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Website: www.virusys.com
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ELECTRON MICROSCOPY SERVICES - FINAL REPORT

I. INVESTIGATOR

Your name and company goes here

II. SERVICE PERFORMED

Catalog #: EM-199

Service: Negative Stain and TEM Analysis of Biological Sample in Suspension

III. SAMPLE INFORMATION

Lot # / Sample I.D.: #07-000-1: Sample A

Virus / Sample Type: Papilloma Virus-Like Particles

Study Dates: April 15 – April 16, 2008

Instructions For Analysis:

1. Examination of the preparations for the presence or absence of papilloma virus-like particles; morphology of virus particles observed, distribution of particles over the grid areas, presence or absence or viral aggregates.

2. Size measurements and mean of papilloma virus particles; observations and morphology of capsomere structures, observations on virus viability; presence or absence of any other material in preparation.

3. Examination for biological contaminants.

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IV. SAMPLE OBSERVATIONS

Lot #07-000-1: Sample A
Micrograph Numbers: 1247 through 1252

1. This sample, prepared diluted 1:3, contained large quantities of papilloma virus-like particles, which were found scattered throughout the grid areas examined. We also observed very small quantities of proteinaceous material in this sample.

2. The papilloma virus-like particles observed in this sample were found as single, individual particles and in various-sized viral aggregates. Single, individual particles were observed in moderate numbers and made up approximately 10% of all the virus particles observed. Virus particles found in aggregate form made up approximately 90% of all the virus particles observed.

3. The viral aggregates observed varied in overall size, from one aggregate to the next. Approximately 70% of the viral aggregates contained more than twenty particles per aggregate. Approximately 30% of the aggregates contained less than twenty particles per cluster. In spite of the aggregation, overall distribution of the particles on the grid areas was excellent.

4. The majority of the virus particles observed appeared to be in good overall condition, with approximately 80% of the particles exhibiting intact, consistently viable forms. The intact, viable virus-like particles observed exhibited spherical or roughly-spherical overall forms. Many of the intact, viable particles did exhibit some erratic structural formation and irregular shapes, but these particles appeared to be viable.

5. Approximately 20% of the particles examined appeared to be exhibiting some structural damage. Most of the damaged particles appeared to be partially intact, but exhibiting some areas where the capsomeres were showing signs of lysis. Most of the damaged particles exhibited irregular structural formation or possible damage to both the interior core regions of the particles, as well as the exterior region of the particles.

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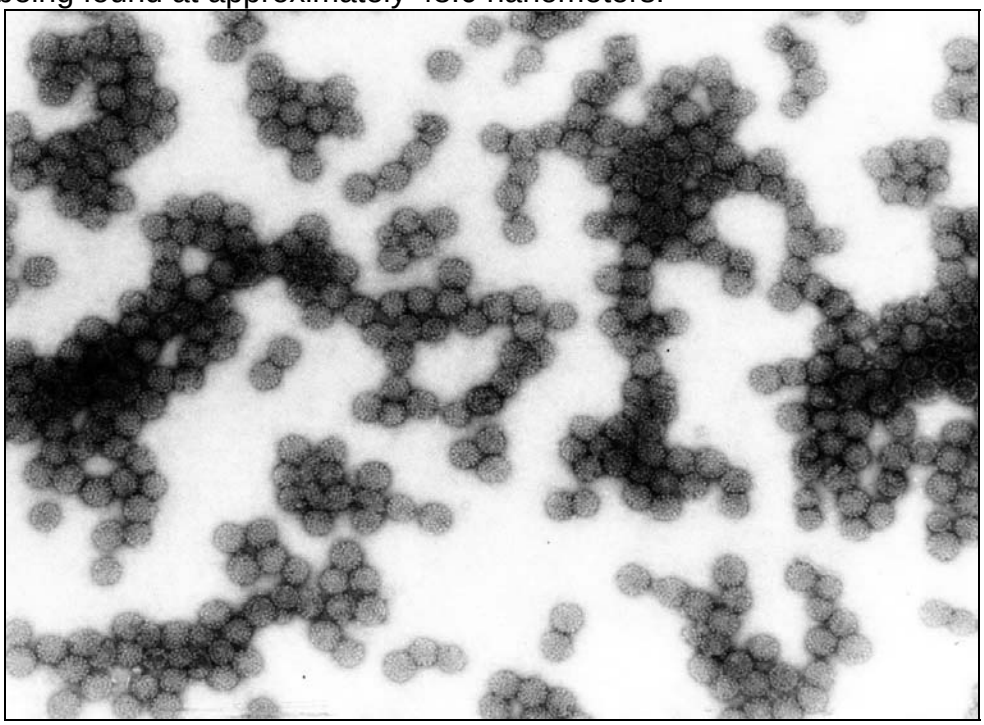
6. The following is the statistical data taken from this sample:

Total number of virus particles examined: 3,550
Intact and viable virus particles: 2,823
Lysed or damaged particles: 727
Particles found in aggregate form: 3,201
Particles found as singles: 349

7. The intact virus particles observed were generally consistent in overall size and measured between 28.5 and 76.0 nanometers in diameter, with the mean of the formed particles being found at approximately 48.0 nanometers.



Five additional high magnification micrographs
are saved to the enclosed compact disk (CD)

8. The papilloma virus-like particles consisted of arrays of capsomere structures, which made up the structure of the virus, overall formation, and subsequent shape of the formed particles. The viral capsomeres examined were consistently spherical in overall shape and measured between 4.0 and 6.0 nanometers in diameter.

9. We did not observe any contaminating biologics in this sample, including bacteria, mycoplasma, or yeast.

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V. MATERIALS AND METHODS

Sample Preparation: Upon arrival to our facility, the frozen sample was held at – 70 C until study initiation. Upon study initiation, the sample was thawed at room temperature for one hour, and then resuspended thoroughly by tube inversion and swirling, before grid preparation.

The sample preparation was prepared multiple times, in the following manner:

#07-000-1: Diluted 1:3, for a final protein concentration of 113 µg/ml.

The sample was resuspended thoroughly by tube inversion and swirling, after diluting.

Grid Preparation/Sample Mounting: 300 mesh copper grids, coated with formvar and carbon, were clasped tightly and secured with forceps. Multiple grids were rinsed with 0.01% Bovine Serum Albumin and the excess solution was then wicked off using filter paper. A 2.0 microliter aliquot of the virus sample was placed on separate grids and allowed to air dry. After ten minutes, any remaining residual material was wicked from the grids.

Sample Fixation/Staining: After the sample had completely air-dried, the sample was fixed and stained with 20 microliters of 2.0% phosphotungstic acid, which was placed onto each grid and allowed to incubate for 30 seconds. Any excess stain was then removed with filter paper.

TEM Examination: The sample was then intensively examined in a JEOL 1200 EX Transmission Electron Microscope, at high magnifications, at 60kV. Numerous areas from each grid were thoroughly examined before micrographs were taken.

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VI. APPROVALS

This report accurately represents Virusys procedures. The results from these samples were obtained according to these procedures. The data are recorded in laboratory logbooks and computer files, at our laboratory. Embedded samples, grids and negatives have been returned, unless noted otherwise.

Electron Microscopy Technologist MSA Certified	Date

VII. REFERENCES

1. Pinteric, L., Taylor, J., The Lowered Drop Method for the Preparation of Specimens of Partially Purified Virus Lysates for Quantitative Electron Microscopic Analysis, 1962, J. of Virology, Vol. 18, pp. 359-371; (modified).

2. Harris, R., Horne, R.; Electron Microscopy in Biology, 1991, Negative Staining, Oxford University Press, New York NY; pp. 203-227, (modified).

3. Hayat, M.A., Principles and Techniques of Electron Microscopy, Biological Applications, 1989, Negative Staining; CRC Press, Boca Raton, FL; pp.328-342, (modified).

4. Doane, F. W., Anderson, N., Electron Microscopy in Diagnostic Virology, 1987, Papovaviruses, Cambridge University Press, New York, NY; pp. 64 - 67.

5. Dalton, A., Haguenau, F.; Ultrastructure of Animal Viruses and Bacteriophages, 1973, Papovaviruses; Academic Press, Inc., New York , NY; pp. 47 - 65.