# Virus Diagnosis with ZEISS EMs

Application Note

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## First Virus Microscope In SEA Installed Here

HEALTH SECRETARY Juan Salcedo Jr. yesterday inaugurated the MSA-procured "electro-static electron microscope" at the Alabang serum and vaccine laboratory in Rizal province. Photo shows Werner Degenhard, German scientist who installed the microscope, explaining how it works to Salcedo as Aubrey Williard, MSA malaria control engineer and Dr. Walfrido de Leon, director of laboratories look on. (MSA Photo Exclusive to the Philippines Herald)

Health Secretary Juan Salcedo, ... predicted "a new era of ...tive medicine and medic... research in the Philippines" ... the installation yesterday of the first "electro-static electron microscope" to be installed and operated in Southeast Asia at the Alabang Serum and Vaccine laboratory in Rizal province.

Bought by the U.S. Mutual Security Agency for the Alabang Serum and Vaccine Laboratory under the PHILCUSA-MSA health program, the $52,000 "electron miscroscope EM 8" has a magnifying power of from 1,600 to 640,000 times.

Werner Degenhard, noted German scientist and doctor of

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### First Virus . . .

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chemistry who installed the AEG-Zeiss microscope, said researchers could observe the molecular structure of both organic and inorganic matter and measure spaces as small as one-one millionth of a millimeter.

In preventive medicine, Dr. Salcedo said the causative agents of diseases could be determined by revealing the physical characteristics of virus and other bacterial bodies.

He said it would also be used to determine properties and characteristics of crystals, proteins and solutions for research into their effects on the human physiology.

ZEISS

We make it visible.

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 documentated 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.

*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 vrirus 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

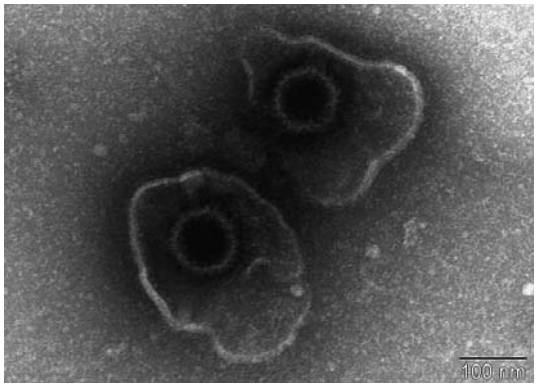


*Fig. 1: Herpes virus*

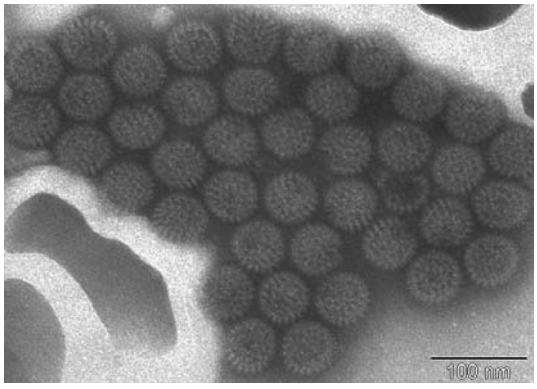


*Fig. 2: Rota virus*

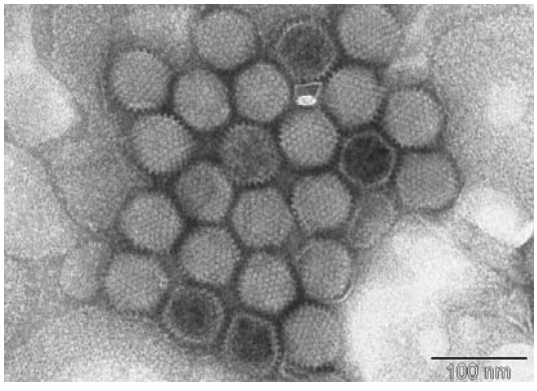


*Fig. 3: Adeno virus*

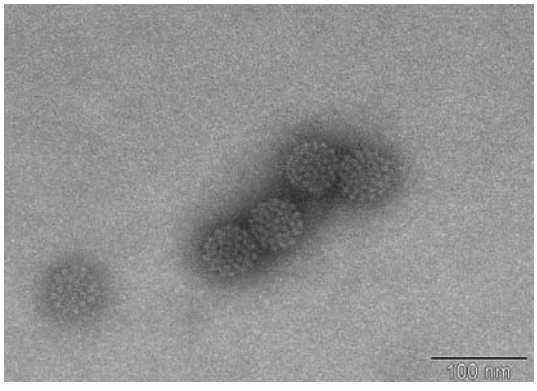


*Fig. 4: Papilloma virus*

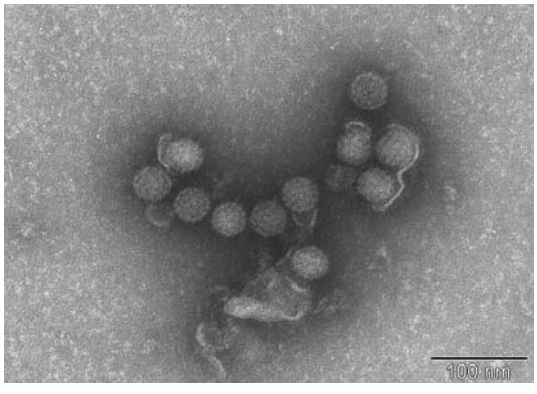


*Fig. 5: Polyoma virus*

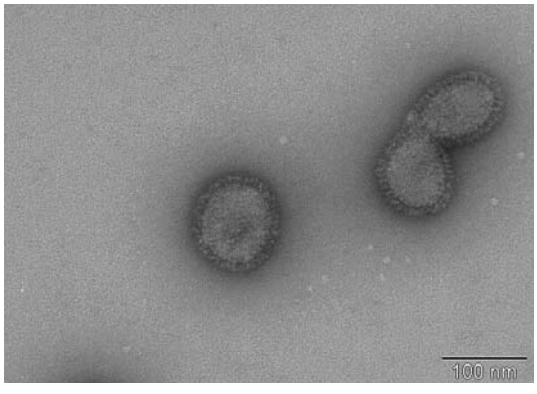


*Fig. 6: Influenza virus*

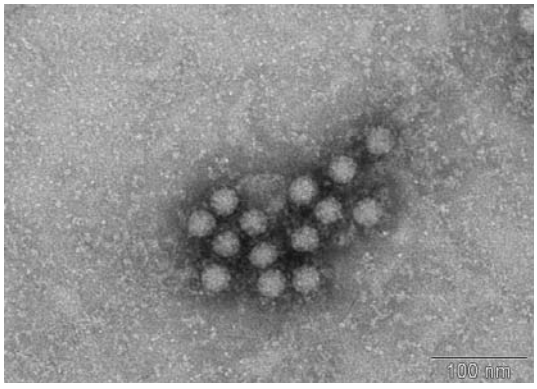


*Fig. 7: Astro virus*

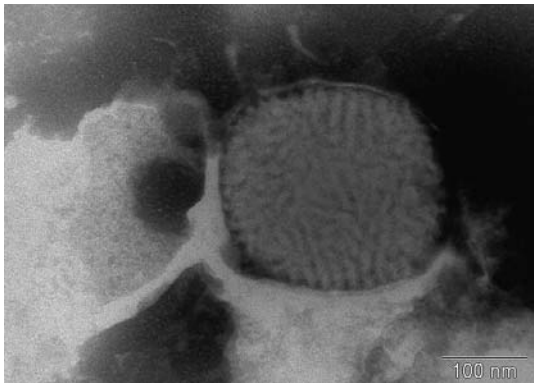


*Fig. 8: Orthopox virus*

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