Adenovirus Manufacturing Platform

Hub Vision and Aim

To advance technologies that will ensure future, uninterrupted vaccine 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

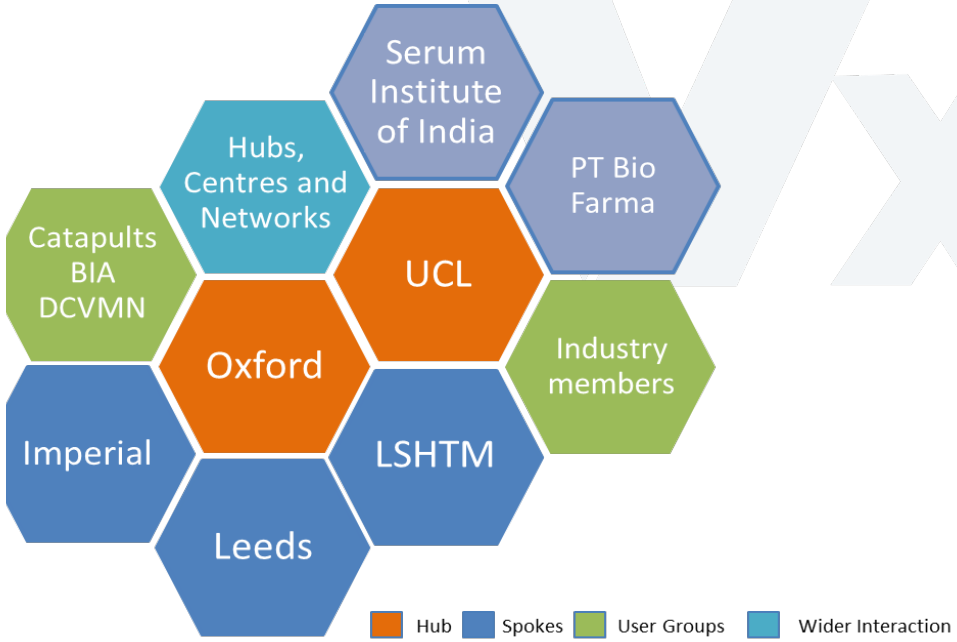
Hub Directors: Professors Sarah Gilbert and Martina Micheletti
£7M, 3 years (April 2018-March 2021)

Two Hubs:

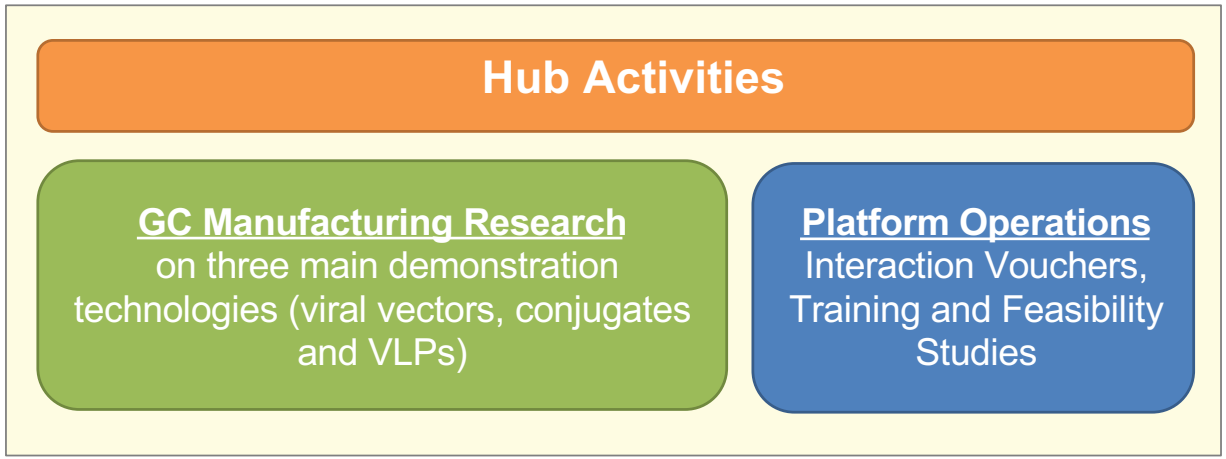
- UCL Biochemical Engineering
- The Jenner Institute, University of Oxford

Three UK Spokes:

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



Hub activities



(1) Grand Challenges Research

Research to focus on established and proven vaccine technologies:

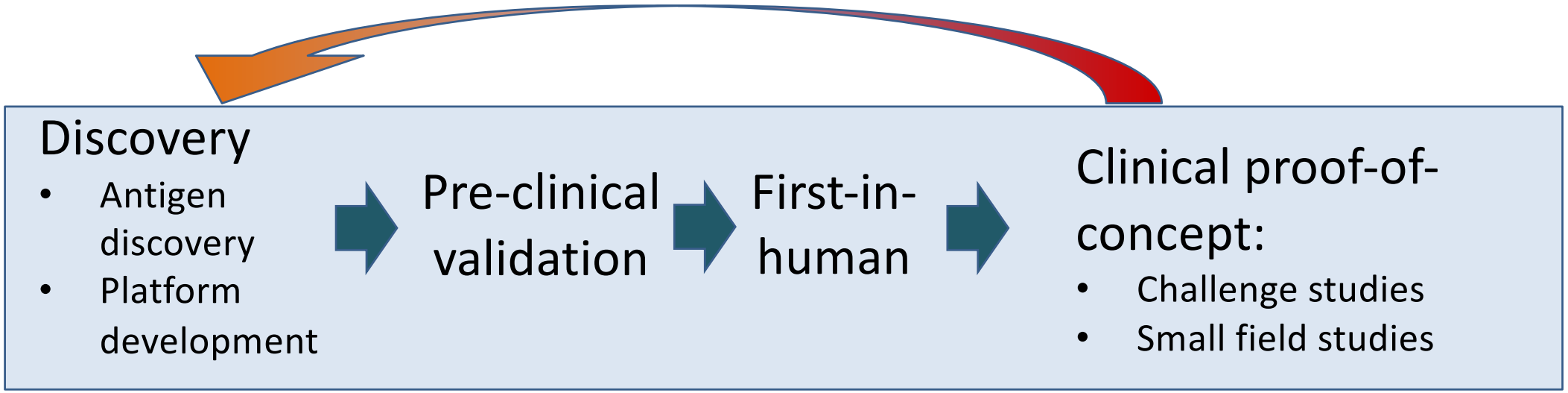
- live viral vectored
- conjugates
- VLP vaccines

(2) Platform Operations

Open to all user group members, beyond the core research team

- Training courses
- Interaction vouchers - vouchers up to £10k, per voucher
- Feasibility studies - demonstration projects, up to £100k per project

University of Oxford, Jenner Institute: scope

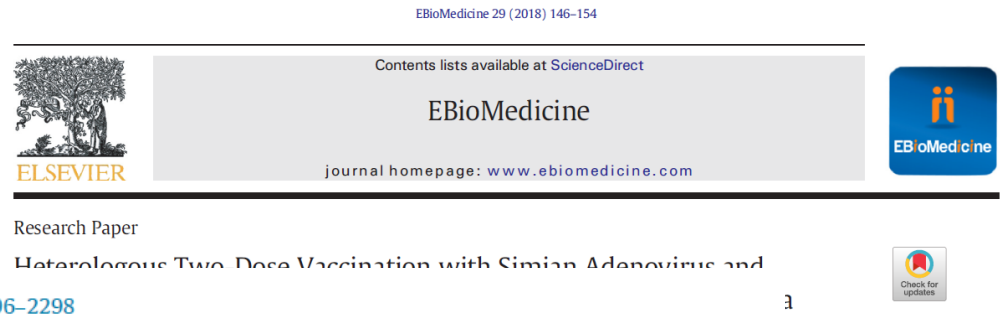


High risk : reward contexts

- Immunologically 'difficult pathogens' – experimental medicine
- Diseases of poverty / niche markets – need proof-of-concept, maybe LMIC manufacturing partners

Need low cost GMP for Phase I

Adenovirus as a vaccine platfc


EBioMedicine 29 (2018) 146–154

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Heterologous Two-Dose Vaccination with Simian Adenovirus and

An effective adenovirus manufacturing an

Vaccine 34 (2016) 2296–2298

Contents lists available at ScienceDirect

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Short communication

Potency of a thermostabil
Fever vaccine in cattle

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involved ZIKV chal-
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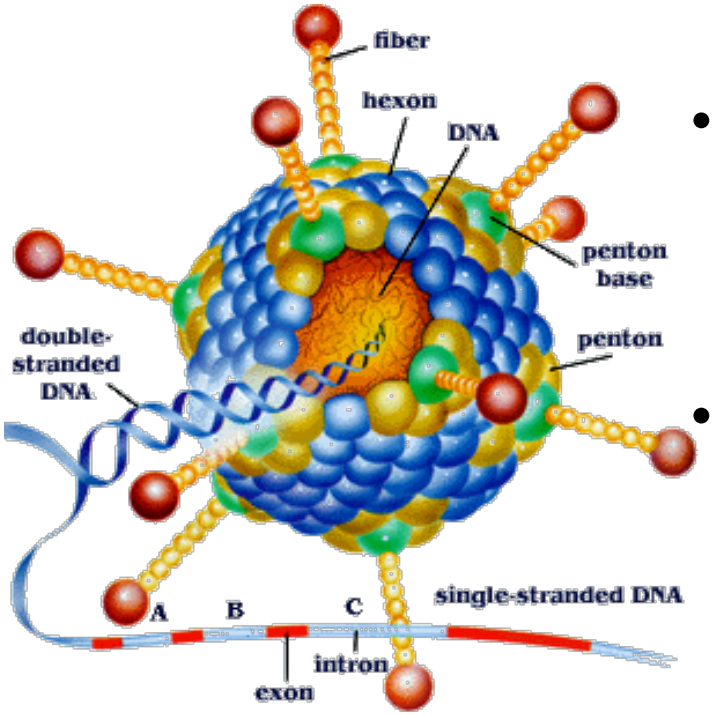
A simian-adenovirus-vectored rabies vaccine suitable for thermostabilisation and clinical development for low-cost single-dose pre-exposure prophylaxis

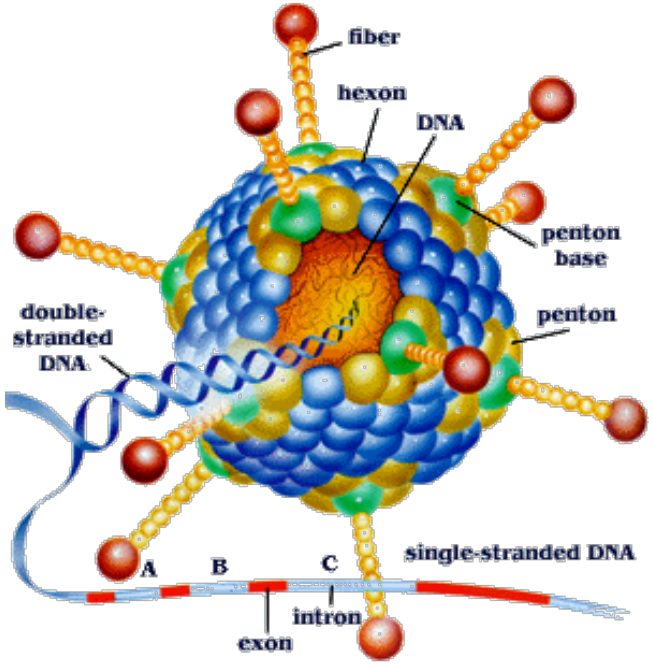
Wang et al., PLoS NTD 2018

Adenovirus biology for manufacturers



- 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 adenoviral 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

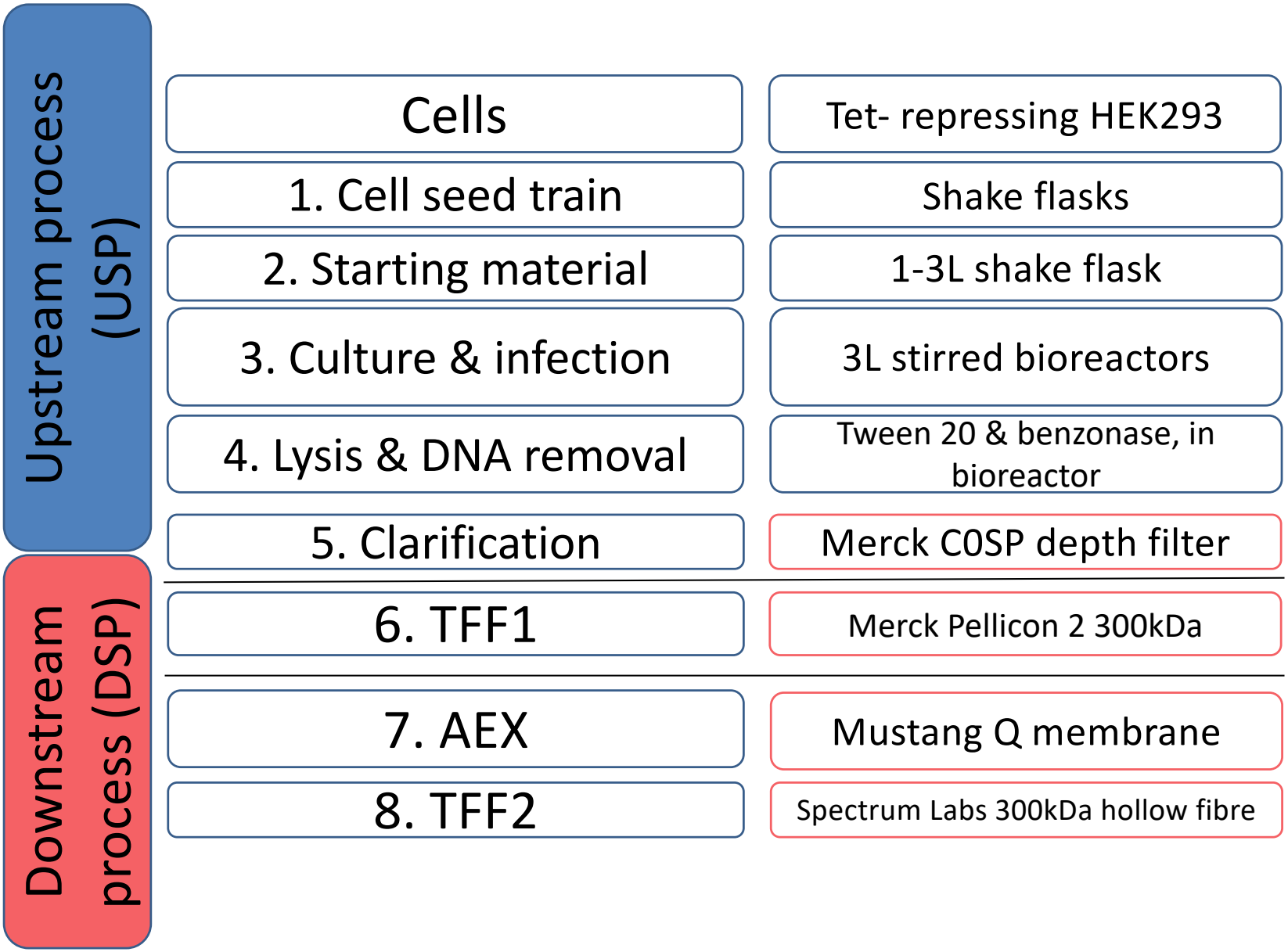
Process requirements for Phase I

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



Small-scale adenov
production proces
overview

FRIDGE OR FREEZE HOLD

FRIDGE OR FREEZE HOLD

Vaccines used as ‘test cases’

ChAdOx2 RabG (rabies vaccine)

ChAdOx1 RVFV GnGc (Rift Valley Fever vaccine)

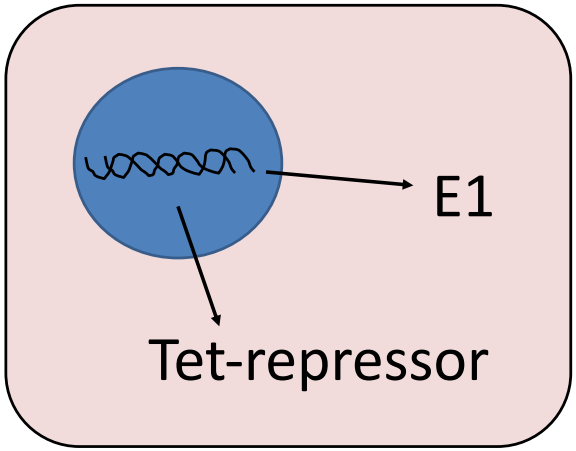
ChAd63 ME-TRAP (malaria vaccine)

Antigen-repressing HEK293 / promoter combination

Provide adeno E1

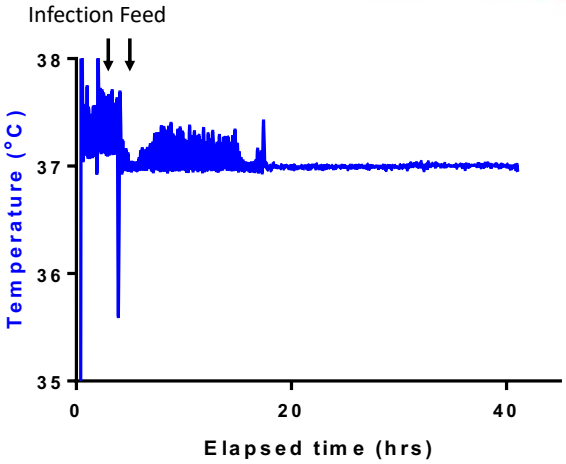
Repress antigen expression in culture
- Consistent viral behaviour regardless of antigen
- ?Increased yields

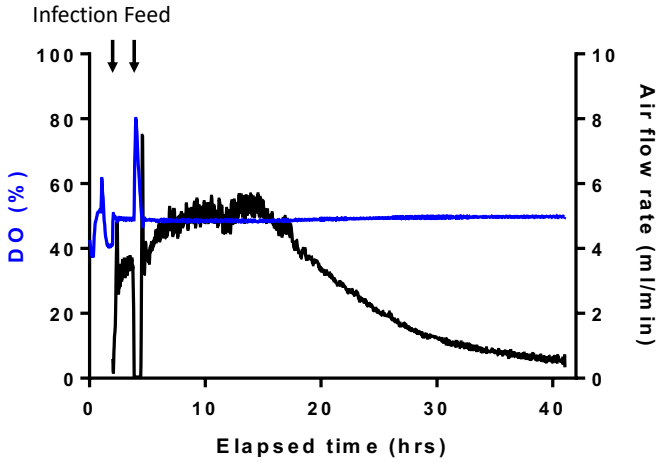
Now:
- Master cell bank
- Suspension growth

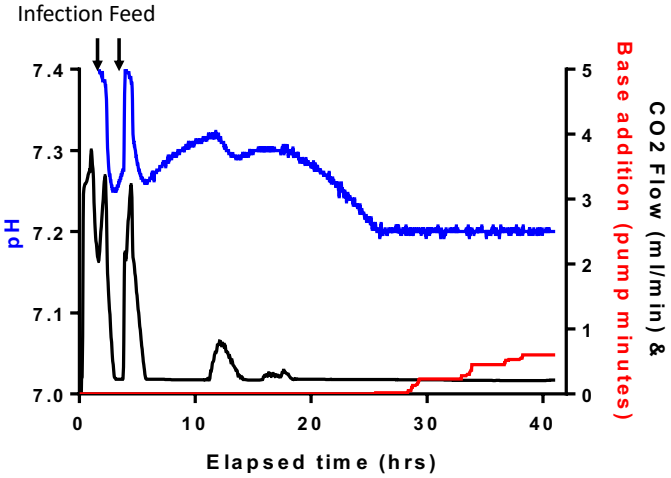


3L single-use stirred-tank bioreactors

- Good results with two different vessels
  - Yield c. 1x10^5 VP per cell
- Simple <48hr batch process
  - Cell expansion in shake flasks
  - Can almost certainly be substantially improved upon!



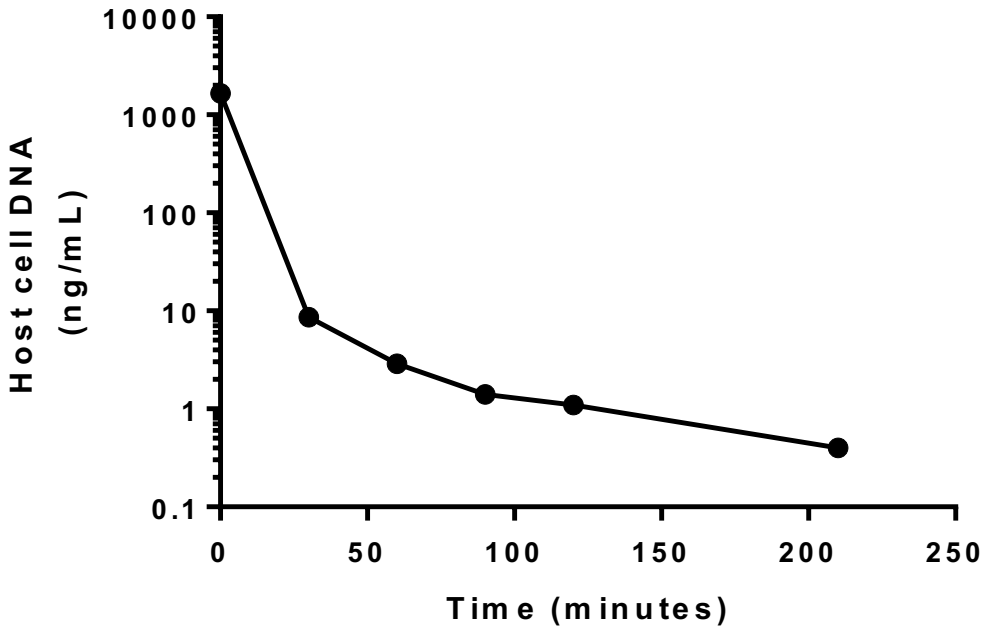




Fedosyuk et al, Vaccine, 2019

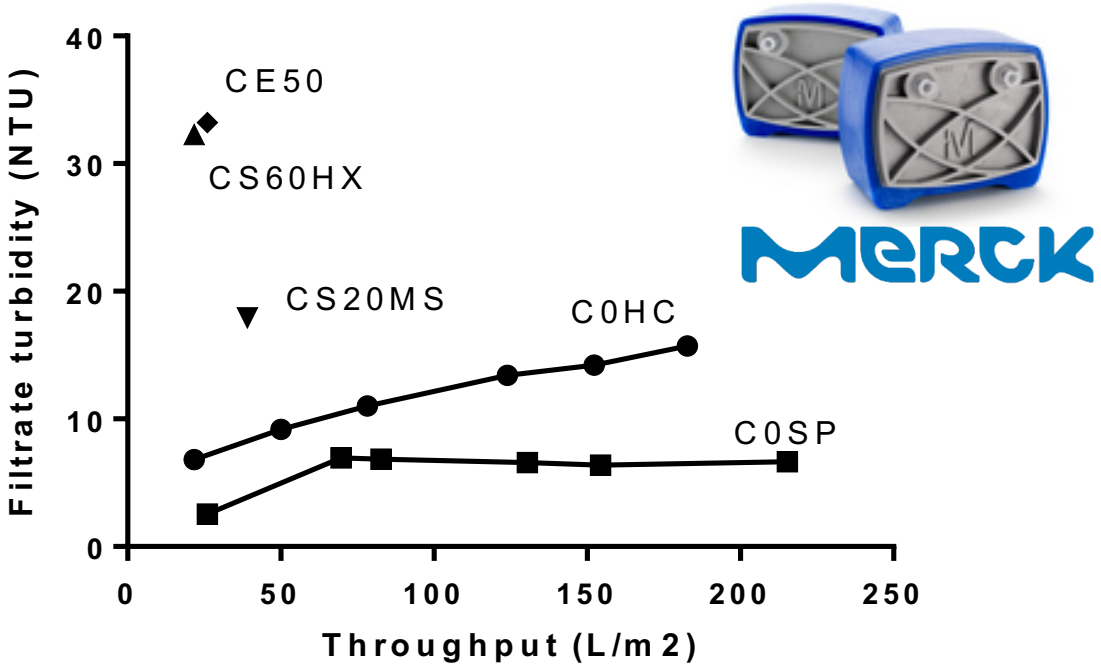
In-bioreactor detergent lysis and benzonase nuclease treatment followed by single-step depth filter clarification

1000-fold host cell DNA reduction with benzonase at 60 u/mL, 2 hours



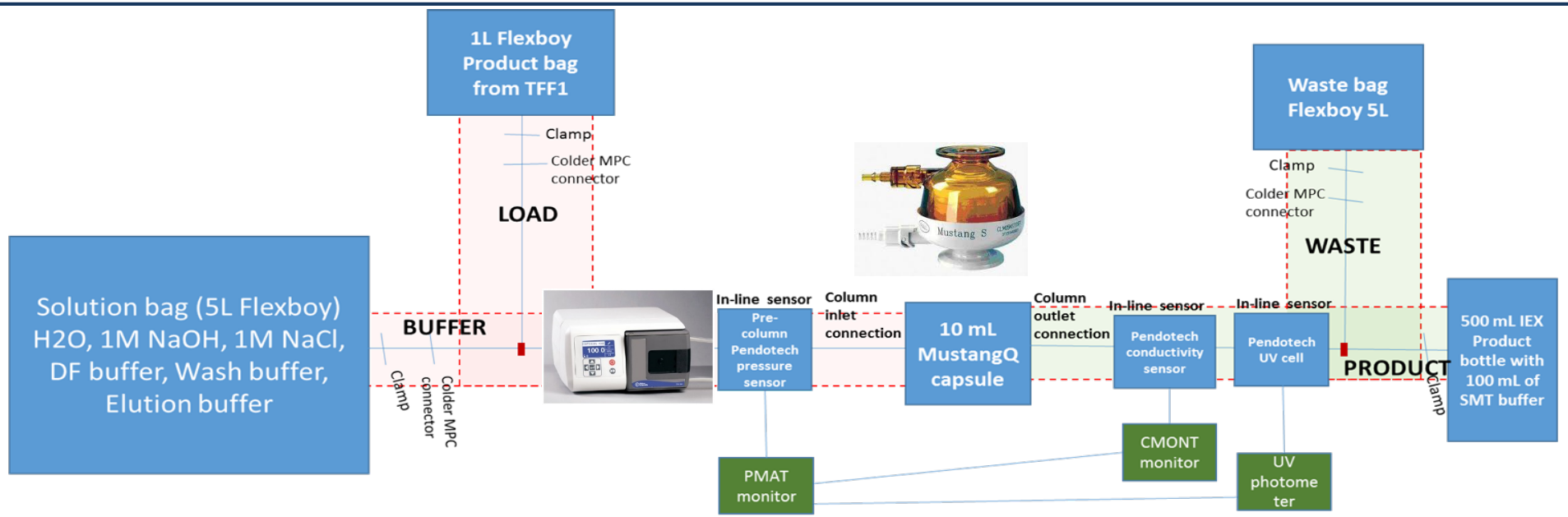
Comparison of depth filters

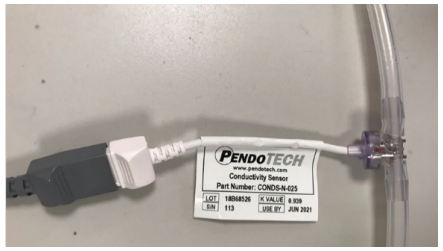
Input lysate turbidity = 90NTU



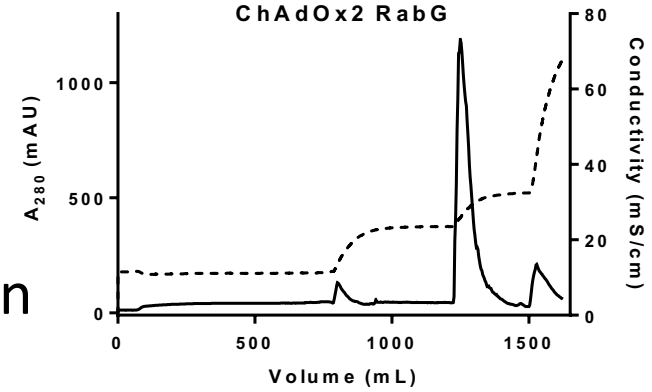
Fedosyuk et al, Vaccine, 2019

Low-cost single-use GMP-suitable chromatography rig





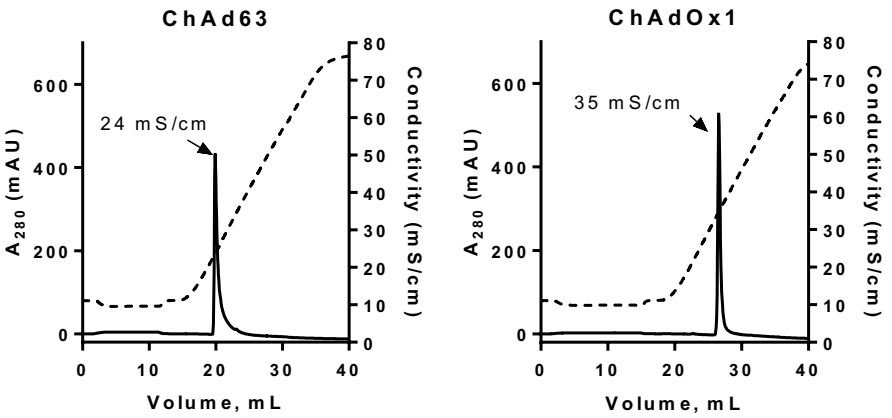
Single-use in-line pressure, conductivity and A260/280 sensors (Pendotech)



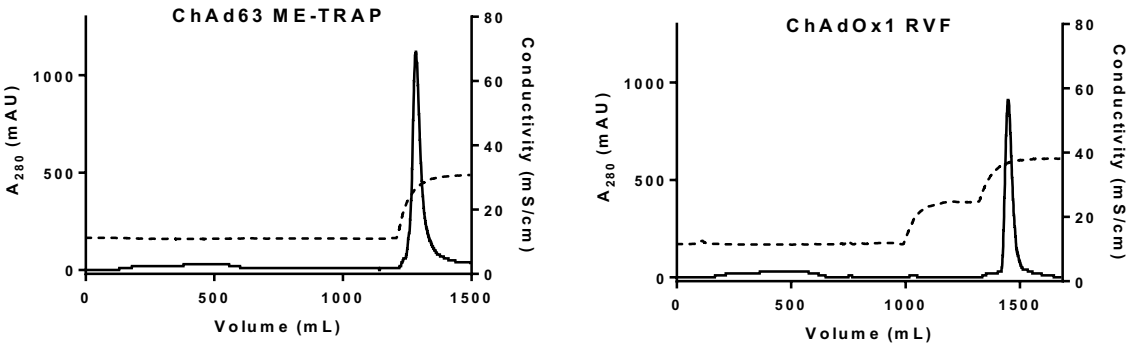
Equipment cost <£25k; consumables cost <£1k per run

Anion exchange with other serotypes

Small-scale identification of elution characteristics



Purification from 3L bioreactor run



Fedosyuk et al, Vaccine, 2019

Formulation, fill & finish

TFF2- diafiltration into formulation buffer
- Spectrum Labs hollow fibre

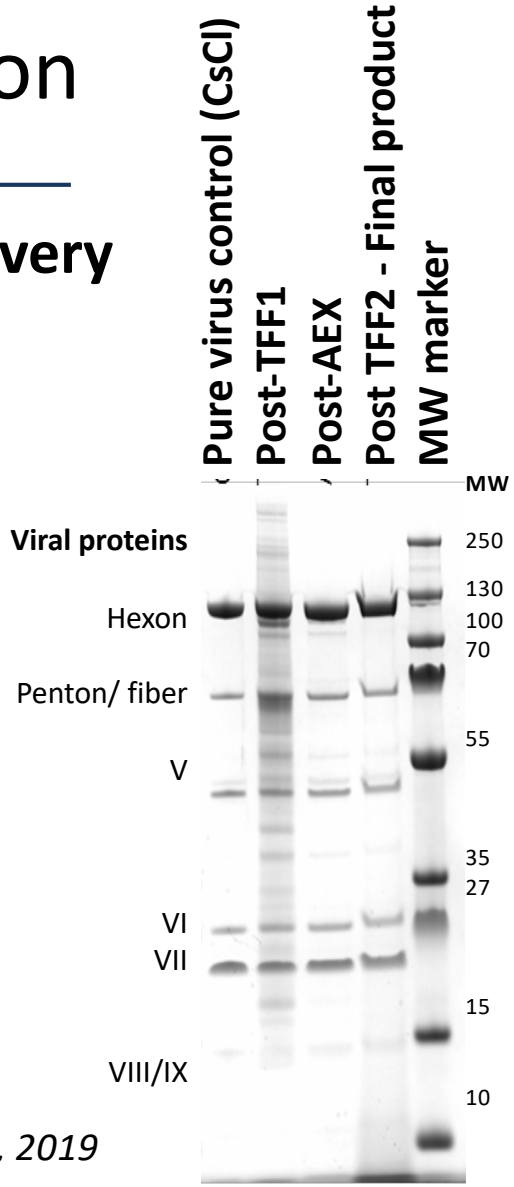
Freeze hold

0.45 & 0.2 µm filtration
- Negligible filtration losses

Step recovery and purification contribution

Median step recovery (n=7)

Step1	• TFF1: 10x conc, 6x diafiltration (Pellicon® 2 Mini)	93%
Step 2	• Anion-exchange (IEX; MustangQ 10mL capsule)	48%
Step 3	• TFF2: concentration adjustment, diafiltration into formulation buffer (Pellicon® 2 Mini or XL50/ Spectrum Labs MidiKros)	90%



Fedosyuk et al, Vaccine, 2019

Performance across three viruses, multiple runs

Virus	Number of runs and vessel type	Culture volume (litres)	USP yield (VP [by qPCR], per litre of culture)	DSP yield (VP [by spectrophotometry], per litre of culture)	Host-cell protein (ng/mL)	Host-cell DNA (ng/mL)	Residual Benzonase, ng/mL	A260:A280 ratio	Empty capsid : VP ratio (n=1 per	Particle: infectivity ratio
ChAdOx2 RabG	1 (2x Mobius vessels)	4	$1.2x10^{14}$	$3.60x10^{13}$	5.2 (3.3-8.6)	<0.1	<0.3	1.3 (1.28-1.34)	0.17	103 *(85-122)*
	3 (BioBlu vessels)	3.2	$8.3x10^{13}$ ($7.9x10^{13}$-$1.4x10^{14}$)	$4.3x10^{13}$ ($1.7x10^{13}$-$6.4x10^{13}$)						
ChAd63 ME-TRAP	2 (Mobius vessels)	2	$6.4x10^{13}$ ($3.7x10^{13}$-$9.0x10^{13}$)	$3.1x10^{13}$ ($1.3x10^{13}$-$5x10^{13}$)	3 (2.9-3.1)	<0.1	<0.3	1.39 (1.35-1.42)	<0.1	56 *(23-88)*
ChAdOx1 RVF	2 (Mobius vessels)	2	$5.5x10^{13}$ ($3.6x10^{13}$-$7.0x10^{13}$)	$1.3x10^{13}$ ($1.1x10^{13}$-$1.5x10^{13}$)	4.9 (3.1-7.9)	<0.1	<0.3	1.38 (1.37-1.39)	<0.1	146 *(99-192)*

Yield >1000 doses per run
(500 – 1700 doses per litre)

Quality satisfactory

Results shown are median (range)

Fedosyuk et al, Vaccine, 2019

Summary:
Plug and play chimp adenovirus process

Fully single-use USP & DSP product-contact components

Applicable to multiple serotypes / multiple antigens
- Minimal product-specific tuning
- Antigen-repressing cells

Simple enough for any GMP facility capable of growing mammalian cells in suspension

Benefits for Hub User Group

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

How to become a member of VaxHub

Academic institutions (Hubs and spokes) have signed a Collaboration Agreement in February 2019

All companies who have provided a LoS will automatically receive a copy of the overarching Collaboration Agreement after this meeting outlining how to become a member and what the process involves

- Standard type Agreement, non-negotiable
- Finance Schedule
- Additional agreement, NDA, MTA, etc might be needed for 1:1 collaborations stemming from the Hub and for feasibility projects

Companies who are new to the Hub – would be great to have you join – please contact Nav (naveraj.gill@ucl.ac.uk) after this meeting to confirm your interest in joining and we will start the process for you