The Future Vaccine Manufacturing Hub: Tools and technologies

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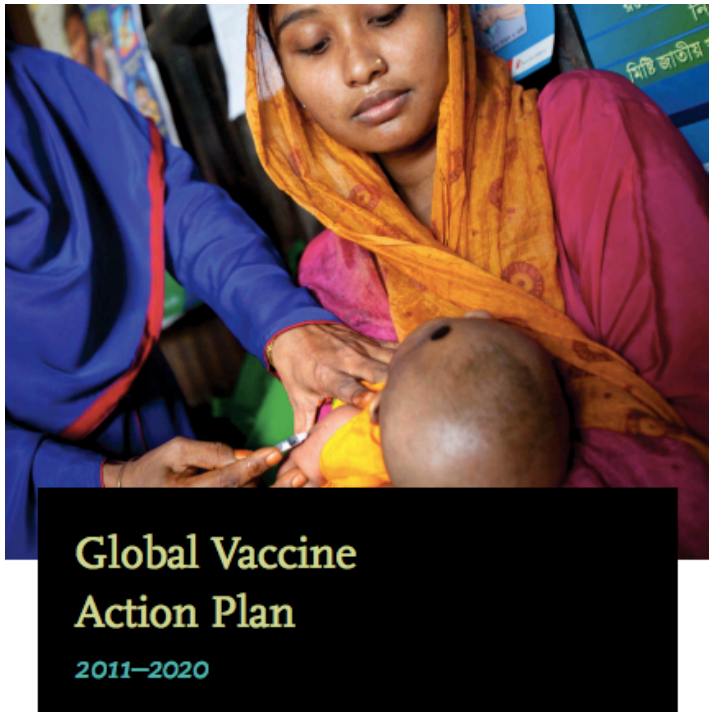
Presentation Outline

- Introduction to Vax-Hub, platform funding and Grand Challenge research overview
- Vaccine technologies
  - Adenovirus manufacturing platform process
  - VLP vaccines: Quality by design
  - Novel glycoconjugate vaccines technologies
- Next steps

The Global Vaccine Action Plan (GVAP)

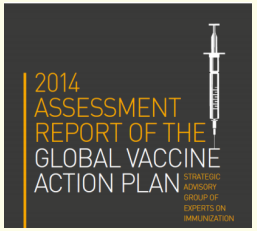


In 2012, the World Health Assembly, representing 194 countries, endorsed the GVAP to ensure that no one missed out on a vital immunisation by 2020.

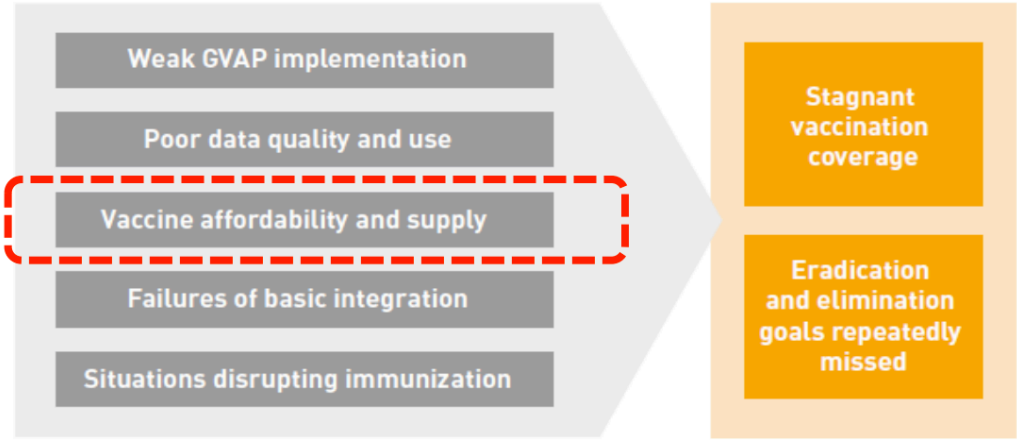
To date, progress towards the GVAP targets is off track. In 2015, more than 19 million children missed out on basic immunisations.

World Health Organization

The manufacturing landscape – supply is failing demand



Vaccine affordability and supply is one of the key priorities identified by the WHO Global Action Plan



...but the most pressing need is to get them to everybody

Vaccines' future is exciting, but the biggest need is in the present. According to the most recent WHO estimate, 1.5 million children die every year of diseases that could be readily prevented by vaccines that already exist[2]. This represents gross inequity. A small proportion of the

Hub Vision and Aim

To advance technologies that will ensure future, uninterrupted supply.

To ensure that these advances translate to LMIC markets and manufacturers.

Ability to support and respond to epidemic threats.

The Hub supports an ambitious programme of innovative research related to the challenges of developing, scaling-up and manufacturing vaccines of benefit to low and middle income countries.

Hub-Spoke model

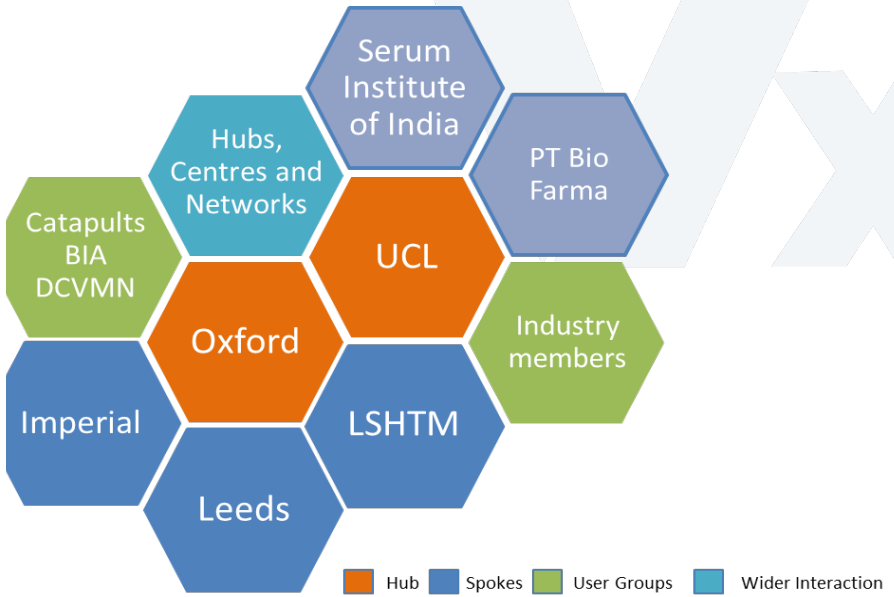
Hub Directors: Professors Sarah Gilbert and Martina Micheletti
£7M, 3 years (April 2018-March 2021)

Two Hubs:

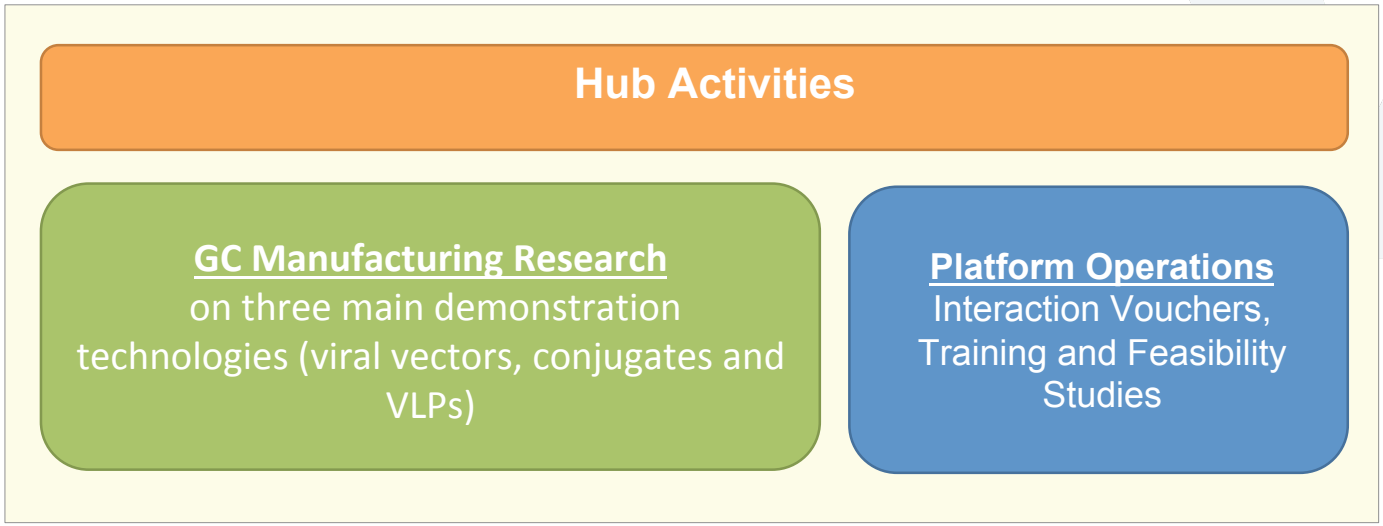
- UCL Biochemical Engineering
- The Jenner Institute, University of Oxford

Three UK Spokes:

- Imperial College London
- University of Leeds
- London School of Hygiene and Tropical medicine



Hub activities



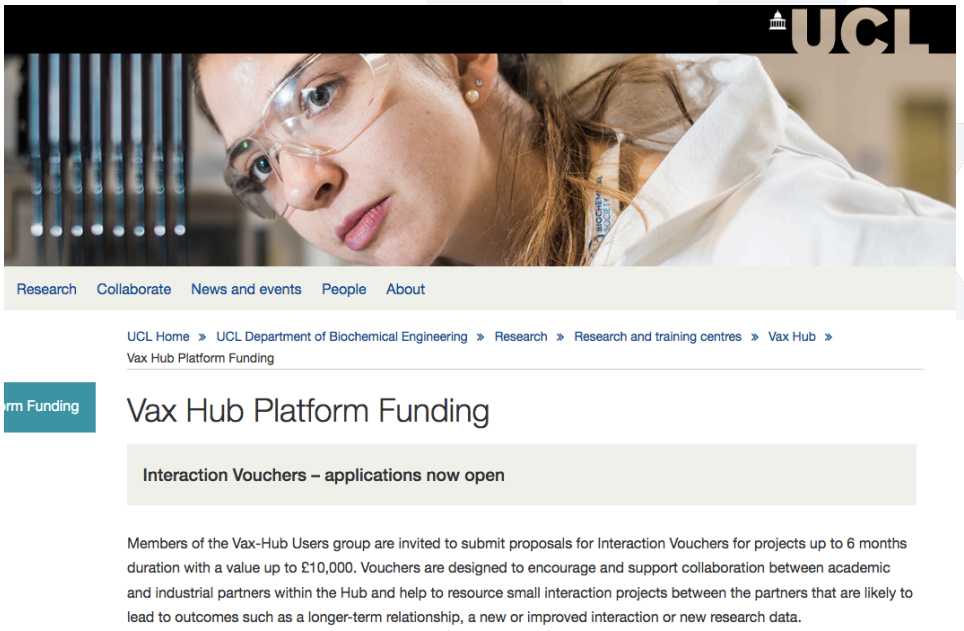
Platform Operations

Interaction vouchers Call

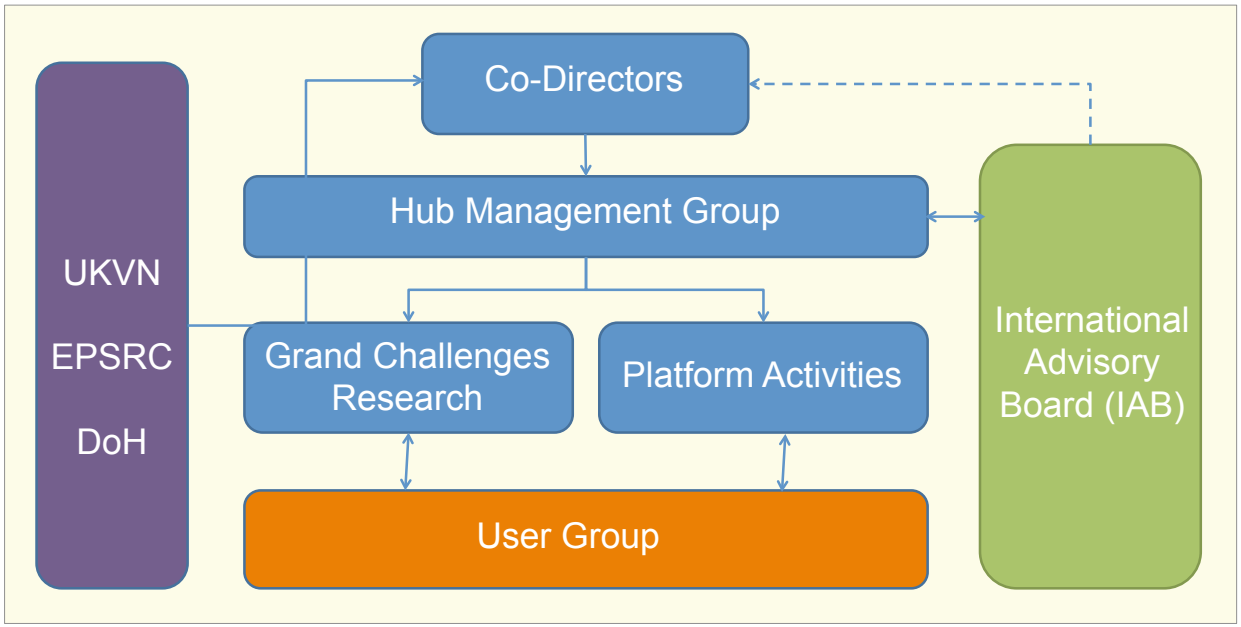
8 vouchers in total, budget of up to £10K per voucher (< 6 months duration)
Available to Users group members only
Must bring together any two organizations (including academia-industry partnerships)

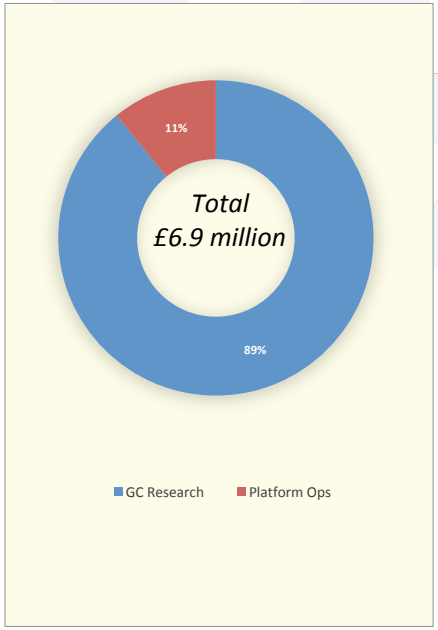
Feasibility projects Call (early 2020)

Minimum of 6 projects, £100K each (< 12 months duration)

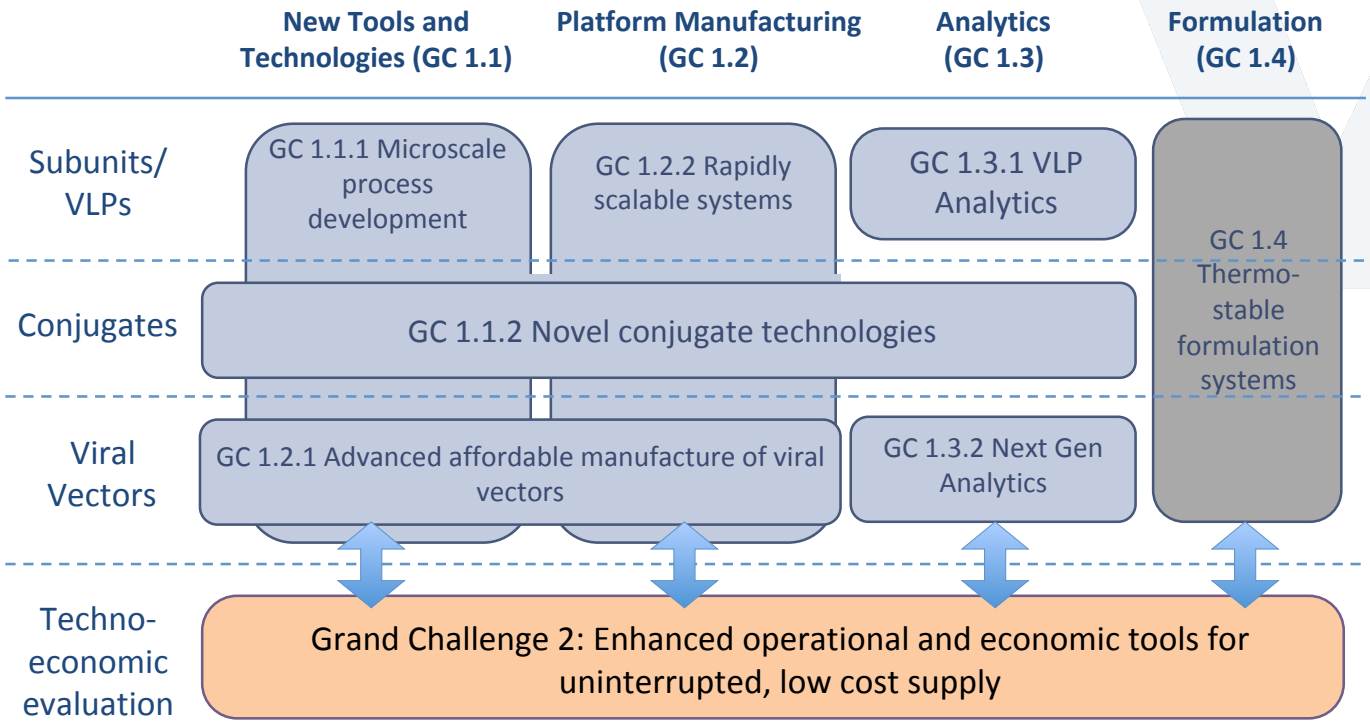


Management Structure

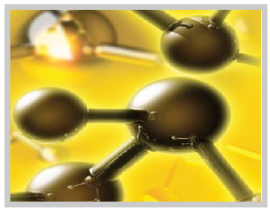




Grand Challenge Research Overview

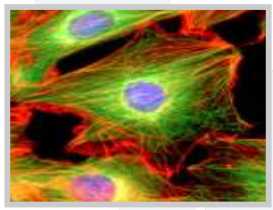


Hub Expertise - UCL



Industrial Biotechnology
e.g. chemicals and pharmaceuticals

Macromolecular Medicines
e.g. antibodies and vaccines



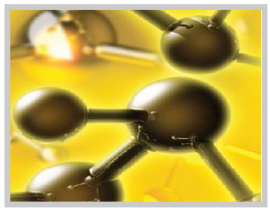
Cell and Gene Therapies
e.g. cells and engineered tissues

High Throughput Bioprocess Design and Scale-up

Synthetic Biology (Protein, Vector and Cell Engineering)

Decisional Tools (Data Mining, Process and Economic Modelling, LCA)

Hub Expertise - UCL



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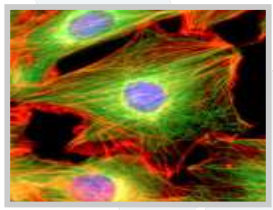


Cell and Gene Therapies
e.g. cells and engineered tissues

High Throughput Bioprocess Design and Scale-up

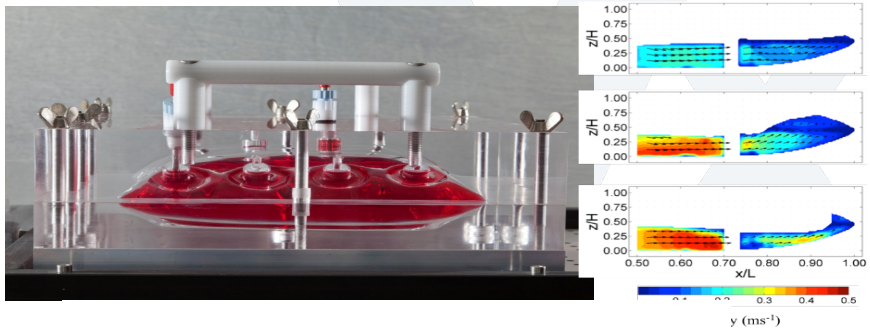
Synthetic Biology (Protein, Vector and Cell Engineering)

Decisional Tools (Data Mining, Process and Economic Modelling, LCA)

Tools for bioprocess design and scaling

Experimental study of flow dynamics, mixing and suspension dynamics

- Laboratory scale bioreactors (rocked, shaken and stirred)
- Single-use and conventional technologies (Ambr250, Sartorius Cultibag, Millipore CellReady, DasBox)
- Impact of environment for different cell types and products
- Robust scaling equations and methodologies



Miniaturisation and development of Scale-Down Tools

- Quasi-perfusion microscale methodologies
- 250 ml perfusion bioreactor
- Microscale tangential flow filtration device
- Integration of mimics within automated platforms to speed up bioprocess development

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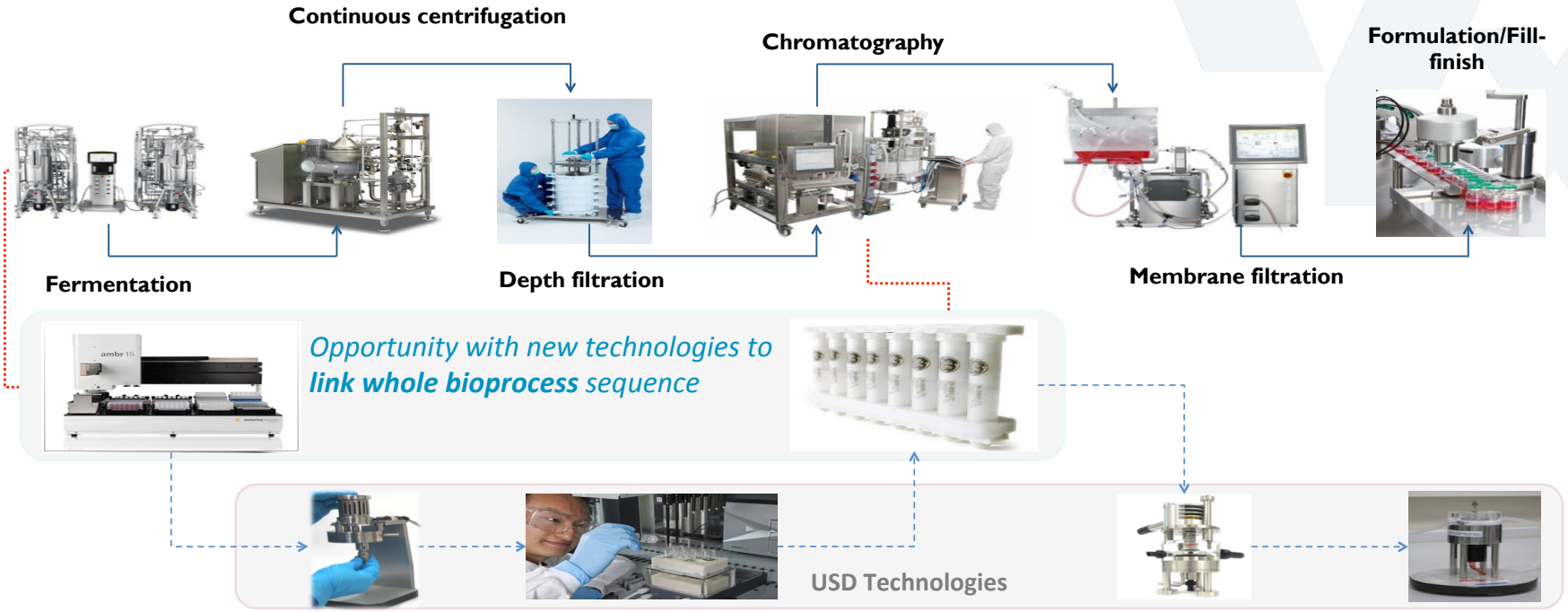
Ultra Scale-Down (USD) Technologies: Manufacturing insight in the lab



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Hub Expertise – University of Oxford

The Jenner Institute

Unique mission to develop innovative vaccines against major global disease and focus on translational research (rapid early stage development and assessment of new vaccines in clinical trials)

Clinical Biomanufacturing Facility (CBF)

The University of Oxford GMP facility – MHRA Authorisation for viral vectored vaccines and ATMPs – providing a link between academic research and clinical drug development

Design and testing of viral vectored and VLP vaccines
Transition from research to GMP
GMP manufacture of viral vectors, VLP and recombinant proteins
Assay development for release and in-process testing
Formulation of drug product
Clinical vaccine development (UK and overseas)

Adenovirus Manufacturing Platform

Process requirements for Phase I

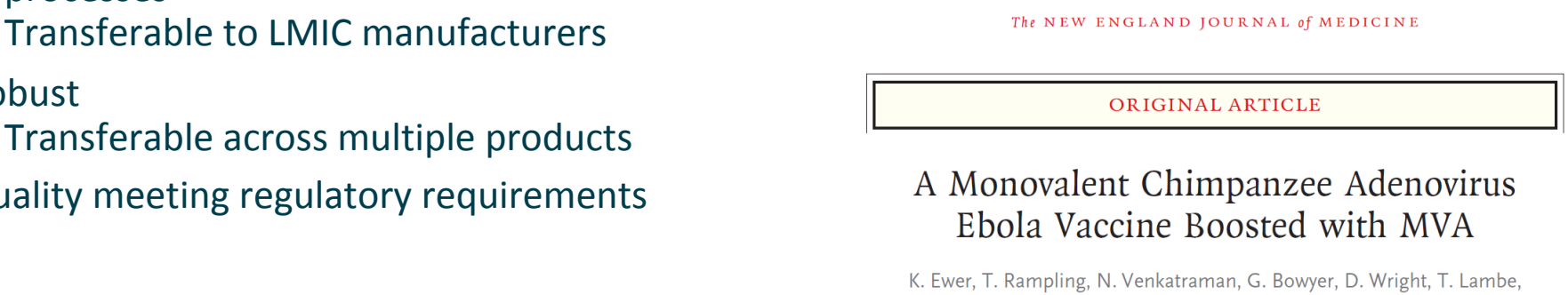
Small
- ≥100 doses

Simple
- Limited staff, one team makes all products
- Limited capital equipment
- Limited capacity to validate new equipment / processes
- Transferable to LMIC manufacturers

Robust
- Transferable across multiple products

Quality meeting regulatory requirements

An effective adenovirus manufacturing approach is likely to be applicable to vaccines against multiple pathogens:
- Emerging outbreak pathogens
- Veterinary
- Antibody & T cells


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA

K. Ewer, T. Rampling, N. Venkatraman, G. Bowyer, D. Wright, T. Lambe,

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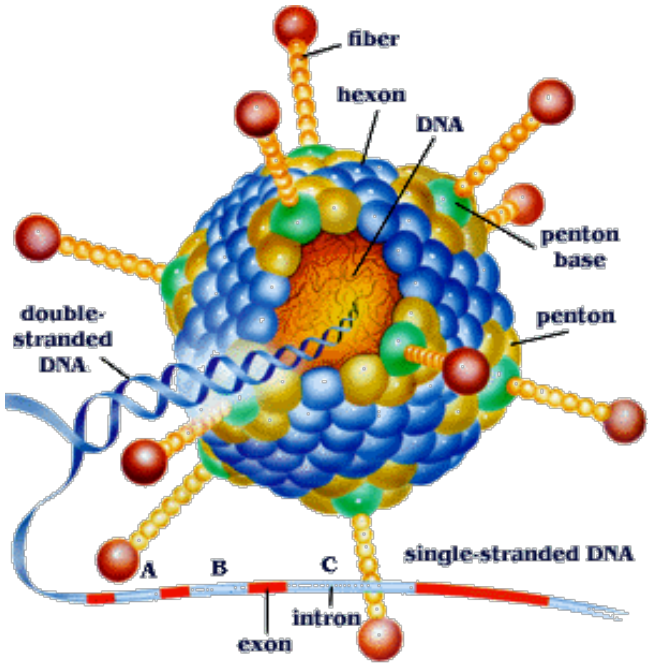
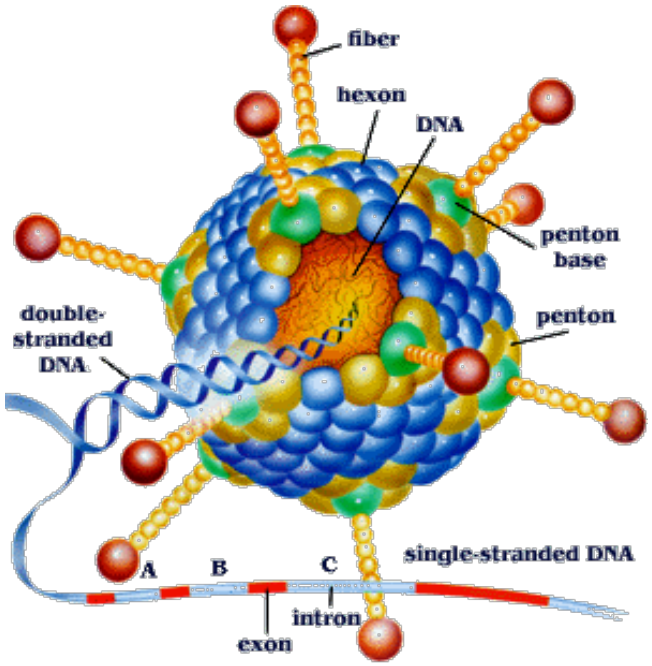
Adenovirus biology



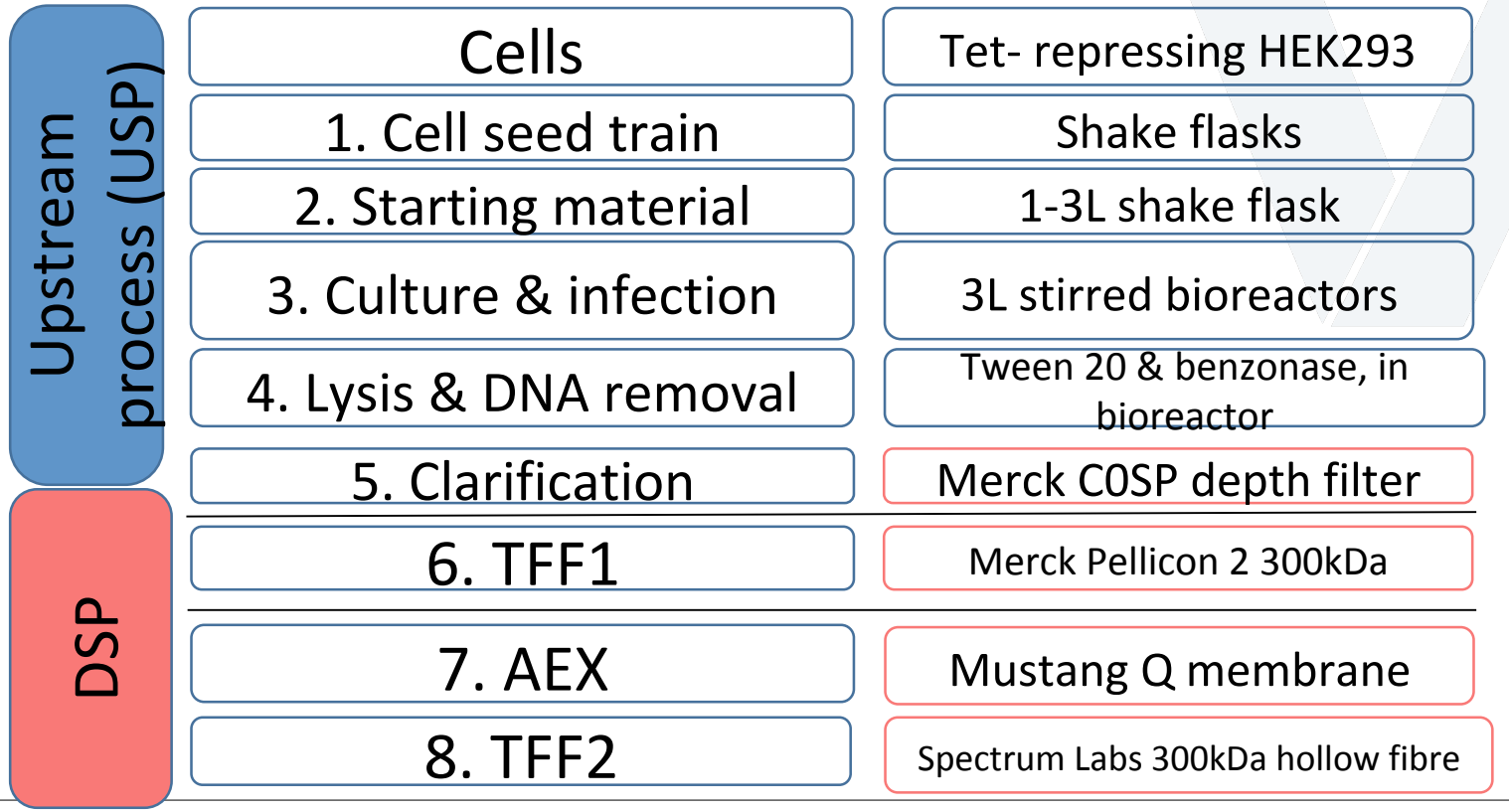
- Non-enveloped dsDNA virus, 90nm
- Non-replicating due to E1 (and E3) gene deletion
  - HEK293 or PERC6 cells supply E1 in *trans*
- Antigen-encoding transgene under strong constitutive mammalian promoter
  - Antigen is not a structural part of the virion → vaccines using a single Ad serotype are structurally the same, regardless of Ag
  - Antigen **is** expressed in culture: can alter growth characteristics, selection pressure for genetic instability

Chimpanzee adenovirus vectors (‘ChAds’)

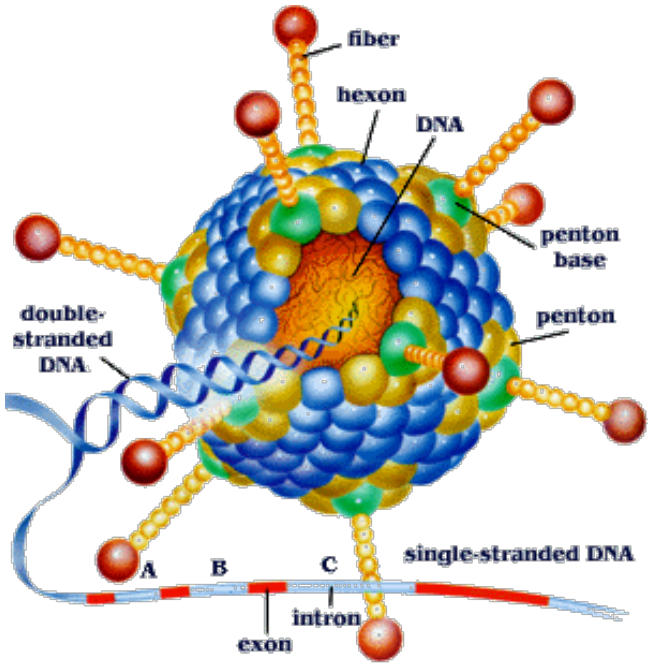


- Minimal pre-existing anti-vector immunity in human population
- Multiple serotypes
  - Different hexon / fiber capsid proteins
- Issue of compatibility with HEK293 Ad5-derived E1:
  - Manufacturing can be enhanced by non-structural gene manipulation

Small scale adenovirus production process



Vaccines used for ‘test cases’



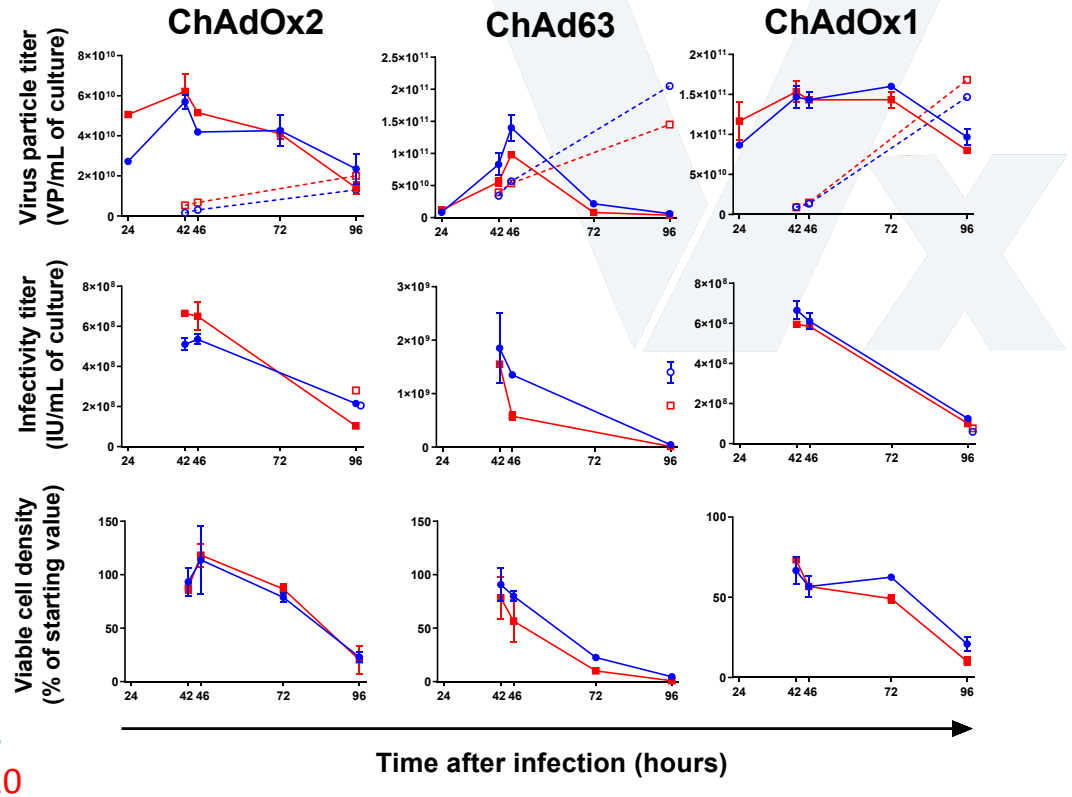
ChAdOx2 RabG (rabies vaccine)

ChAdOx1 RVFV GnGc (Rift Valley Fever vaccine)

ChAd63 ME-TRAP (malaria vaccine)

Vaccines used for ‘test cases’

In Ag-repressing HEKs, infection at MOI 3, harvest at ~42h gives good yields of all three viruses



Fedosyuk et al, Vaccine 2019

MOI 3
MOI 10

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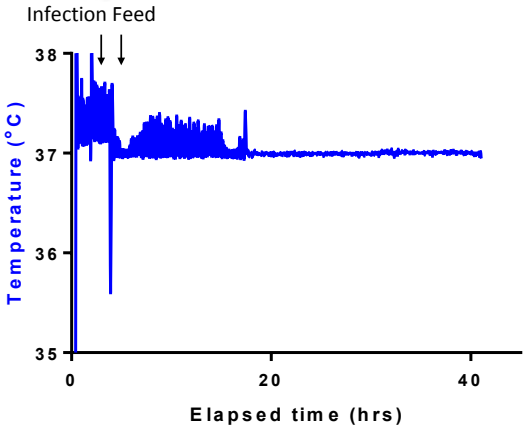
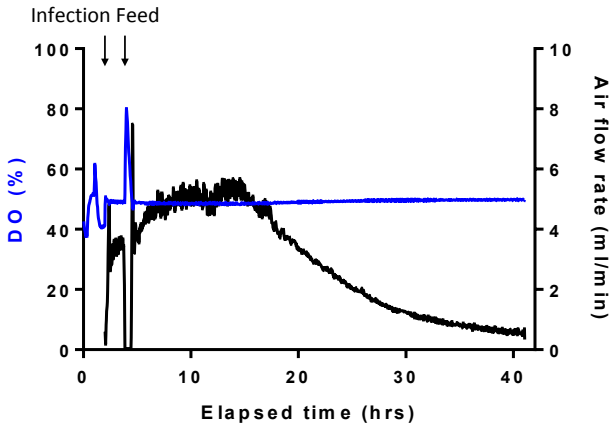
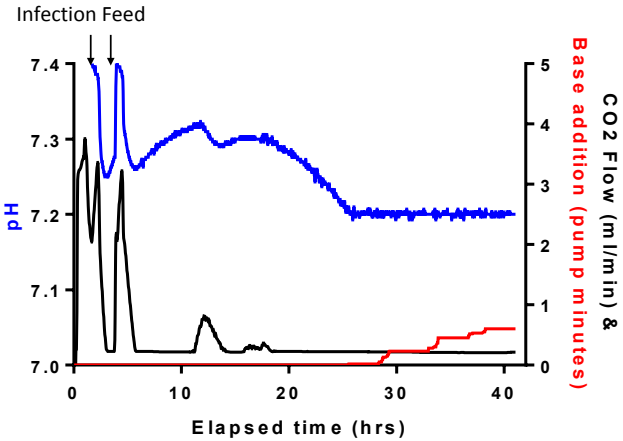
3L stirred tank bioreactor

- Good results with two different vessels
  - Yield c. 1x10^5 VP per cell
- Simple <48hr batch process
  - Cell expansion in shake flasks

Fedosyuk et al, Vaccine, 2019







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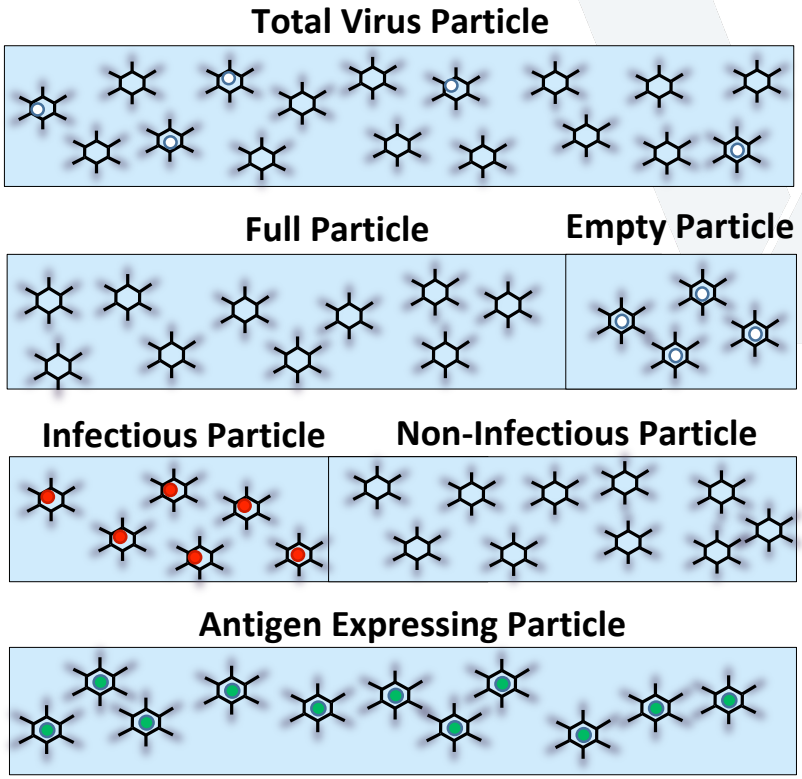
Separation of adenoviral product variant

Total Virus Particles
Lowry Protein Assay
Dynamic Light Scattering
SPR
UV Measurement

Full Particles
Genome Quantitation Assay (GQA)
Reverse-Phase HPLC Assay
CsCl Gradient Analysis
(% full)UV Absorbance Assay (UV-SDS)

Infectious Particles
TCID50
Q-PCR Based Potency Assay

Antigen Expressing Particles
Western blot
In vitro Antigen Expression Assay

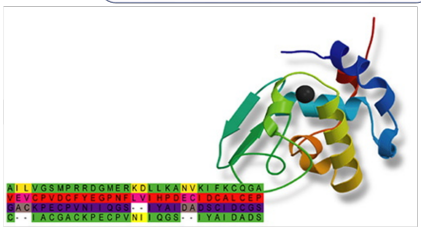


VLP Vaccines: Quality by Design

VLP Vaccines: Understanding VLP assembly and quality attributes

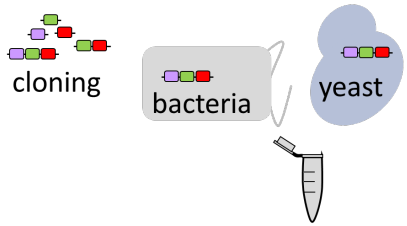
Design and Production of high quality Foot-and-Mouth Disease (FMD) virus-like particles (VLP)

I. *In silico* Analysis



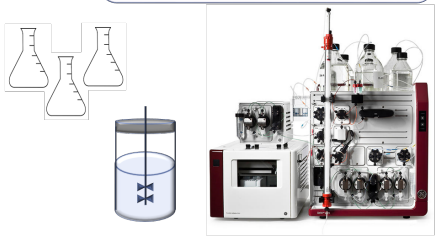
structural information

II. Design & Expression



Cell free

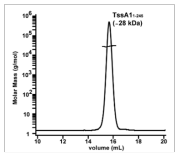
III. Production



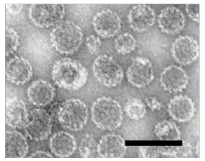
Scale up

purification

IV. VLPs Purity & Quality



biophysical methods



electron microscopy

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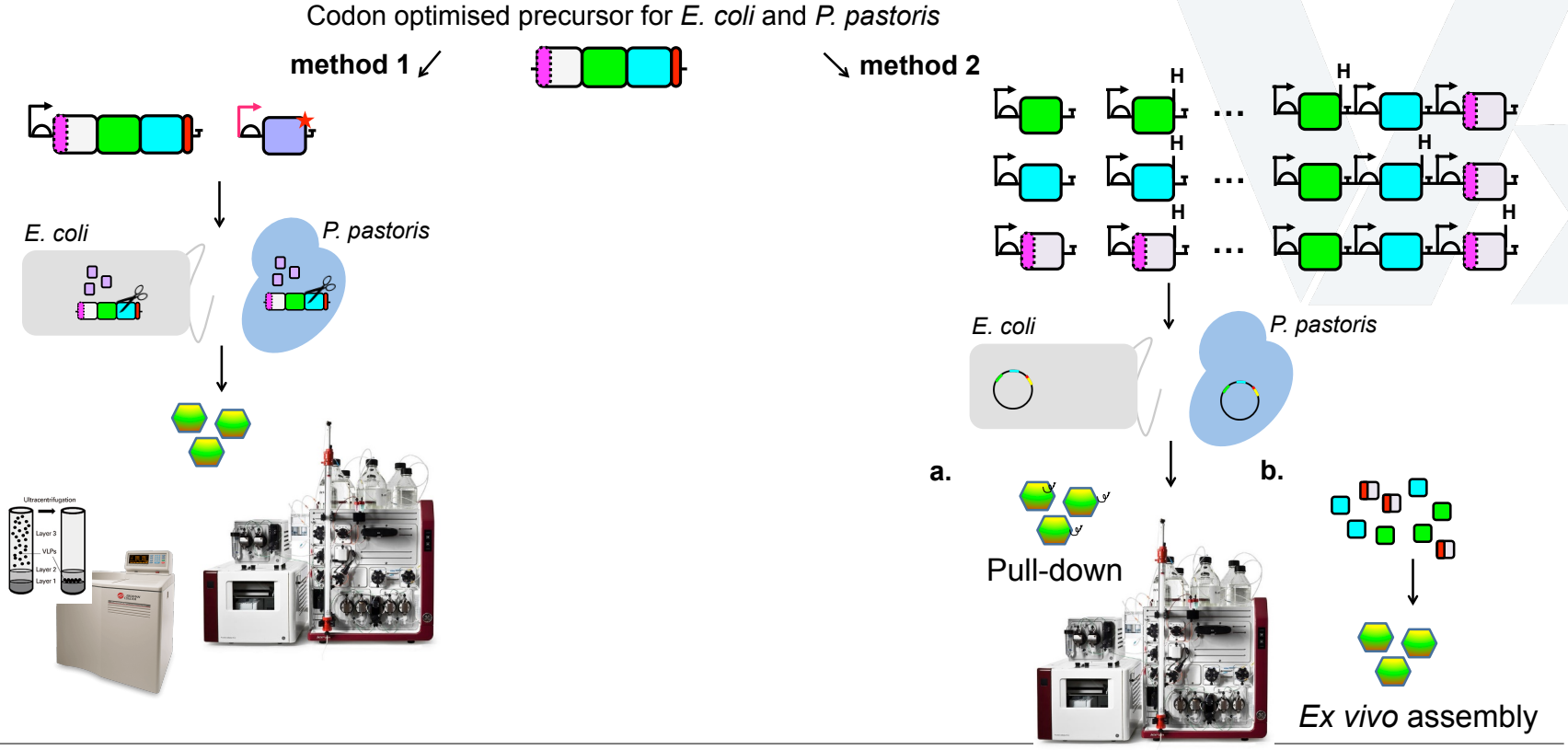
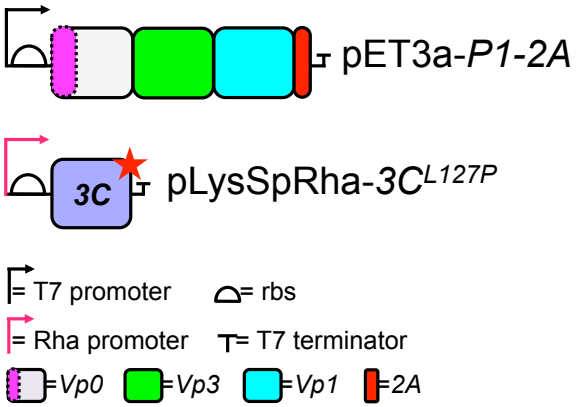
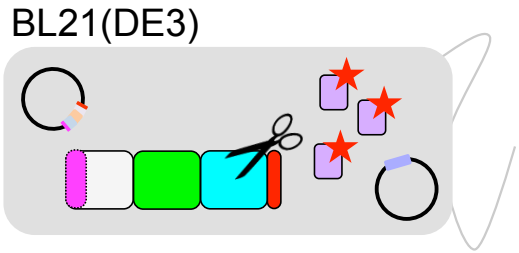
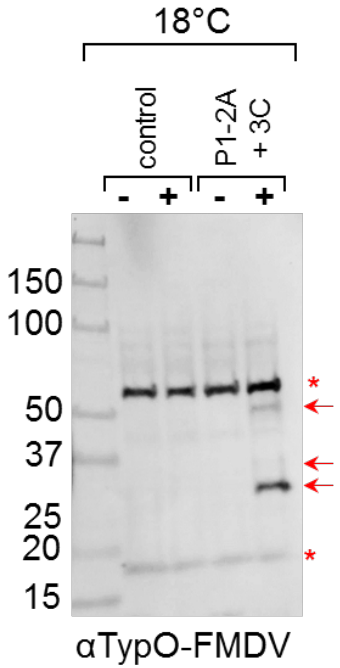
VLP Vaccines: Design (I)

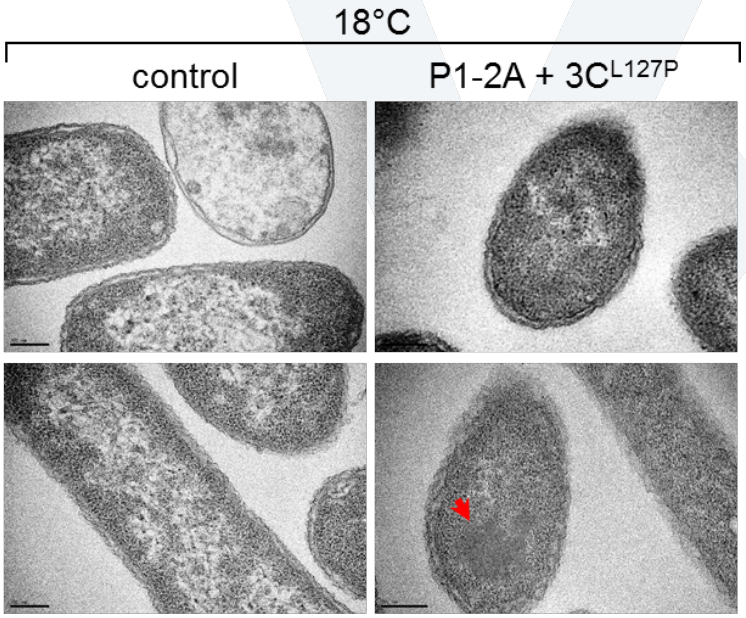


VLP Vaccines: Cloning/Expression (II)



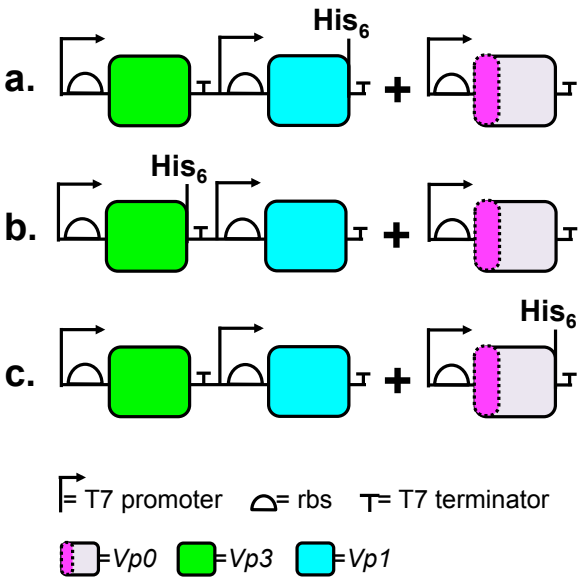


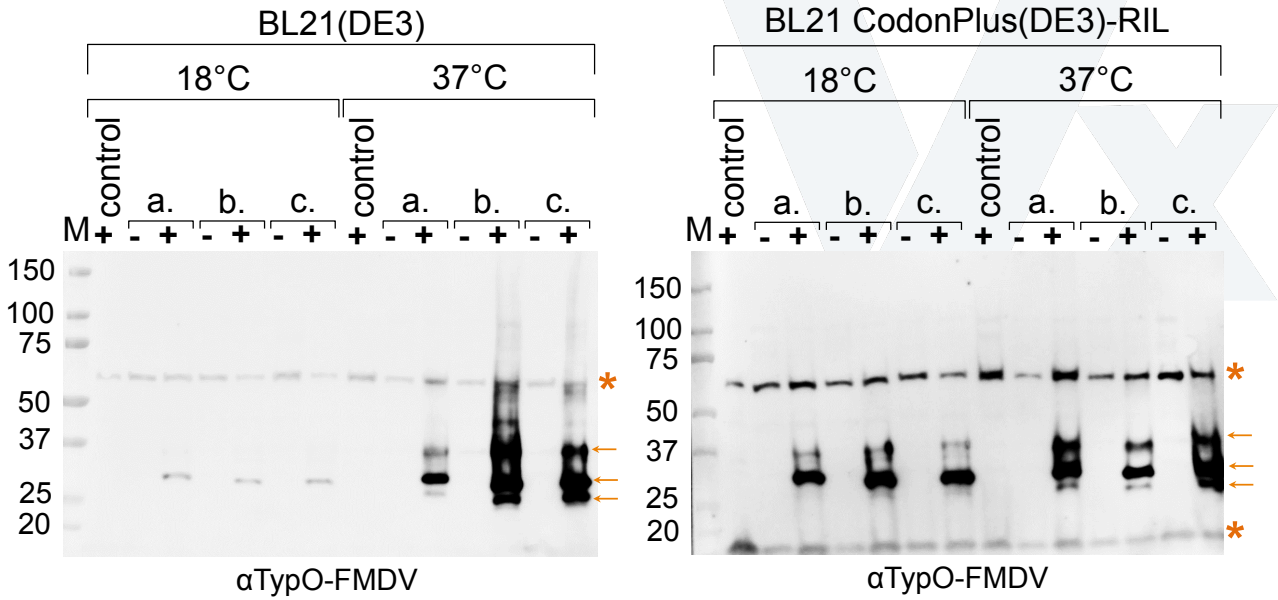




* non-specific binding

VLP Vaccines: Cloning/Expression (II)

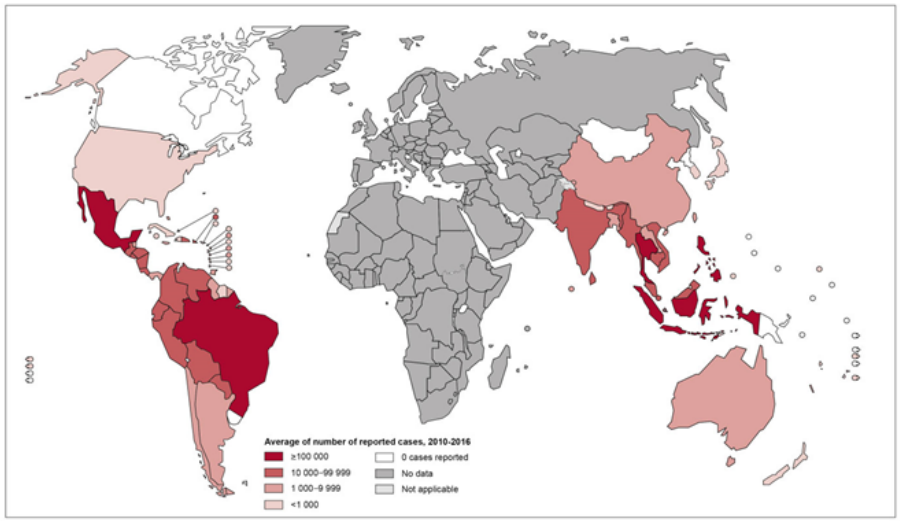




$Vp1_{his6}$~ 25 kDa
$Vp3_{his6}$ ~ 27 kDa
$Vp0_{his6}$ ~ 35 kDa

* non-specific binding

VLP Vaccines: Platform Approaches for Dengue vaccine



390 millions infections per year
Asymptomatic to severe acute febrile disease

- Control measures targeting mosquito vectors have very limited effectiveness
- Vaccination is an important part of an integrated dengue prevention and control strategy
- One licensed dengue, Dengvaxia® (CYD-TDV) and only effective in seropositive individuals

↓

Development of a cost-effective platform for the delivery of a multicompetent Dengue vaccine

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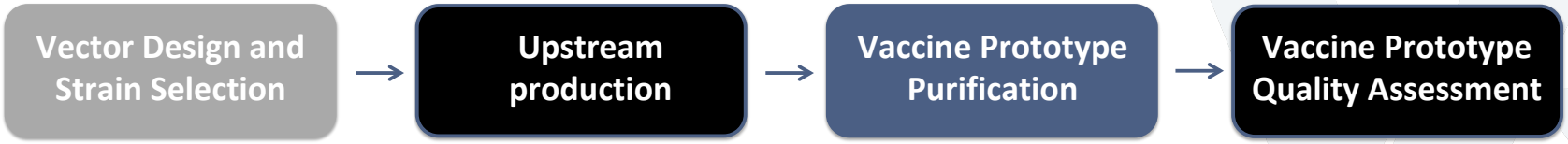
Imperial College London
LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
UNIVERSITY OF LEEDS
EPSRC Engineering and Physical Sciences Research Council
Department of Health & Social Care
UKaid

University College London
The Jenner Institute
London School of Hygiene & Tropical Medicine
Imperial College London
University of Leeds

Proposed Platform



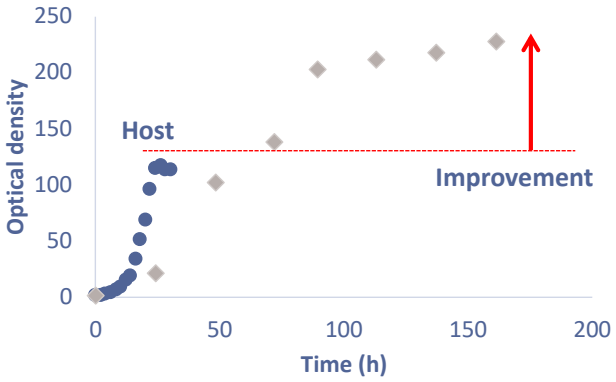
To be performed at Biofarma (Indonesia)

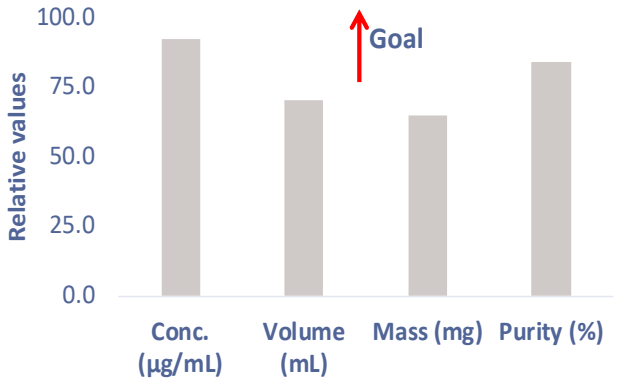
Development of a representative upstream dengue vaccine, VLP form, production platform in *Pichia Pastoris*

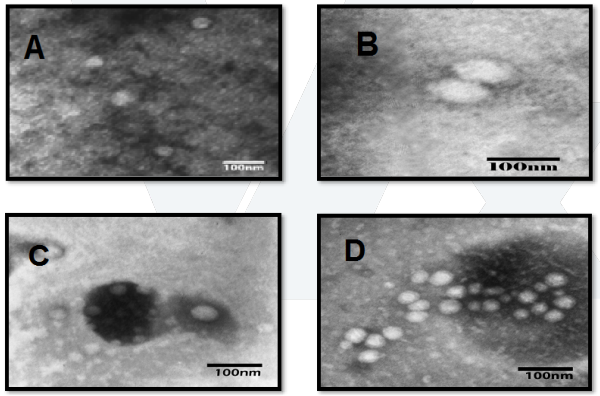
- Studying optimal strategy in medium, feeding, induction technologies and VLP effective recovery
- Development of robust analytics for product characterisation and quality evaluation

Development of a strategy for scale-up and technology transfer

Preliminary results







Growth profile of *Pichia pastoris* expressing the dengue vaccine vector (DENV prM/E).

Significant improvements were achieved in biomass yield by fermentation protocol optimisation. However, **higher densities are desired to achieve desired titers.**

Purification and preliminary characterization of recombinant prM/E dengue serotype using anion-exchange chromatography (AEC) and hydrophobic interaction chromatography (HIC).

Virus-Like Particle (VLP) prM/E dengue serotype 1 (A),2 (B),3 (C) and 4 (D) using transmission electron microscopy (TEM).

The particle diameters varied between 29-35nm depending on the serotype and are smaller than the natural virus (40-50nm).

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Novel Glycoconjugates Technologies

Relevance of Glycoconjugate Vaccines

Glycoconjugates favour T-cells dependent response (memory)

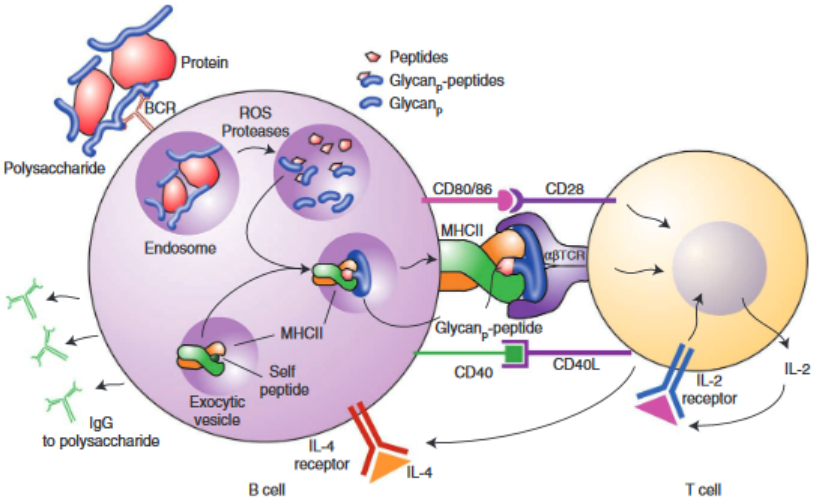
Polysaccharides-based vaccines (Glycan only)

↓

T cells independent immune response

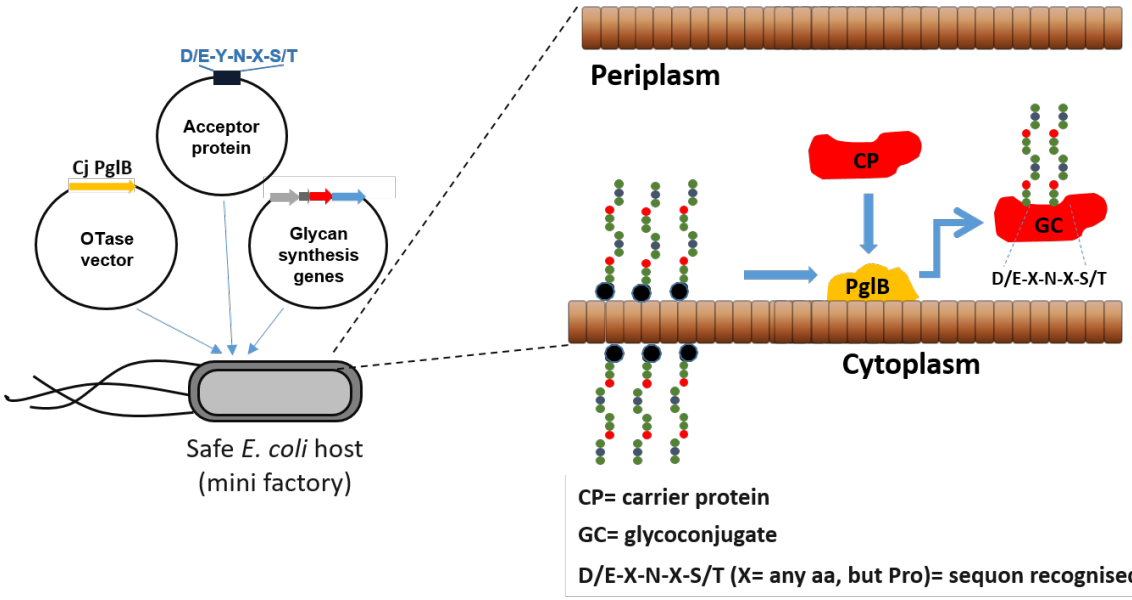
Examples of successful human glycoconjugate:

1. H*aemophilus influenzae*
2. N*eisseria meningitidis* (except type B)
3. S*treptococcus pneumoniae* (some serotypes)



Berti and Adamo *Chem Soc. Rev.* 2018

Protein Glycan Coupling Technology (PGCT) for low cost glycoconjugate vaccines



CP= carrier protein

GC= glycoconjugate

D/E-X-N-X-S/T (X= any aa, but Pro)= sequon recognised by the OTase PglB

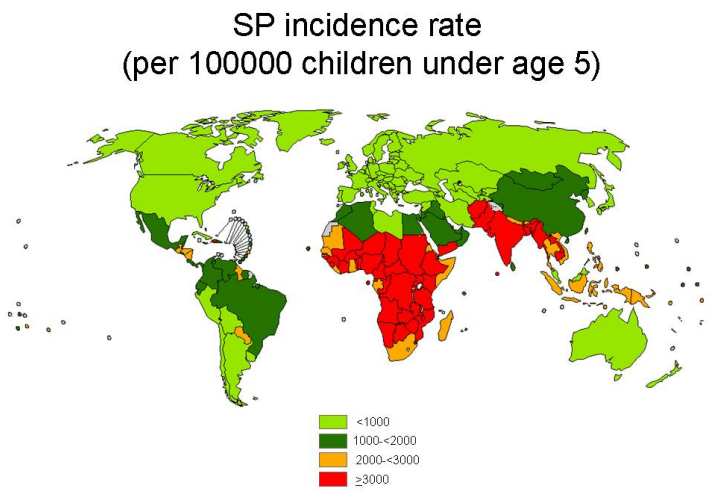
- PGCT is cheap, safe and flexible in design
- It can be applied to improve existing vaccines (pneumococcol)
- or develop new ones (*Francisella*)
- Or enter new markets (veterinary)

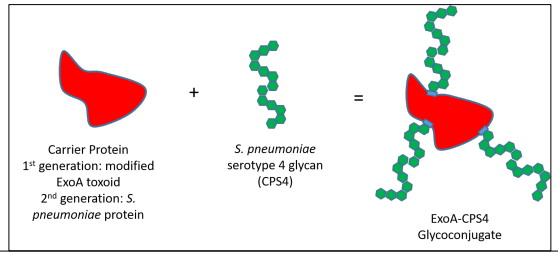
PGCT review: Kay, Cuccui and Wren, npj Vaccines 2019

Development of a vaccine against *S. pneumonia*

Streptococcus pneumoniae:

- Gram +, alpha-haemolytic diplococcus, commensal and respiratory pathogen
- **Over 95** different **serotypes**
- Causes **pneumonia, meningitis**, conjunctivitis, bacteraemia and otitis media
- Estimated that globally **0.5 million children under five die** of pneumococcal disease **each year** (mostly in developing countries)
- **Efficacious vaccines** (eg. PCV13) are **available, but expensive** and often unaffordable for developing countries





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Strategies for glycoconjugate vaccine production

Biological conjugation

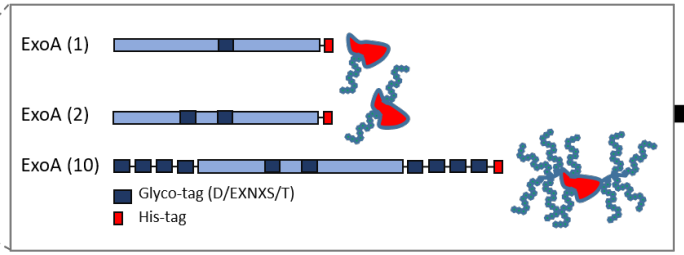
- Engineered **safe** laboratory ***E. coli*** strain
- **Single purification step** of the glycoconjugate
- **Homogeneous** prep
- Manufacturing expected to be **cheaper and less time consuming**

Chemical or enzymatic conjugation

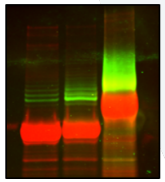
- Requires separate purification of the glycan and carrier protein
- Strain used for glycan purification may be **unsafe**
- **Multi-step heterogeneous** preparation
- **Expensive and time consuming**

Design and ongoing work

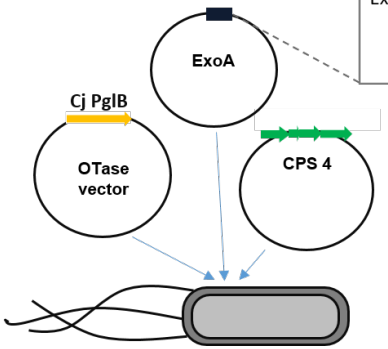
1. Test the most efficacious glycoconjugate in a preclinical trial
(Collaboration with Jerry Brown Lab, UCL)



PGCT-produced glycoconjugates

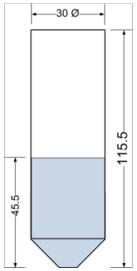


Glycan (CPS4)
Protein



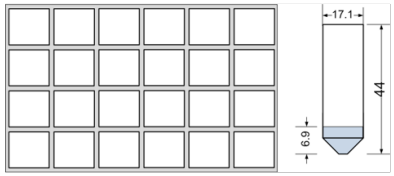
Novel *E. coli* glycoengineering strains with detoxified endotoxin A

2. Downscale to upscale most:
screening variables at a small scale to identify the best parameters to scale up
(Collaboration with Jasmin Samaras, Micheletti Lab UCL)



24-DSW Microwells

Scale down robotic platform



Scale up

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Vaccines in development

Kay, Cuccui and Wren, npj Vaccines review 2019

Table 1. Current glycoconjugate vaccines is developed using PGCT

Organism	Glycan	Protein carrier	Status	Manufacturer	References
Streptococcus pneumoniae	Capsule-multivalent	rEPA	Phase I clinical trials	Limmatech Biologics	NCT03303976[a]
Streptococcus pneumoniae	Capsule-serotype 4	piuA	Development	Academic- UCL/LSHTM UK	Reglinski et al.[36]
Staphylococcus aureus	Capsule-Type 5 and 8	rEPA	Development	GlycoVaxyn	Wacker et al.[28]
Shigella dysenteriae	Capsule-Type 1	rEPA	Phase I clinical trials	Limmatech Biologics	Hatz et al.[71]
Shigella flexneri	Capsule- 2a	rEPA	Phase I clinical trials	Limmatech Biologics	Riddle et al.[72]
Escherichia coli	O-antigen-ExPEC serotypes 01, 02, 06, 025	rEPA	Phase Ib clinical trials	Limmatech Biologics/ J&J	Huttner et al.[79]
Francisella tularensis	O-antigen	rEPA	Development	Government/ Academic -DSTL/ LSHTM UK	Marshall et al.[31]
Burkholderia pseudomallei	O-PSII	AcrA	Development	Government/ Academic- DRDC/ University of Alberta Canada	Garcia-Quintanilla et al.[42]

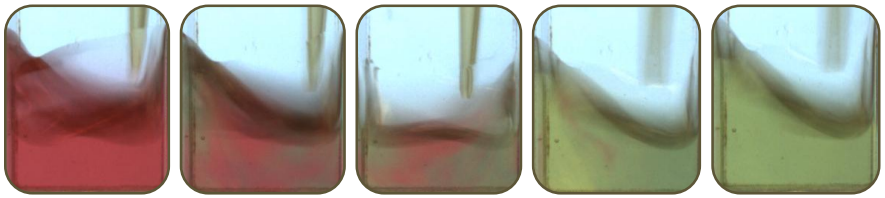
PGCT-adapted from Micoli[1]
[a]ClinicalTrials.gov Identifier

Automated Microscale Process development

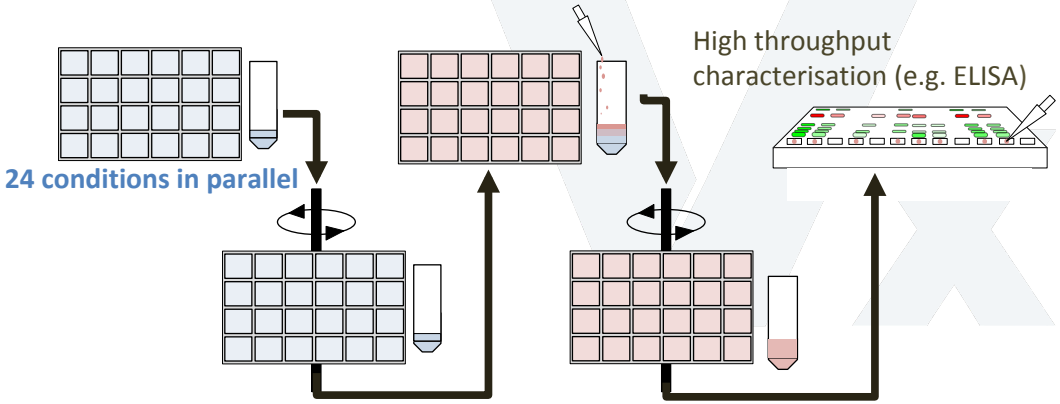
AIM: To scale original 20 mL culture to 2 mL

Operating conditions at the microscale based upon engineering fundamentals.

- Scaling based upon matching mixing characteristics at both scales



- Scaling based upon matching the oxygen transfer coefficient, $k_L a$



Conditions for screening:

- Media composition
- DO, pH, temperature
- Harvest times/culture duration
- *E.coli* strain
- Carrier protein and enzyme alternatives

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Next steps

Benefits for Hub Users

Driving the research agenda

Access to internationally-leading academics and top researchers with expertise in process development, vaccinology, analytical development, GMP manufacturing and decisional tools
Ability to steer the research agenda over the next 2 years, aligned to your organization's priorities and the hub vision and remit

Access to funding, outputs and skillset

Early access to Hub outputs (new methodologies and technologies) via the Collaboration Agreement
Participation in vouchers or feasibility studies to evaluate Hub outputs using your systems and processes
Leverage funding for greater impact via industry-led Innovate UK projects
Opportunity for wider collaboration via the Engineering Doctorate (EngD) studentships
Access to highly skilled graduating doctorate and researchers

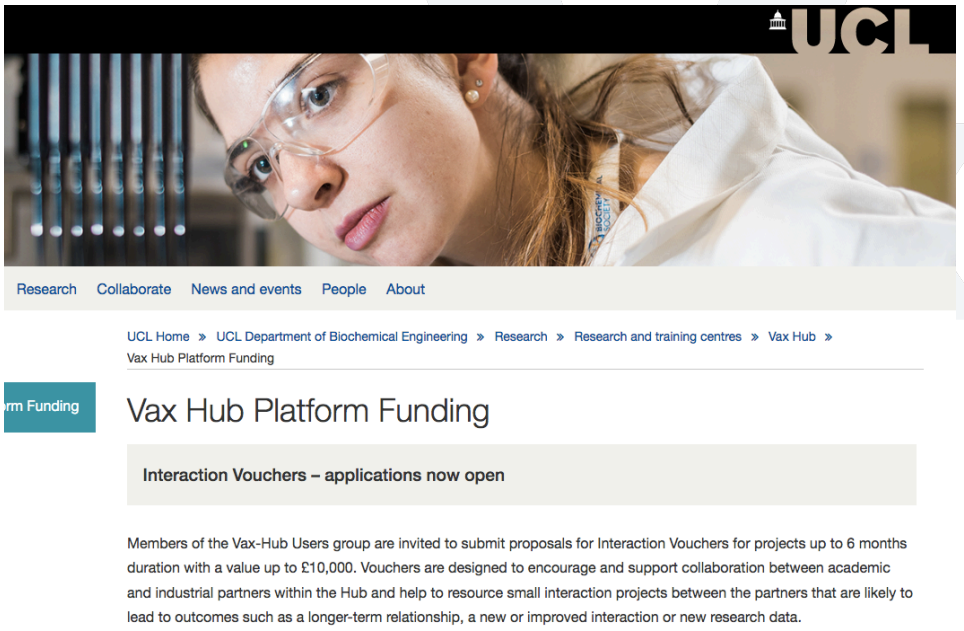
Platform Operations

Interaction vouchers Call

8 vouchers in total, budget of up to £10K per voucher (< 6 months duration)
Available to Users group members only
Must bring together any two organizations (including academia-industry partnerships)

Feasibility projects Call (early 2020)

Minimum of 6 projects, £100K each (< 12 months duration)



Upcoming events

Vax-Hub Users Group meeting – 8th November

Vouchers Interaction Submission deadline – 4th November

For more information and news

Biochemical Engineering Department Website

@VaxHub

For how to become a member

Please contact Dr Nav Gill (n.gill@ucl.ac.uk)

Acknowledgements/ Thank you

Adenovirus platform manufacturing

Dr Sandy Douglas

Professor Sarah Gilbert

Dr Fatemeh Vahid Dastjerdi

VLP Vaccines

Dr Sara Placemente, Dr Steffi Frank

Dr Salome De Sa Malaghaes, Prof Eli Keshavarz-Moore

Glycoconjugates

Dr Marta Mauri, Prof Brendan Wren

Dr Jasmin Samaras

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