Transmission Electron Microscopy for Morphology Characterization of Virus like Particles

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Transmission Electron Microscopy for Morphology Characterization of Virus like Particles

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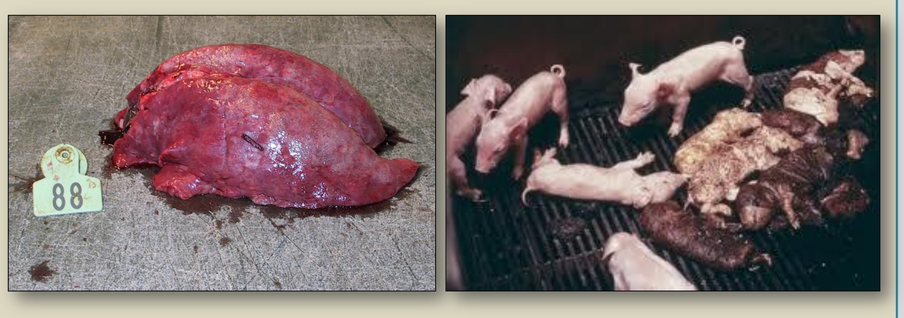
Research Area

Denmark produces about 4500 tons pig meat per day and about 90 % of this is exported. This results in an annual export of 4 billion EUR and represents about 5% of the total Danish export. Porcine reproductive respiratory syndrome virus (PRRSV) causes a loss of about 43.6 million EUR per year in Denmark, and therefore controlling the virus is a top priority.
The commercially available PRRSV vaccines are not effective in the prevention and extermination of the virus and therefore new development routes, such as virus like particles (VLPs) are approached for obtaining efficient and safe vaccines, capable of addressing prevalent strains.
The project is a consortium between 8 partners from academia and industry (for details see www.pigvac.dk). DTU Cen's role in the project is to apply electron microscopy for morphological characterization of VLPs.

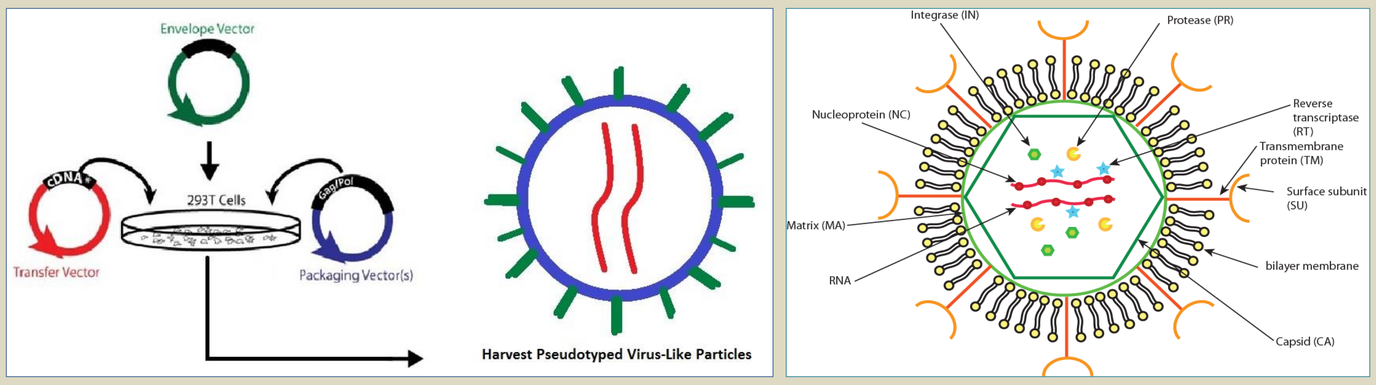


PRRSV infected pig lung and piglets born from a PRRSV infected sow. Images from Charlotte Sonne Kristensen, Danish Pig Research Center.

Virus like Particles (VLPs)

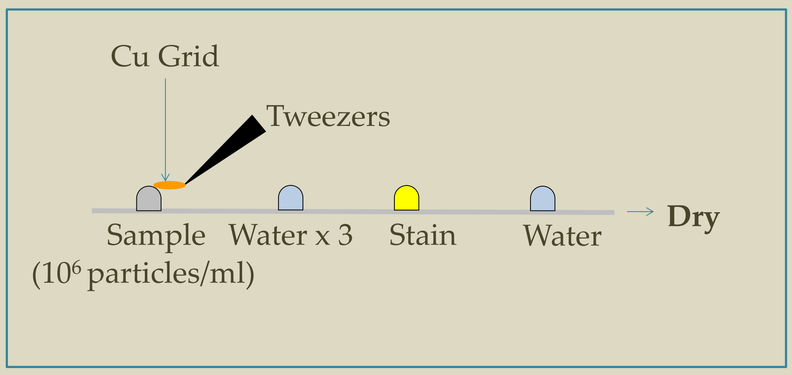
Virus like Particles are composed of a subset of structural proteins and are attractive because:
1. They present high immunogenicity- they induce a humoral and/or cell mediated immune response similar to that produced for a virus.
2. They are safe; they do not contain genomic material.
3. They are high adaptable for inclusion of new viral proteins.

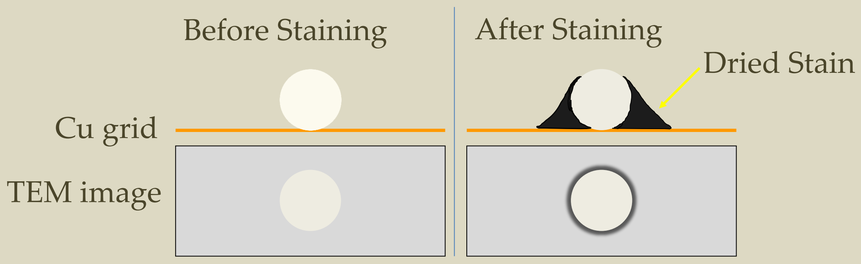
In the current project we use pseudotyped VLPs: Murine Leukemia Virus pseudotyped with PRRSV GP5 .



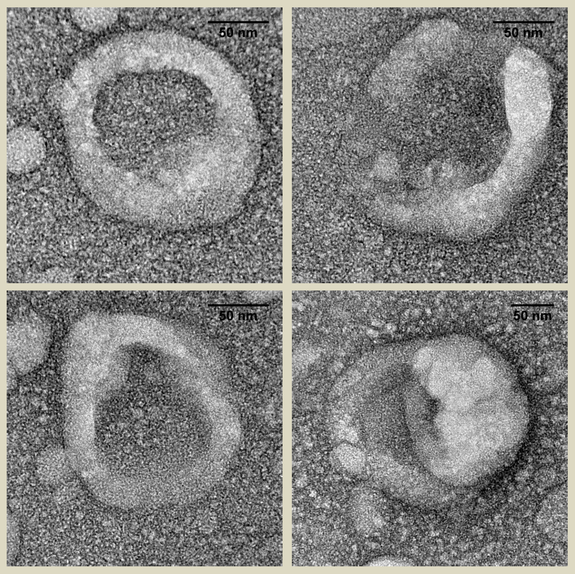
Schematic drawing showing the pseodotype synthesis of VLPs and a Murine Leukemia Virus like particle pseudotyped with PRRSV envelope particles.

Negative Staining

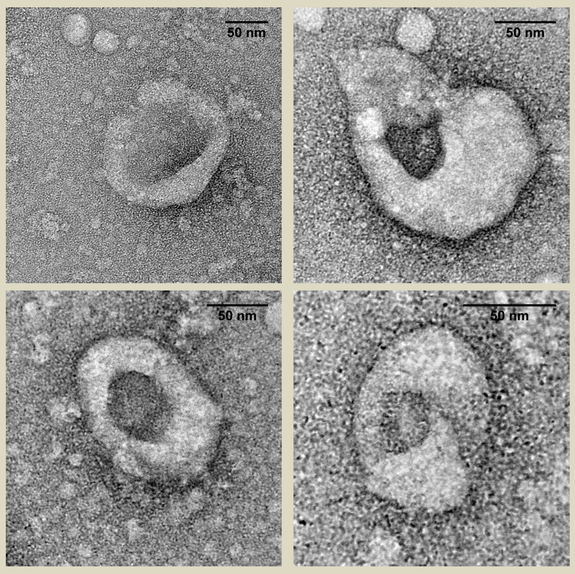




Electron Microscopy of Virus like Particles

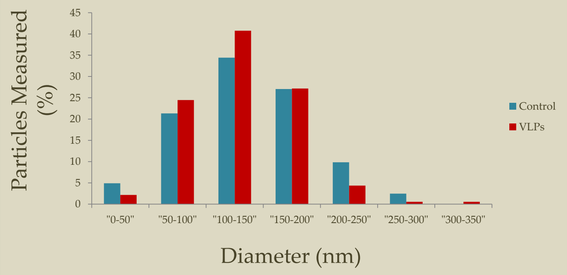


Control stained with 2% uranyl acetate on Collodion film.



VLPs stained with 2% uranyl acetate on Collodion film.

Size Distribution



Conclusions

Negative staining is a quick and easy sample preparation method for morphological electron microscopy analsyis of VLPs. However, the VLPs are not stabilized enough by the uranyl acetate and the particles collapse. Moreover, the control sample contains structures with size comparable to that of VLPs, and therfore specific labelling is needed in order to identify the VLPs by TEM.

Future sample preparation methods aim at using immulabelling, which will allow for the identification of the VLPs, and cryo electron microscopy, which will ensure the preservation of the particle morphology.

We are looking for

I would like to meet other people currently using electron microscopy for visualization and characterization of viruses and large proteins.

Acknowledgments

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