

Virus Diagnosis with ZEISS EMs

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Application Note



One of the first biological applications in Electron Microscopy (EM) was the imaging of viruses.

Thus it was possible to identify viruses according to their size, shape, and fine structure.

Carl Zeiss was involved in this technique from the very beginning which is documented by the delivery of a **ZEISS-AEG EM 8** to the Philippines in 1953 (see front page).

Application

Despite the introduction of powerful new molecular methods electron microscopy is still an important tool for the investigation of infectious diseases. Because of its unbiased approach and its “open view” it remains essential in the detection of new emerging pathogens. In cases of unusual outbreaks or bioterrorism attacks it is still a front line diagnostic tool. A viral pathogen can sometimes be morphologically identified within 10 minutes. Electron microscopy provides information that can not be produced by alternative methods.



ZEISS LIBRA® 120

Sample preparation

- Stool is diluted by 1:5 to 1:10 depending on the consistency of the sample.
- The sample is clarified in a standard centrifuge at 5000 rpm.
- The supernatant is transferred into another tube and centrifuged in an ultra- centrifuge at 20.000 rpm for 1 h. This provides a sufficient enrichment of particles.
- Discard the supernatant and resuspend the pellet in 100 µl of distilled water.
- Put 30 µl of the sample on parafilm.
- Put up to 3 grids with a Formvar/Carbon film upside down on the drop.
- Let them sit for 10 min. The drop must not dry out.
- Place the grid on a drop of 2% PTA (pH 7,2) or on a drop of saturated uranyl acetate for 1 min.
- Soak the excessive staining liquid with a piece of filterpaper.
- Inactivate the sample to avoid any contamination of the EM. The method depends on the hazard of the sample (e. g. UV light or Osmium smoke).
- Screen the grid for 15 min.
- After that time the diagnosis can be made.

Equipment

The samples can be observed in every Zeiss EM starting with the EM 9 which is still in operation in some labs. The high end microscopes are the LIBRA® 120 and 200 which provide the best contrast because of their integrated energy filter and better electron sources.

All microscopes are equipped with a sheetfilm camera that is very often used for this application.

Parallel to sheetfilm a digital camera can be used for documentation which really makes the virus diagnosis a fast and easy to communicate method.

The major preparation equipment is the ultracentrifuge or an Airfuge which allows an enrichment of particles in a reasonable time.

The grids with a Carbon coated plastic film are commercially available. If there is a Carbon Coater, the supporting films can be prepared in the lab.

The lab must be certified for this application and the location must fulfill the safety aspects.

Results

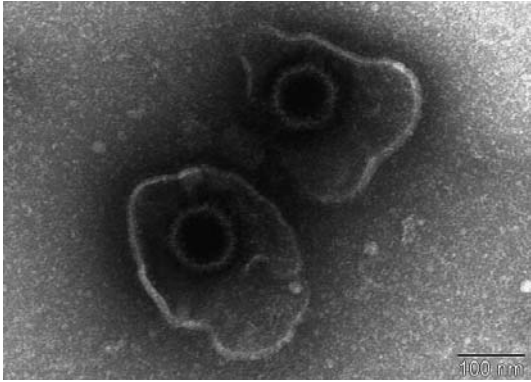


Fig. 1: Herpes virus

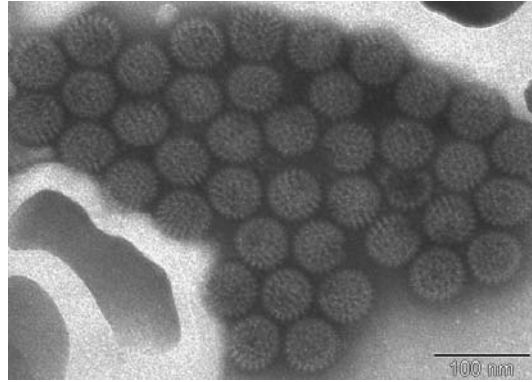


Fig. 2: Rota virus

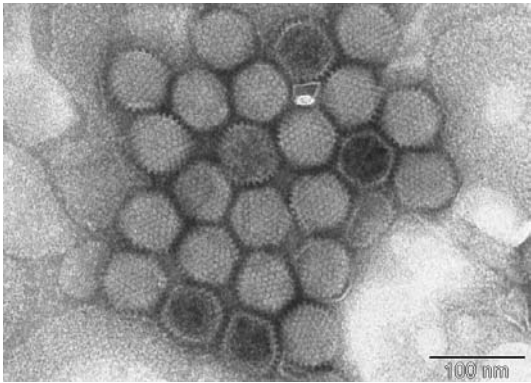


Fig. 3: Adeno virus

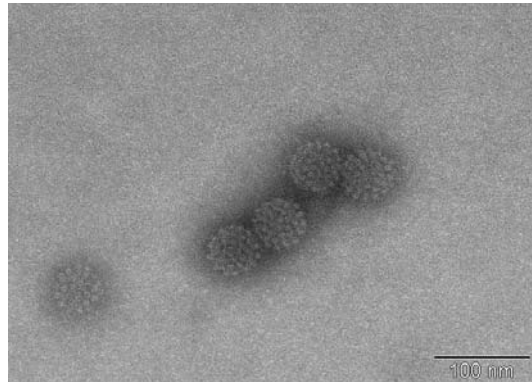


Fig. 4: Papilloma virus

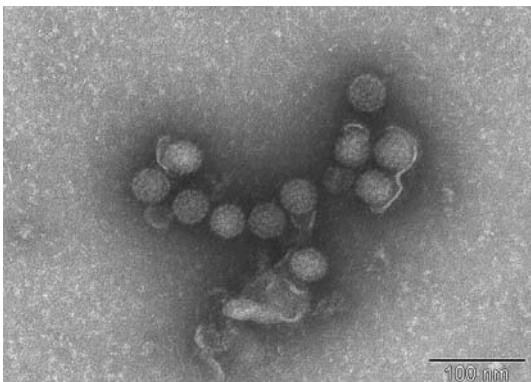


Fig. 5: Polyoma virus

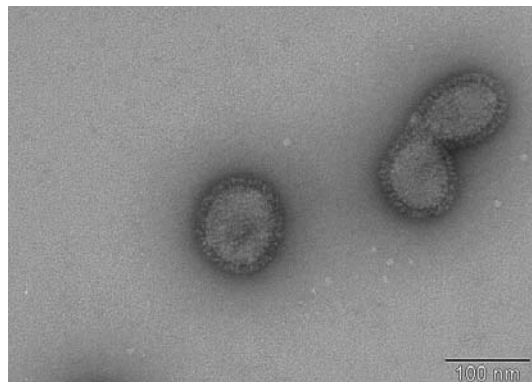


Fig. 6: Influenza virus

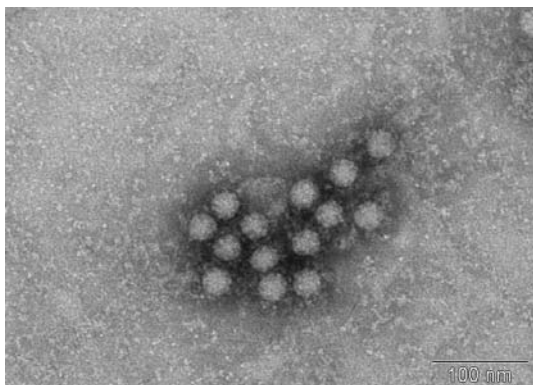


Fig. 7: Astro virus

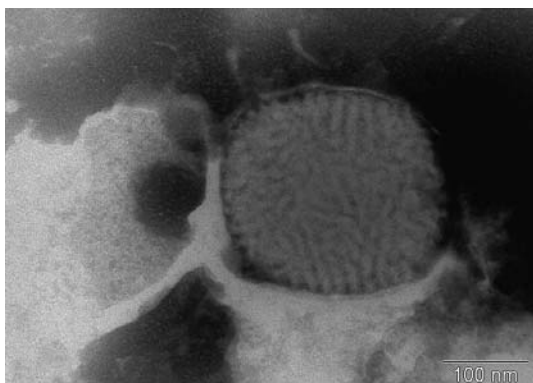


Fig. 8: Orthopox virus

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Emerging Infectious Diseases Vol. 9, No. 3, March 2003
Biel SS, Gelderblom HR.
Diagnostic Electron Microscopy is still a Timely and Rewarding Method.
Journal of Clinical Virology 13 (1999) 105 - 119

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Global Solution Provider

The Nano Technology Systems Division of Carl Zeiss SMT provides its customers with complete solutions featuring the latest leading-edge EM technology. The company's extensive know-how, meticulously acquired over 60 years in the field of e-beam technology, has brought many pioneering innovations to the market. Our global applications and service network ensure fast, reliable and high quality support sharply focused on customer requirements. Combined with dedicated upgrade strategies, this will protect your investment for its entire lifetime. The core technology embedded in our innovative products enables us to provide solutions which add value to our customers' businesses.

Customer feedback is always welcomed and gathered by collecting valuable information at trade shows, in workshops, in user meetings and upon instrument installation. The Division's business services also include outstanding support from sales consultancy to technical service options even when the warranty has expired.

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