent particles containing DNA whereas the ones with light internal staining are empty.



S1: Empty

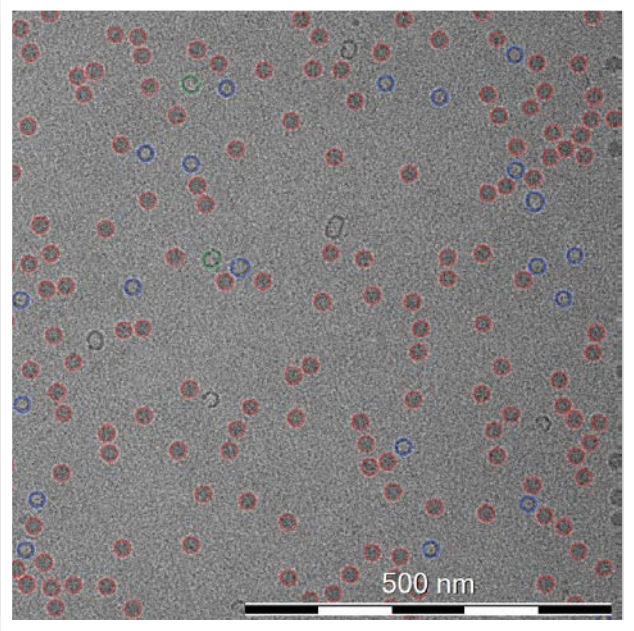


S3: 51% full

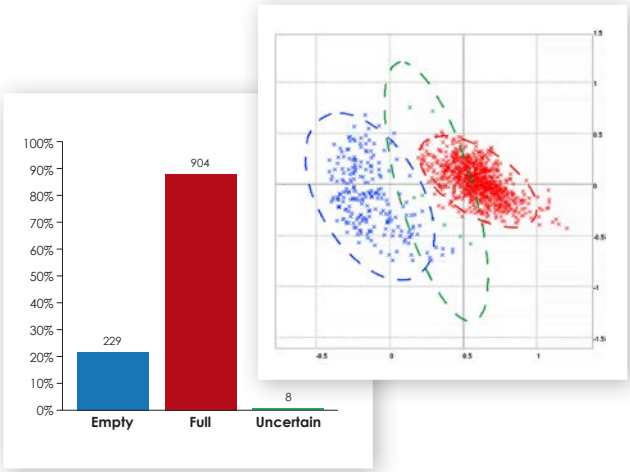
The quantitiative image analysis of 3 imaged samples defined as empty (S1), 51% filled (S3) and a mix of S1 and S3 (S2) performed with VAS is shown in the graph in figure (A). The imaging results are shown in figure (B) and (C) below.

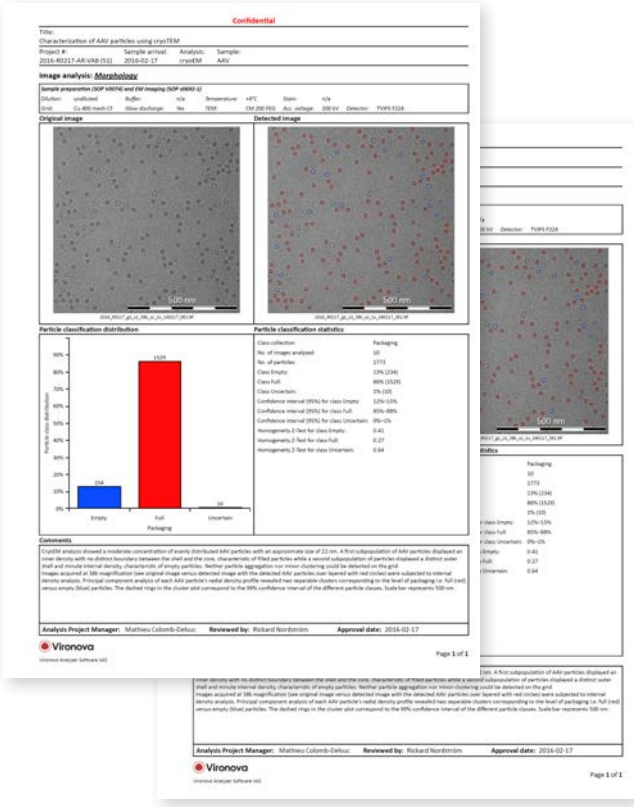
Validated AAV full/empty analysis service

Virus particle packaging analysis with cryoTEM in combination with VAS is recommended for batch release testing and QC of AAV-based gene therapy products. The automation that VAS provides allows Vironova EM services to deliver high quality images and timely and cost-effective accurate quantitative data.



Scatter plot showing 2 distinct populations of particles; full in red and empty in blue. Particles that don't cleary belong to any of these groups and which appear between the red and blue clusters are identified as uncertain.





Simply send your sample to the TEM experts

Our experts will tailor the analysis to address your specific questions. The report will contain:

- Representative EM-images
- Quantitative analysis
- Concluding summary with comments from the expert team

VAS – Vironova Analyzer software

Vironova Analyzer Software (VAS) is designed to analyze transmission electron microscopy (TEM) images of nanosized particles.

VAS enables reproducible and semi-automated particle detection and classification. For QC testing of capsid percentage full/empty of AAV samples Vironova offers services using cryoTEM and VAS-based image analysis that can be validated according to GMP.

Detect

The software works by algorithmically identifying particle characteristics based on selected criteria, such as the size or shape.

Explore

Each detected particle is stored together with the meta data. You can use the collected data to qualify particles into separate classes or groups, such as filled or empty.

Report

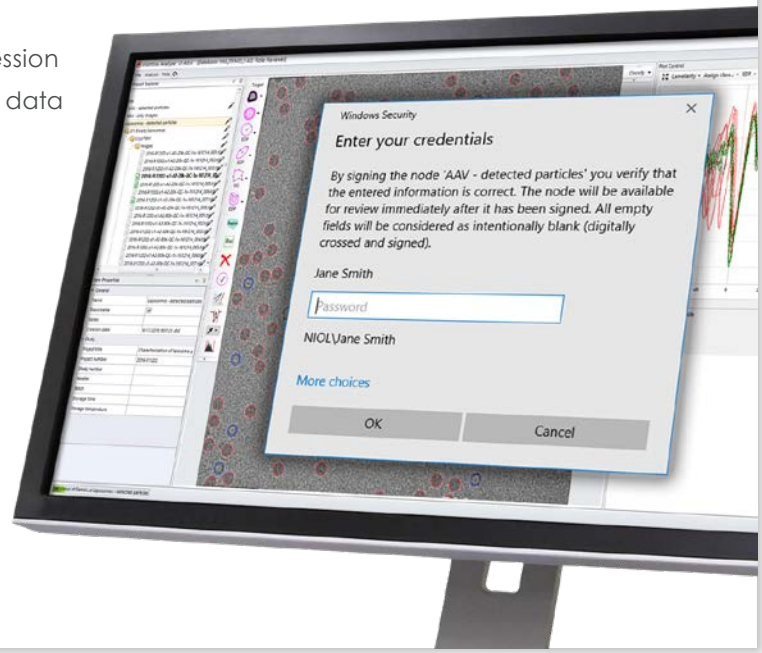
After the analysis VAS generates a report of the results with particle data presented in histograms, with statistics, together with representative microscopy images.

Complicance with GMP

The software is 21 CFR part 11 compliant and has access control, electronic signature, full traceability and audit log. VAS is suitable for regulatory controlled environments that work under GMP.

Full traceablility

- Database architecture for safe archiving
- Instant import of images from live microscope session
- Pre-defined roles for access and right to process data and sign off
- Electronic sign off



About Vironova

Vironova is founded on combined expertise in areas of virology, image analysis, electron microscopy, and mathematics. This expertise has enabled Vironova to offer a world-leading nanoparticle characterization analysis service with specialized expertise in vaccines, gene therapy and drug delivery particles such as viruses, virus-like particles, and liposomes. Experience from providing an EM service for gene therapy vectors, and of the type of features that need to be characterized frequently and in high volume, has inspired Vironova to develop comprehensive hardware and software solutions. Vironova revolutionizes access to transmission electron microscopy–based image analysis in biopharmaceutical development. Our solution enables automated analyses for faster and better informed decisions to secure robust bioprocessing and final product quality.

Vironova Analyzer Software (VAS)

The part 11 compliant Vironova image analysis software, VAS is designed for the analysis of transmission electron microscopy (TEM) images of nanoparticles. The software automates the extraction of morphological data and measurements from the TEM images and transfers them into graph-plotting and report-generating tools. Using VAS for automated nanoparticle detection can save up to six hours per sample compared with manual approaches. The VAS software in the hands of highly expert microscopists allows Vironova EM services to be cost effective and enables fast delivery of both high quality images and reliable quantitative data.

MiniTEM

MiniTEM is a low-voltage, tabletop microscope that can be placed in any standard laboratory. The system software enables control of the microscope and automatic image acquisition, particle detection, and classification. MiniTEM provides bioprocess workers with their own in-house solution that enables non-experts in electron microscopy to acquire meaningful nanoparticle characterization data quickly and easily.

The hardware technology of MiniTEM is assembled, manufactured, tested and serviced by DELONG INSTRUMENTS a.s. Palackého třída 3019/153 b, 612 00 Brno Czech Republic

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