A Refined Approach to Producing Polyclonal Antibodies in Chickens

Completely Replacing All Invasive Elements by Combining Immunizations with Routine Aerosol-based Vaccinations

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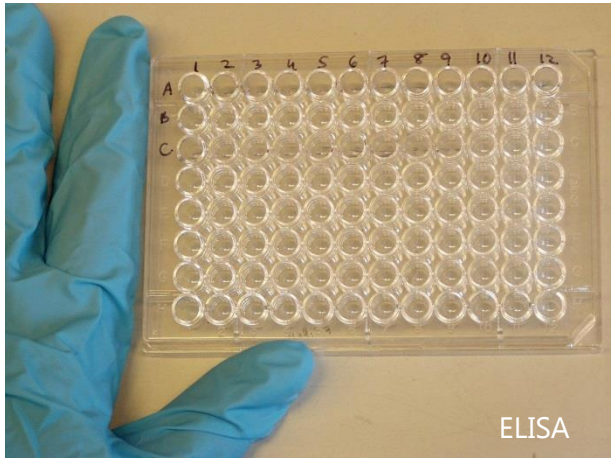
Antibodies in basic and applied science

- Used in
  - Basic research
  - Clinical analyses
  - As pharmaceuticals
  - Home test kits
- A growing market worth billions (~€2 billion)
- Leads to a considerable use of laboratory animals
- … but we could use fewer and inflict less suffering

Humira (Adalimumab)

http://www.truemedcost.com/humira-price/



Pictures retrieved from Wikipedia

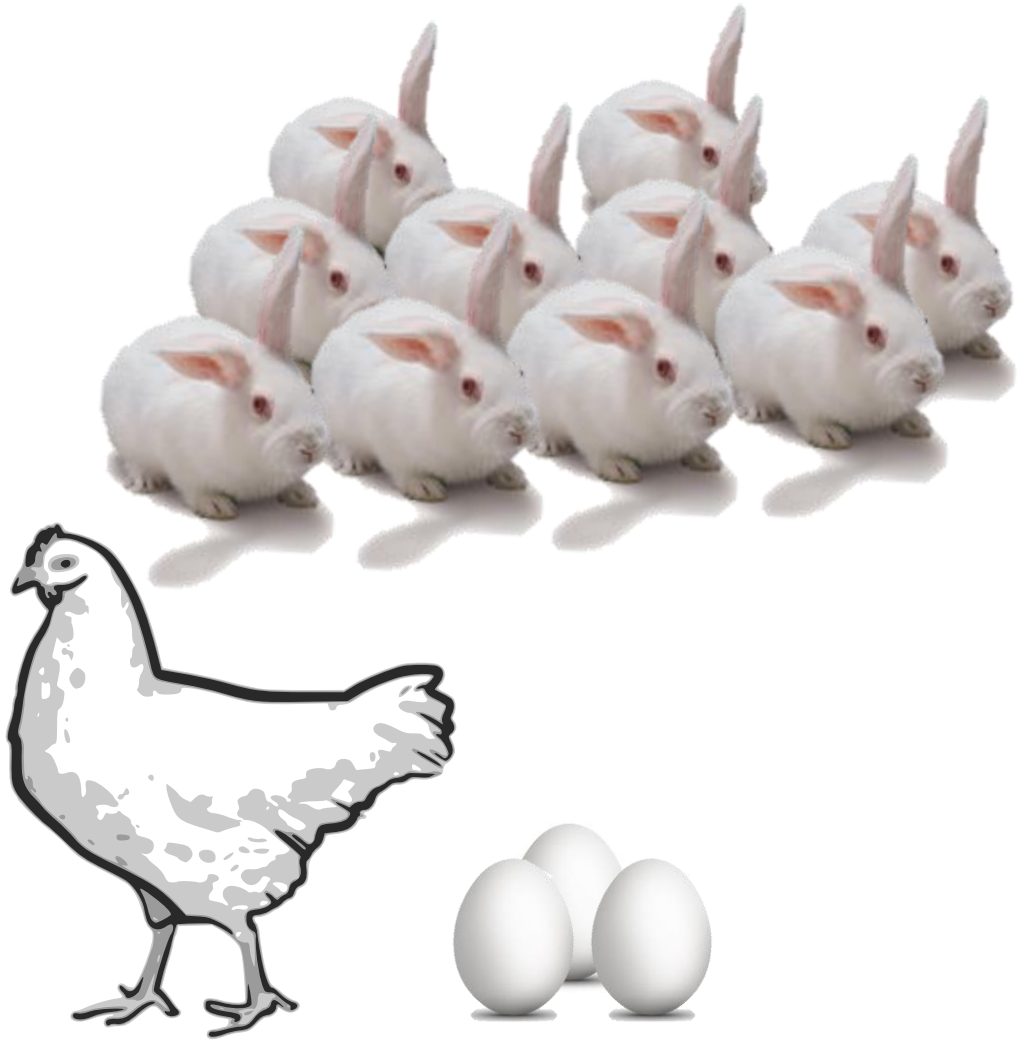
Home pregnancy test

http://www.newkidscenter.com/images/10401080/image001.jpg

Hospital laboratory with automated analyzers

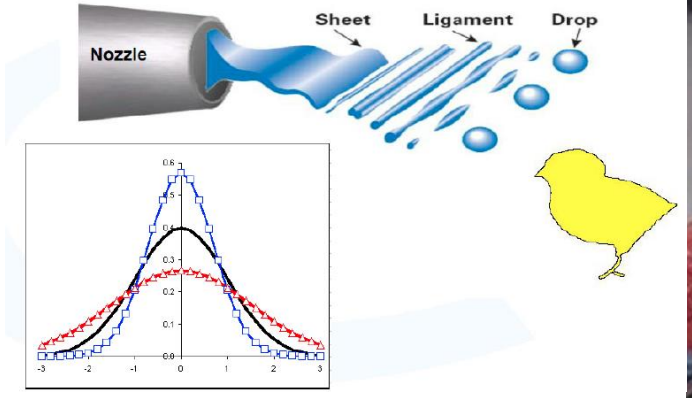
Why chickens?

- Antibodies can be isolated from the chicken egg
- In one month more antibodies can be isolated from one hen's eggs than can be obtained from a whole year's blood samplings from a rabbit
- The avian IgY antibodies have virtually identical properties to the mammalian IgGs



Our aim

- To ultimately remove **all** the invasive elements in antibody production
- If immunizations can be made a hands-off procedure there is no need to ever restrain or break the chicken's skin
- And we are already doing just that with vaccinations

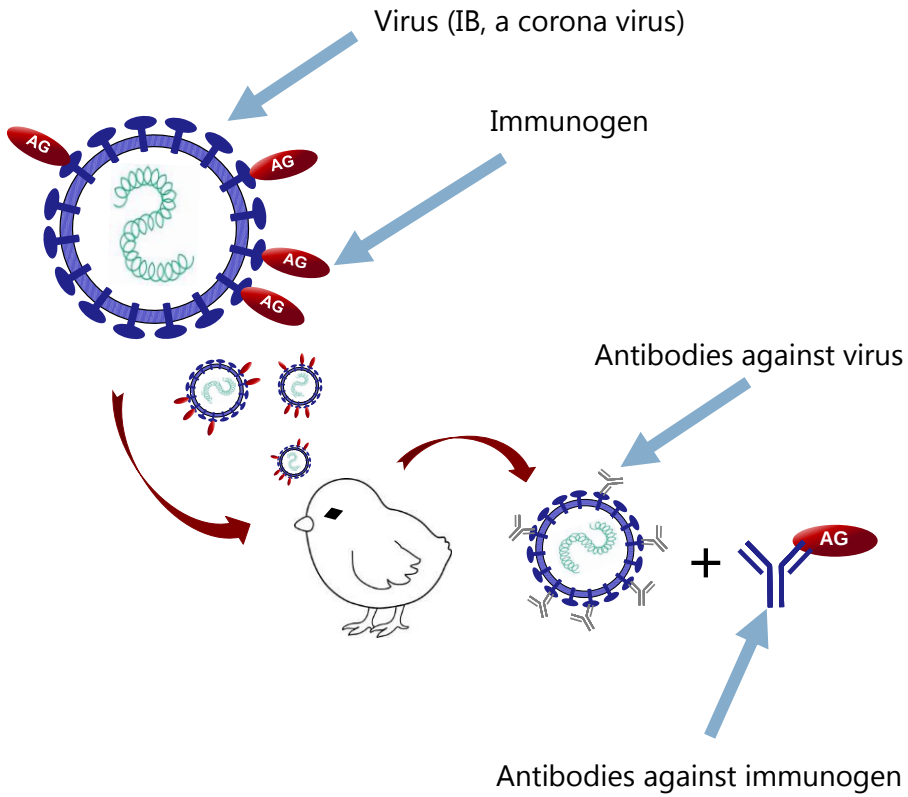


https://www.vectormune.com/Educational-Symposia/New-Technology-Vaccine-Symposium-2013-Miami/Vaccination-Equipment-Status-and-Future-Expectations

http://www.pulsfog.de/sources/header/header-img-application-aerosol-vacination.jpg

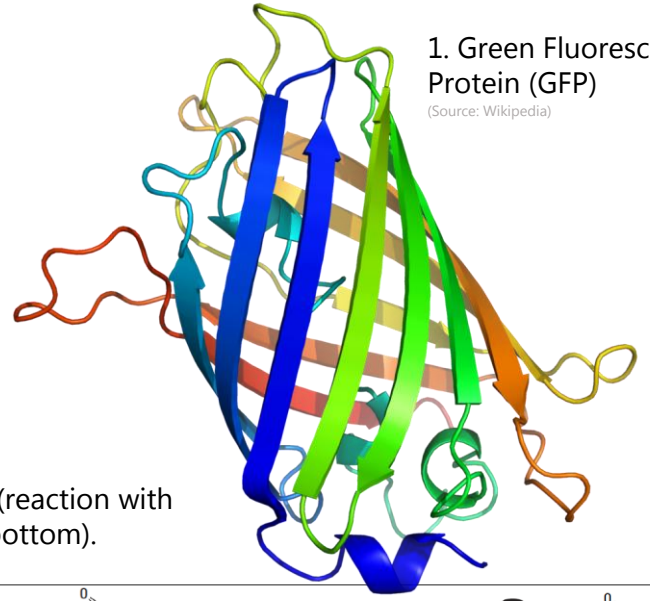
Project outline

- Attach ("conjugate") an immunogen to our model vaccine (IB 4-91)
- Find a means of delivering the modified vaccine in aerosol form
- Develop assays for detecting immune responses to both the vaccine and the immunogen
- If a successful response is found: isolate antibodies from eggs



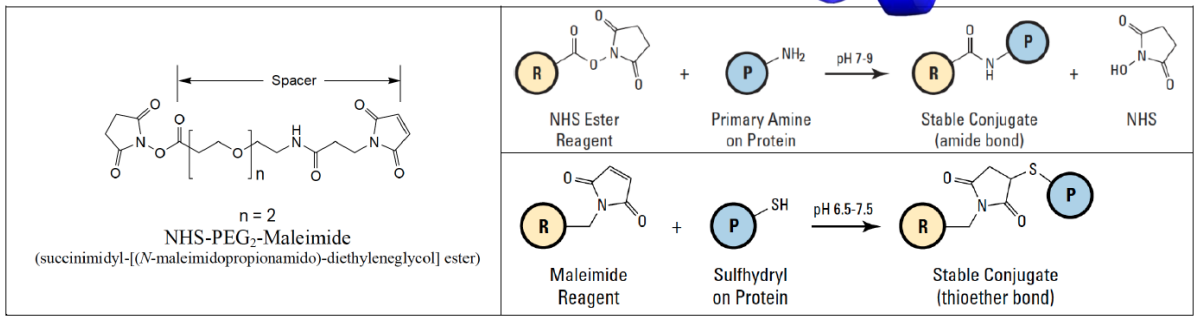
Attaching a foreign protein to the virus

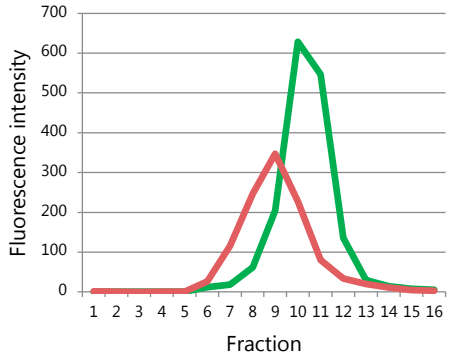
- For convenience we used Green Fluorescent Protein (GFP)
- We developed two candidate conjugation methods
- Opting for the simplest solution we could verify that the GFP was reacting with the vaccine
- Now for a delivery method…



1. Green Fluorescent Protein (GFP)
(Source: Wikipedia)

2. The chosen conjugation method (reaction with virus on top, reaction with GFP on bottom).





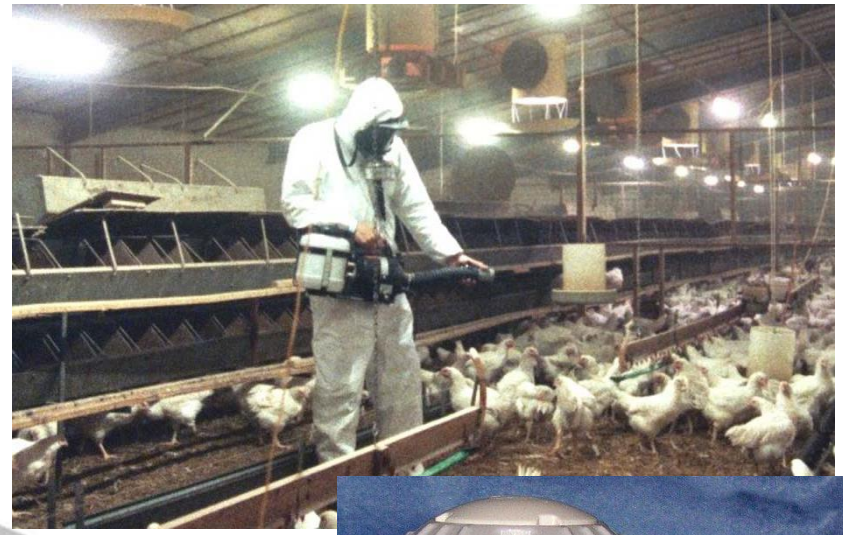
3. Size-exclusion chromatogram verifying successful conjugation – Green: pure GFP; Red: GFP following conjugation with vaccine solution.

Conclusion: GFP has been coupled to a much larger structure.

Creating a microliter scale aerosol

- Equipment used on farms are loaded with liters of vaccine solution
- We can make a few microliters
- Lab-scale equipment is prohibitively expensive
- Conclusion: We need a cheaper homemade solution

Equipment used on farms – cheap but require liters of vaccine solution



https://www.mspcorp.com/aerosol-instruments/flow-focusing-monodisperse-aerosol-generator-1520/

https://www.piceramic.com/en/applications/pumping-dosing/aerosol-production/

Lab-scale equipment – can use small volumes but are costly

Aerosol immunizations, MacGyver-style

Method 1: Spray nozzle from perfume bottle

Method 2: Modified e-cigarette

Project outline revisited

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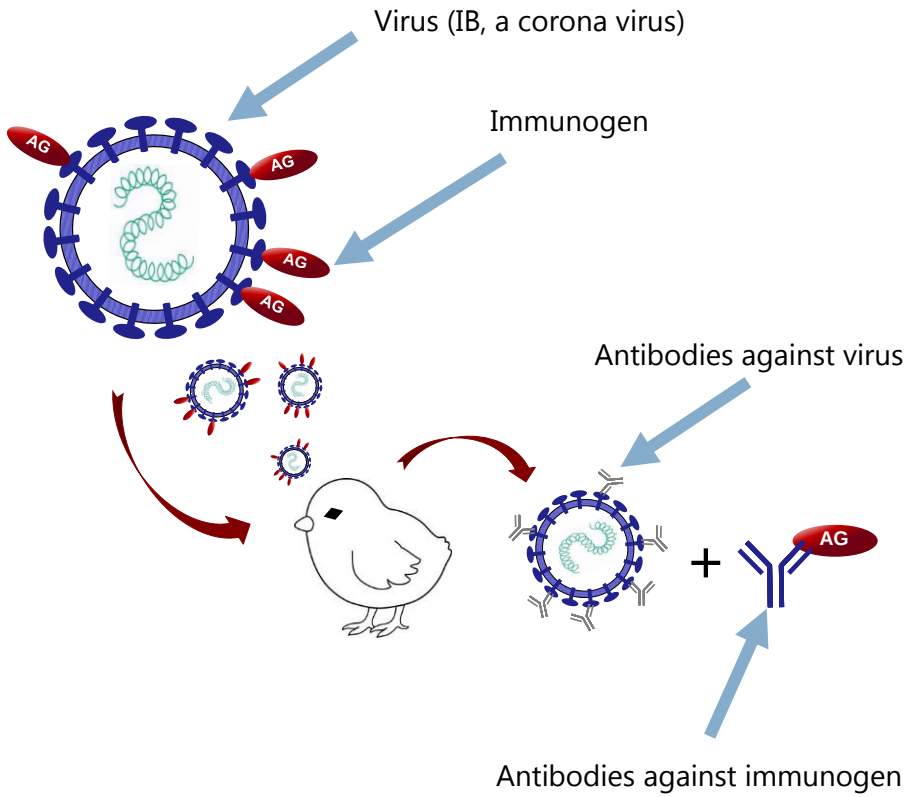
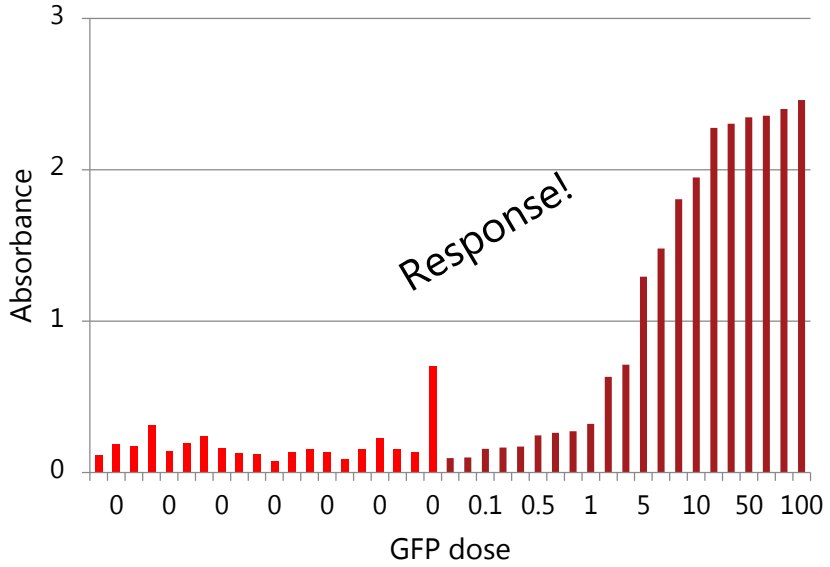
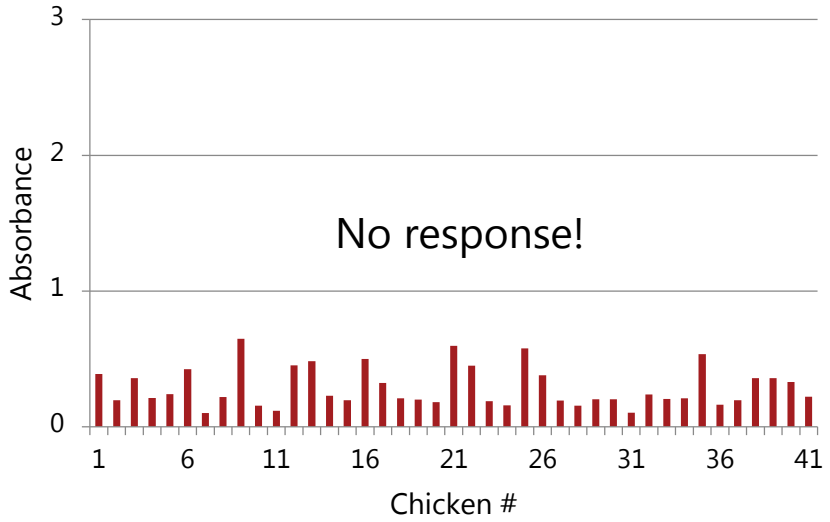


The best laid plans of mice and men…

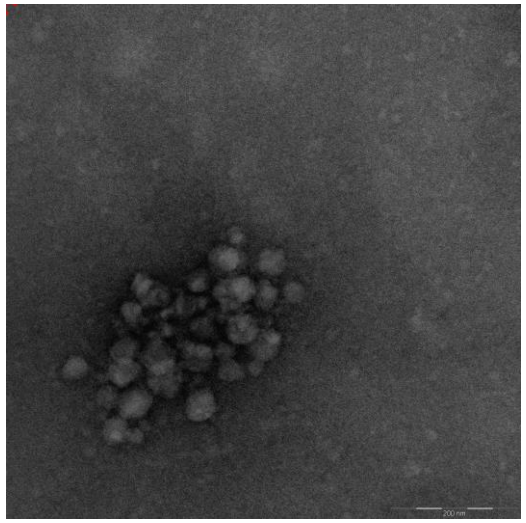
- Our vaccine is not giving an immune response
- Our immunogen seems conjugate with the vaccine – but not the virus particles!
- The composition of the vaccine is unknown and the manufacturers are not taking our calls
- What is wrong?

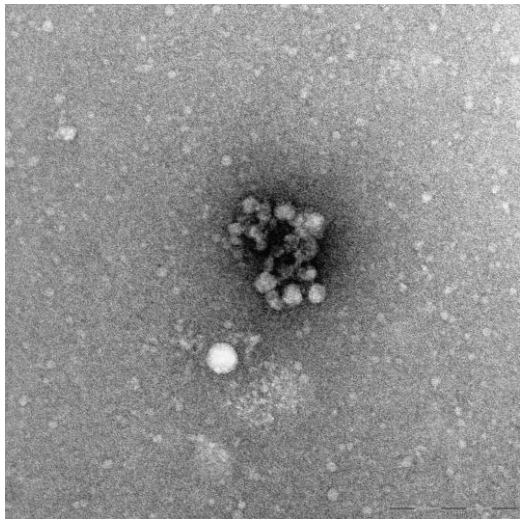


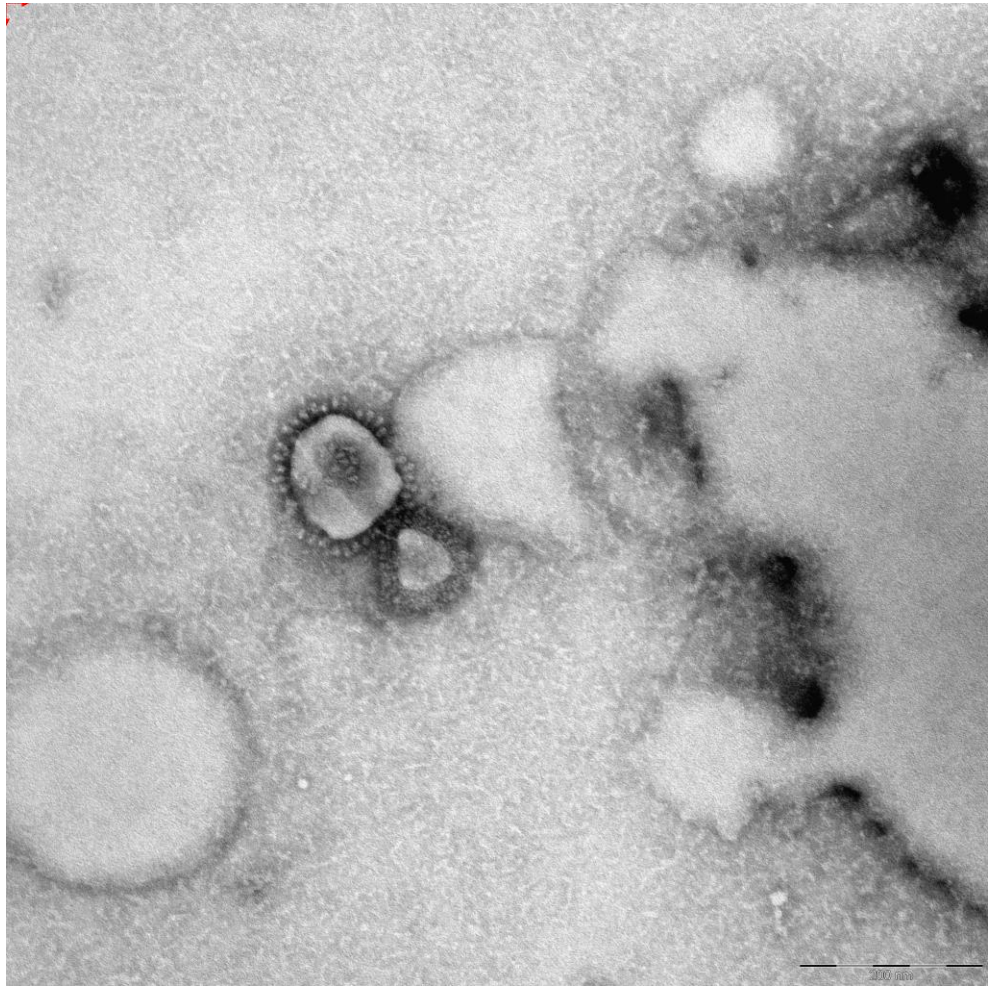


Electron microscopy to the rescue!

- With the help of the Core Facility for Integrated Microscopy (CFIM) we were able to image our vaccine
- We even found virus particles…
- … but only in miniscule amounts

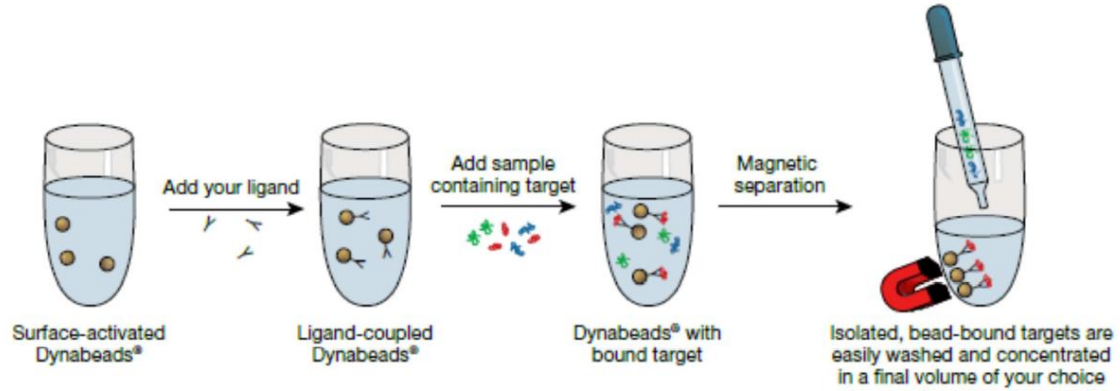






How to proceed?

- To test the original hypothesis we need to utilize a virus that infects through the airways
- The only consistently utilized aerosol vaccine in Europe is IB 4-91
- Even if we purify the virus from the vaccine solution we cannot obtain enough for conjugations and vaccinations



We developed a method for isolating the virus from the solution using antibody-coated magnetic beads.

However, the method can only be used to obtain small amounts of virus.

Conclusions

- We have developed all the techniques needed to combine vaccinations and immunizations…
- … but to test the hypothesis we would need the collaboration of a commercial vaccine manufacturer…

- … in the meanwhile: Our original vision still lives!

A big thank you to the Danish 3R Center for supporting our eccentric idea!

And to my collaborators and the animal caretakers at the Department of Experimental Medicine