From bioassay method development to commercial testing at pandemic speed: the story of AZD1222 (ChAdOx1 nCoV-19)

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25 minutes

Abstract

- The global public health response to SARS CoV-2 created the opportunity to develop, manufacture and distribute novel vaccines at an unprecedented speed. AZD1222 (ChAdOx1 nCoV-19), the adenovirus-vectored vaccine, was codeveloped between Oxford University and AstraZeneca. As part of the world-wide distribution plan, a comprehensive analytical control strategy, for both vaccine substance and vaccine product, was constructed and methods validated and tech transferred to commercial QC labs in a total of 3 months. In addition to key physicochemical methods, including measurement of viral particles by AEX (Anion Exchange chromatography), two bioassays were implemented for characterization, release and stability: an infectivity assay and a transgene expression assay. A review of the history of the bioassay method development reveals several key points. Significant technical hurdles were overcome in the short time available. Close coordination was needed between clinical method development and testing teams, and the commercial QC team to move rapidly from clinical trial support to global distribution. Proactive communication with regulatory agencies, to address questions and align expectations, was also critical to the success of the program. Given the large, global manufacturing network utilized for AZD1222, demonstrating lot and site comparability was essential, requiring good reproducibility of the methods, as well as close collaboration with statistical analysis colleagues to set appropriate criteria. With over 2.6 billion doses of AZD1222 released for global delivery so far, the analytical method development team remains engaged in assay life-cycle management, while the Covid-19 vaccine field adapts to the evolving dynamics of the disease, including the emergence of variants.

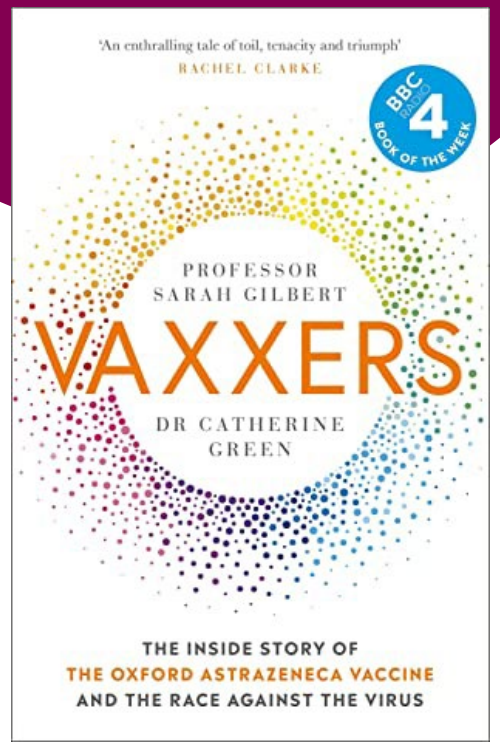
Acknowledgments

- **Biopharmaceutical Development**
- Purification Process & Analytical Sciences
  - Bioassay, Biosafety & Impurities (BB&I)
  - Physico-chemical Development
  - Analytical Quality and Logistics
  - Purification Process Sciences
- Cell Culture and Fermentation Sciences
- Manufacturing Sciences
- Dosage Form and Design Development
- Logistics and Clinical Supply
- Scientific Writing and CMC Project Management
- **Vaccines and Immunotherapies Development**

Outline

- Brief history of ChAdOx1 nCoV-19(AZD1222, Vaxzevria)
- Challenges of developing and implementing analytical methods and control strategy at “Warp Speed”
- Very brief Clinical phase with parallel transfer to Commercial
- Managing large, global network of CMOs/CROs
- Regulatory challenges: eventual emergency use and/or full licensure in >180 countries (as of June 13, 2022)
- Bioassay
- Biosafety
- Impurities
- Lessons learned

Brief history of AZD1222

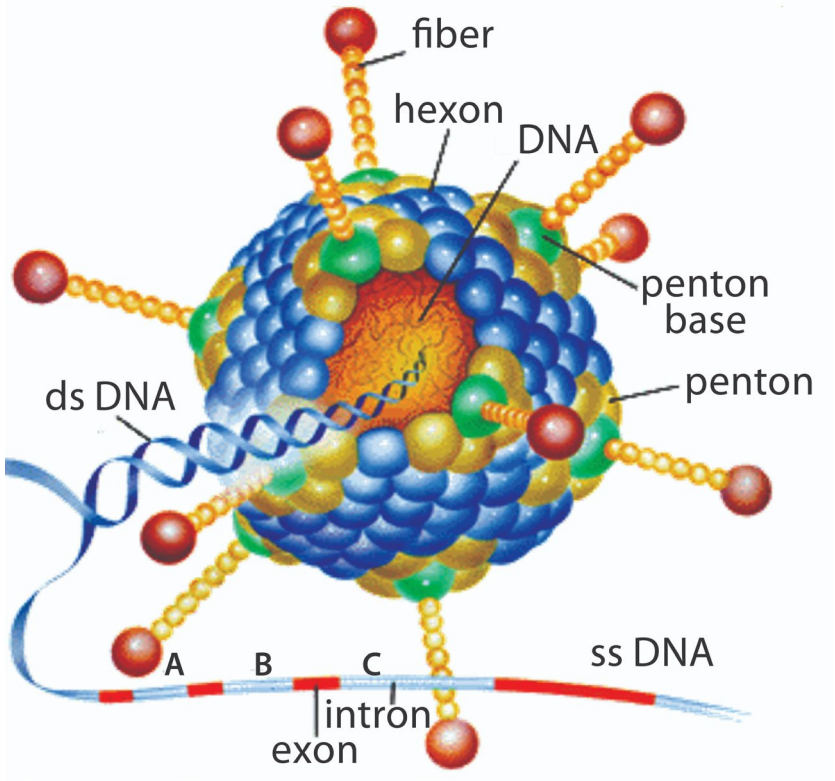


- Originally developed at Oxford University by Sarah Gilbert
- Based on previous work with ChAdOx1 vector in SARS-1 and other diseases.
- Replication-deficient simian adenoviral vectored vaccine expressing nCoV-19 Spike
  - Non-replicating due to E1 (and E3) gene deletion
    - HEK293 cells supply E1 in *trans*

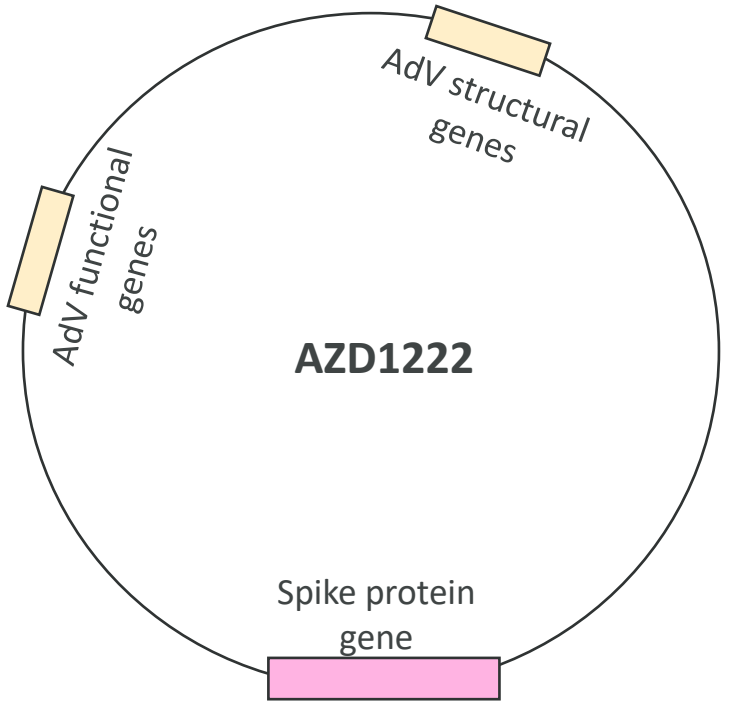
- Clinical scale, GMP manufacturing and analytics under development by Oxford, use of CMO Advent
- AstraZeneca agreed to assume responsibility for late-stage clinical and commercial, global distribution, along with other licensees (e.g. Serum Institute of India)

AZD1222 vaccine project

AZD1222 is a novel recombinant simian adenovirus, expressing the SARS-CoV-2 Spike surface glycoprotein under the control of a strong promoter.

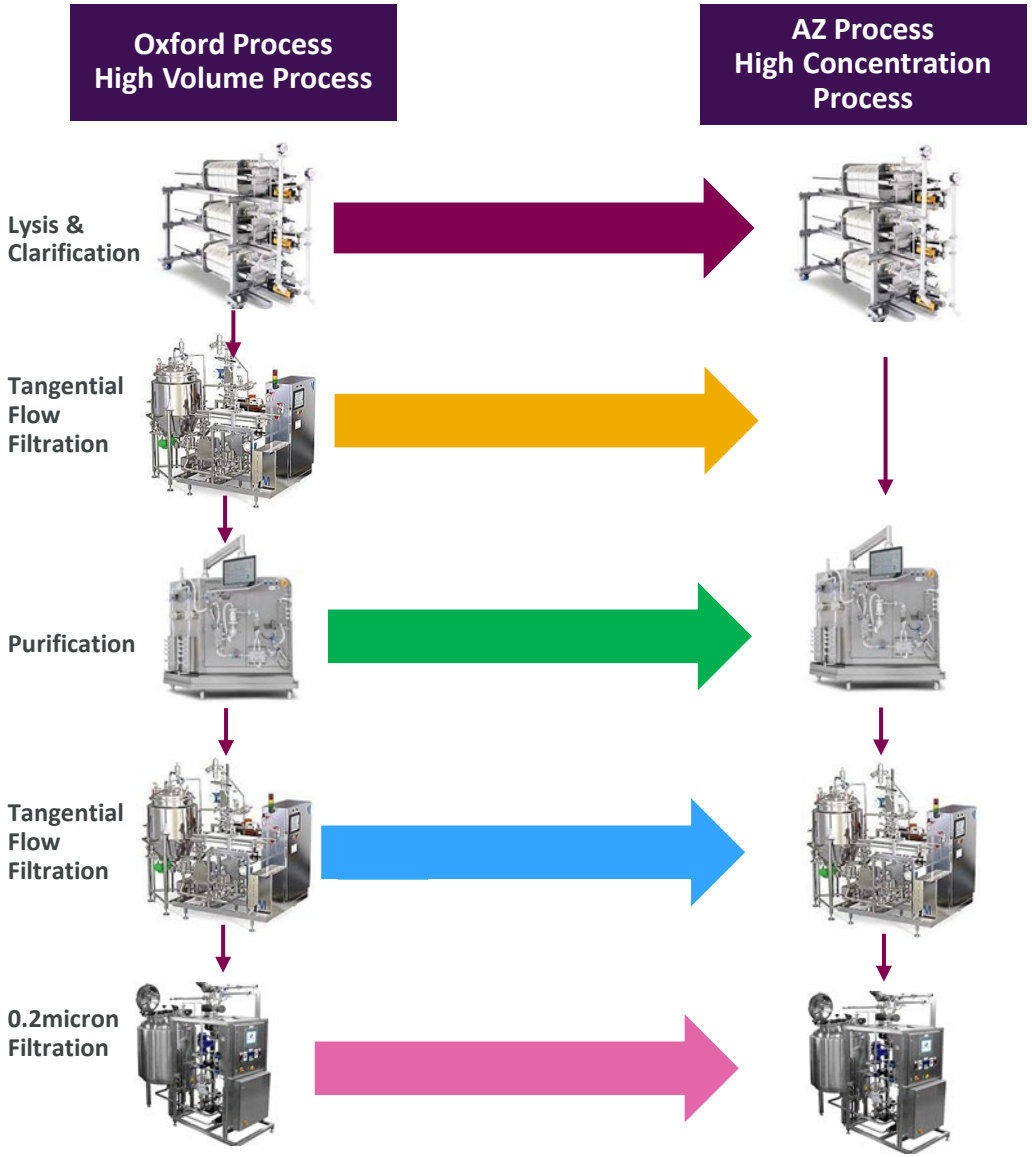


Typical Adenovirus vector

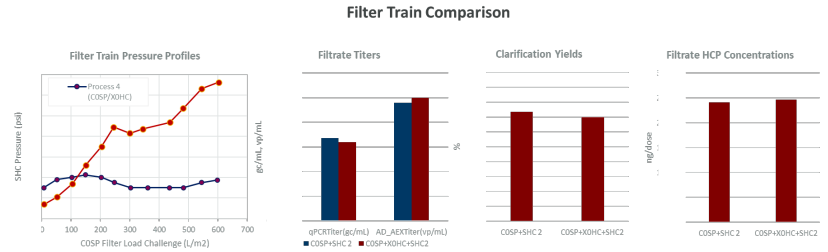


Warp Speed

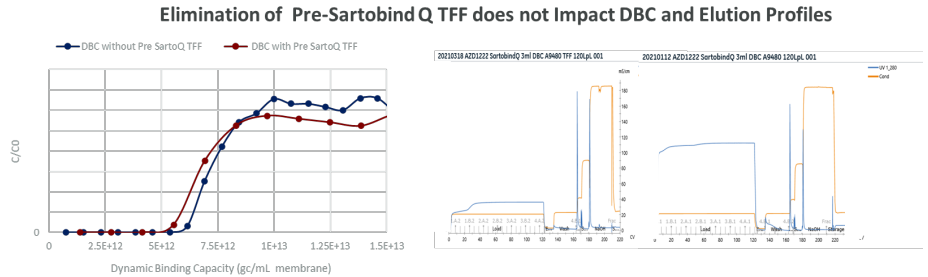
Streamlined Process Enabled Scale Up & Global Manufacturing of More Than 2 Billion Vaccine Doses



- **50% increase in vaccine supply with new filter train**
  - No impact on yield or product quality



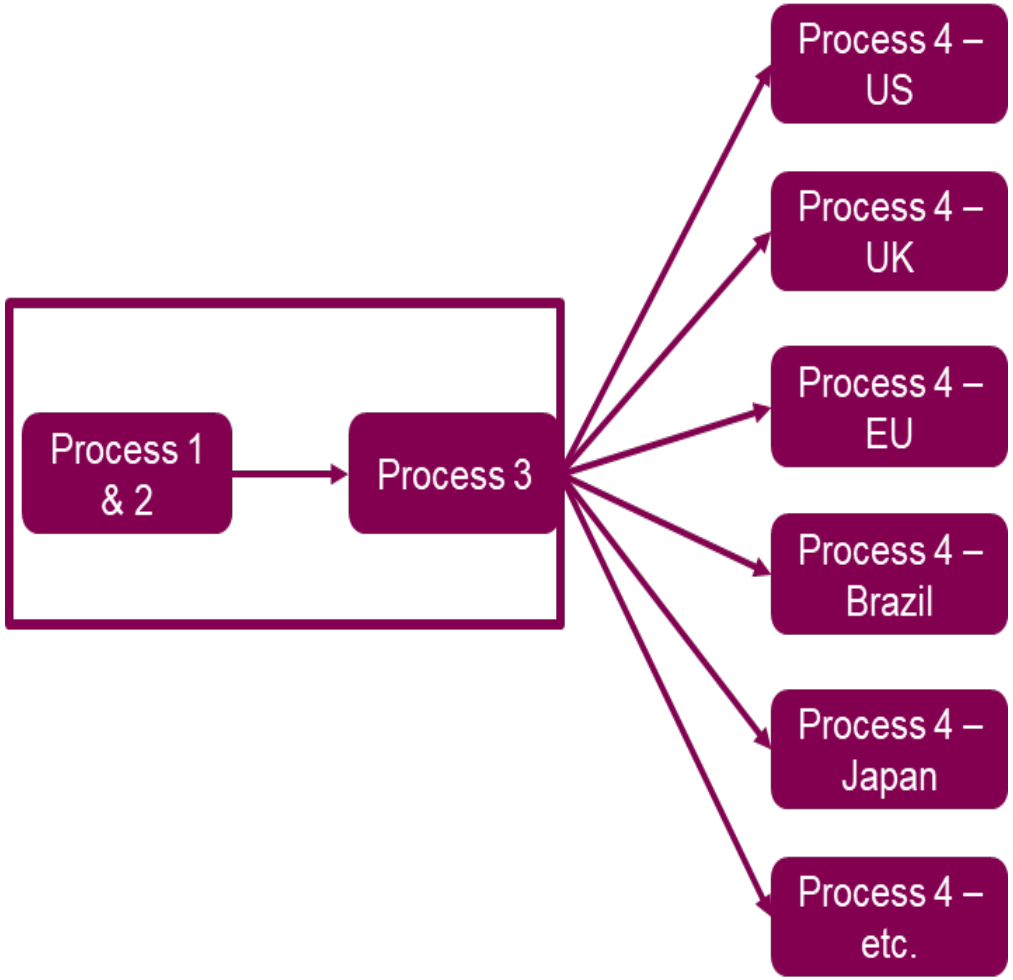
- **20% increase in manufacturing throughput without TFF**
  - No impact on capture step performance



- **10-fold reduction in drug substance volume eliminated cryo bag, shipping and storage constraints**
  - >7-fold higher drug substance concentrations

Analytical Control System

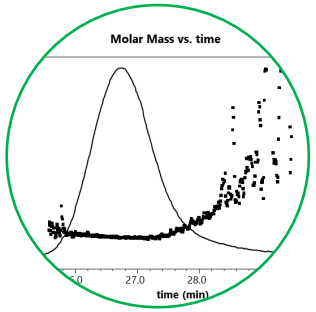
The Approach



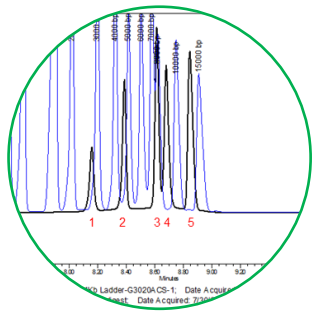
Anchor to clinical process materials as the common comparator to all commercial manufacturing nodes, current and future

Targeted characterization tests based on criticality of structural and functional attributes and likelihood of variation by differences in manufacturing sites

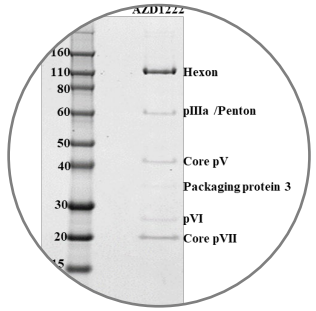
Molar Mass



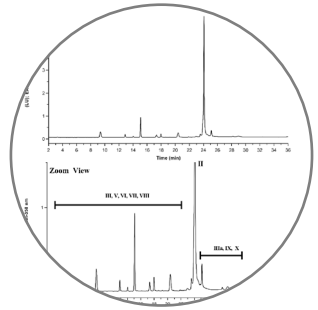
Viral Genome Integrity



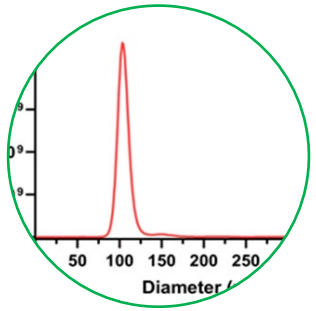
Capsid Profiling

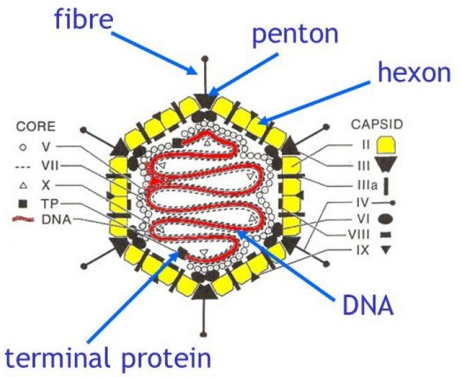


RPLC-MS

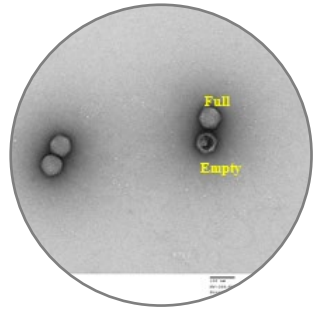


Particle Size

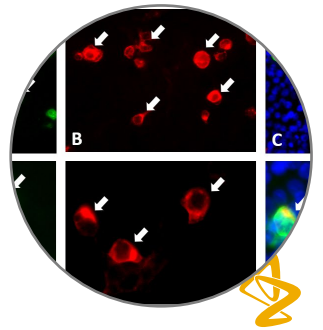




Transmission Electron Microscopy



Immunocytochemistry



Viral Genome Conc.

Vector Aggregates

Transgene Expression

Transgene Sequencing

Dynamic Light Scattering

Size Exclusion Chromatography

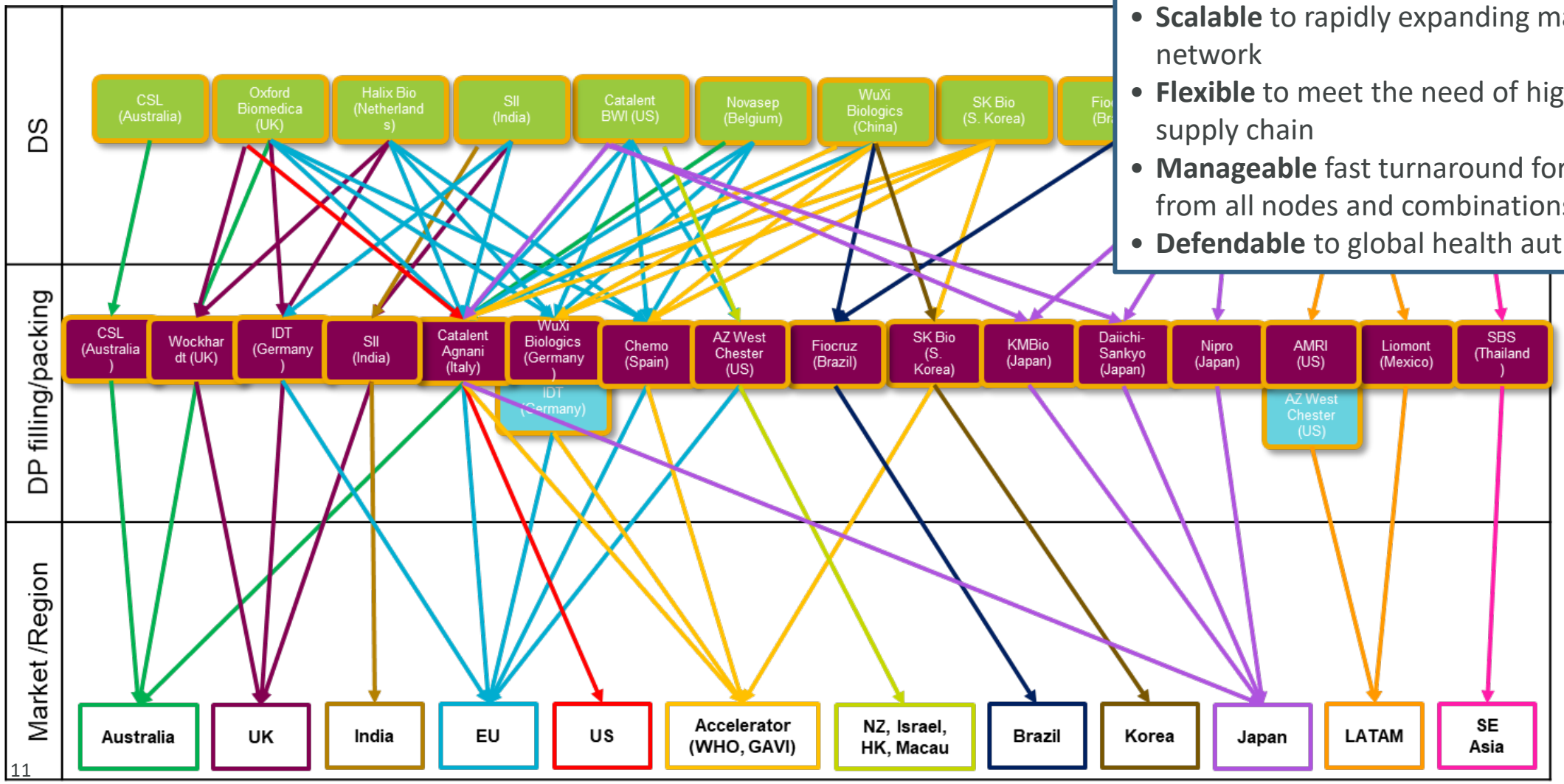
Analytical Ultracentrifugation

The Challenge

Analytical Comparability for a Complex AZD1222 Global Supply Chain

Pre-approved comparability protocol with defined acceptance criteria

- **Scalable** to rapidly expanding manufacturing network
- **Flexible** to meet the need of highly dynamic supply chain
- **Manageable** fast turnaround for hundreds of lots from all nodes and combinations
- **Defendable** to global health authorities



The Impact

- **A right sized comparability approach based on scientific understanding of product attribute criticality and accepted across global health authorities**

- **High quality data at unprecedented speed**
  - Total of ~5000 test results to date (December, 2021)
  - Only 10 data points (0.2%) outside of comparability criteria limits, which have all been scientifically justified

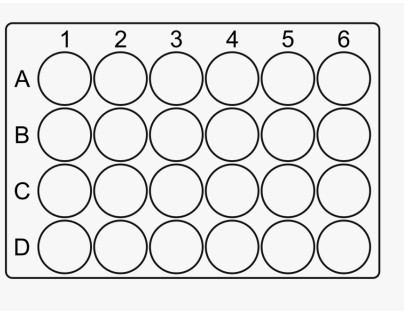
- **Analytical Comparability demonstrated for:**
  - 127 DS lots and 97 DP lots

- **Supporting global regulatory acceptance of commercial manufacturing at:**
  - 15 DS sites, 16 DP sites, 39 DS/DP node combinations

3 billion doses distributed to >180 countries

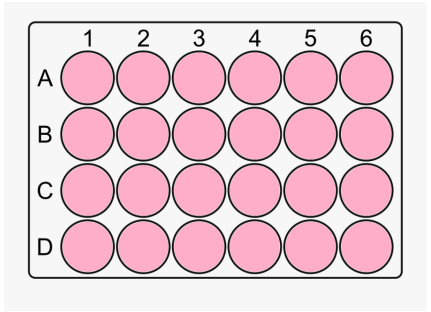
Infectivity

Infectivity Assay Workflow

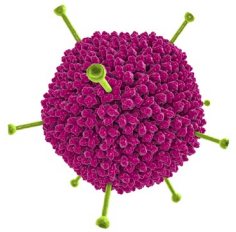


Poly-lysine pre-coated 24-well plate

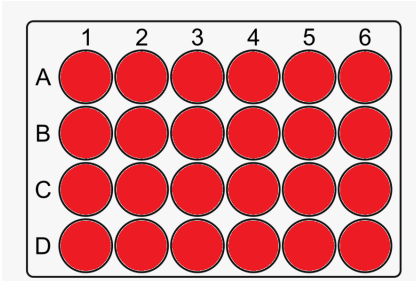
Seed HEK293



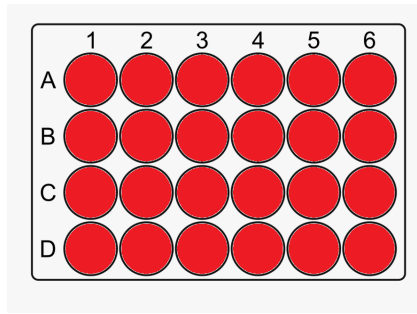
Immediately followed by viral infection



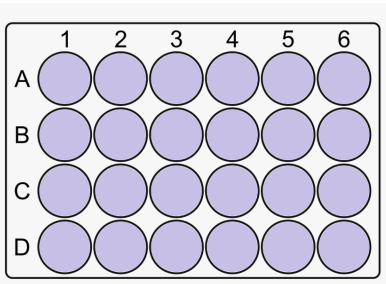
AZD1222 infection



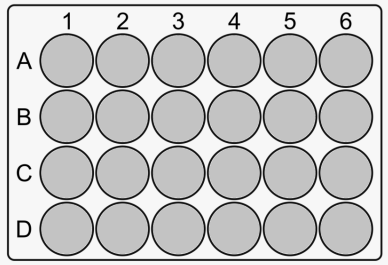
46-48 hour incubation



Fix in methanol

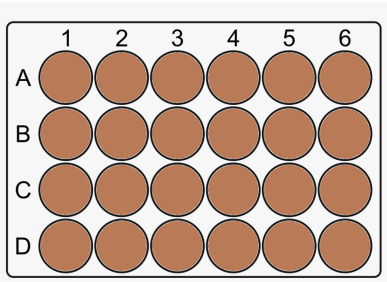


1st Ab (1:1000) for 1h at RT



2nd Ab (1:2000) for 1h at RT

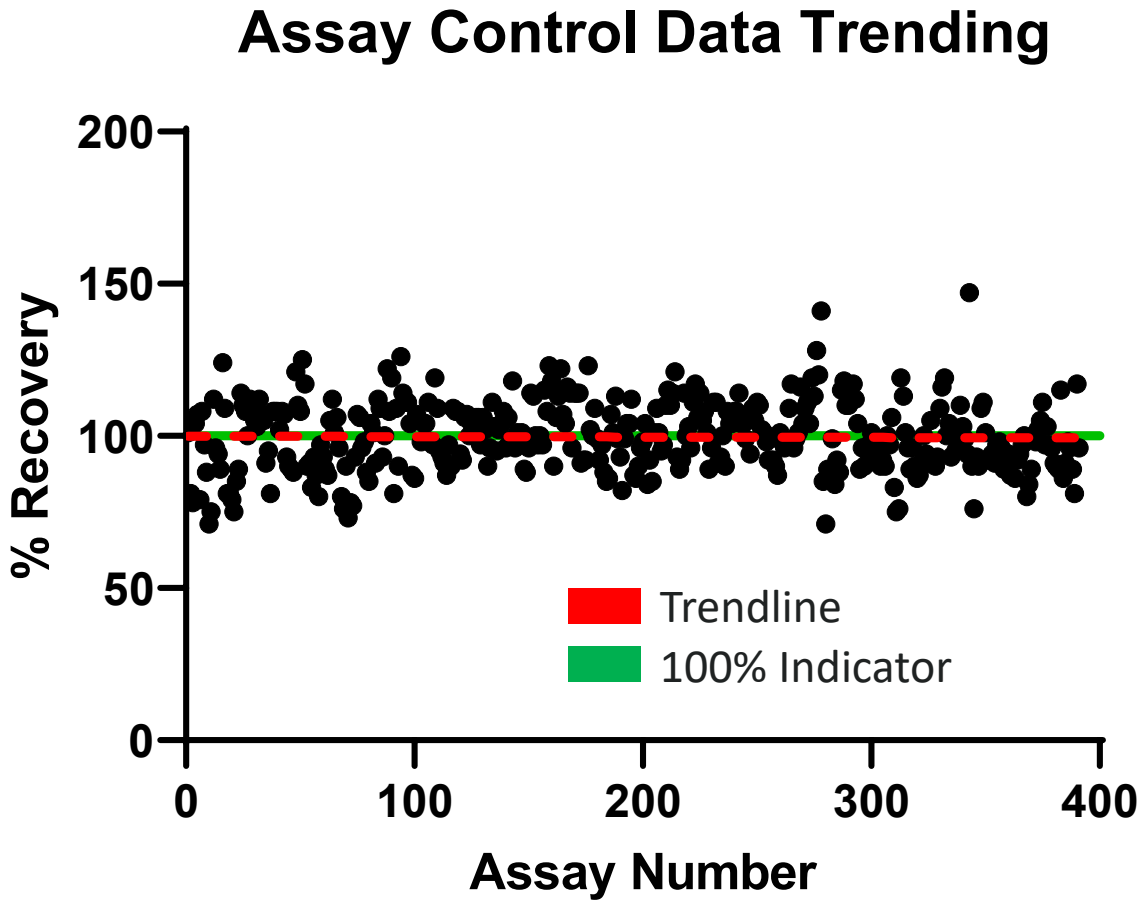
DAB reaction 10min at RT



Cell counting:

- 5 fields/ well
- 2 wells/ sample

24w Assay Control Data Trending



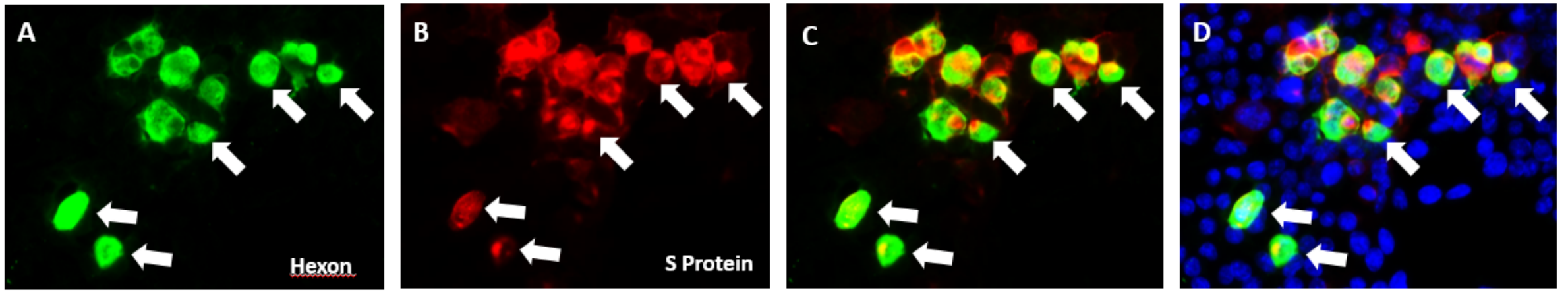
Average % Recovery	%CV
100%	11%

- N=391 plates
- 5 main analysts
- Robust Assay
- Low variability

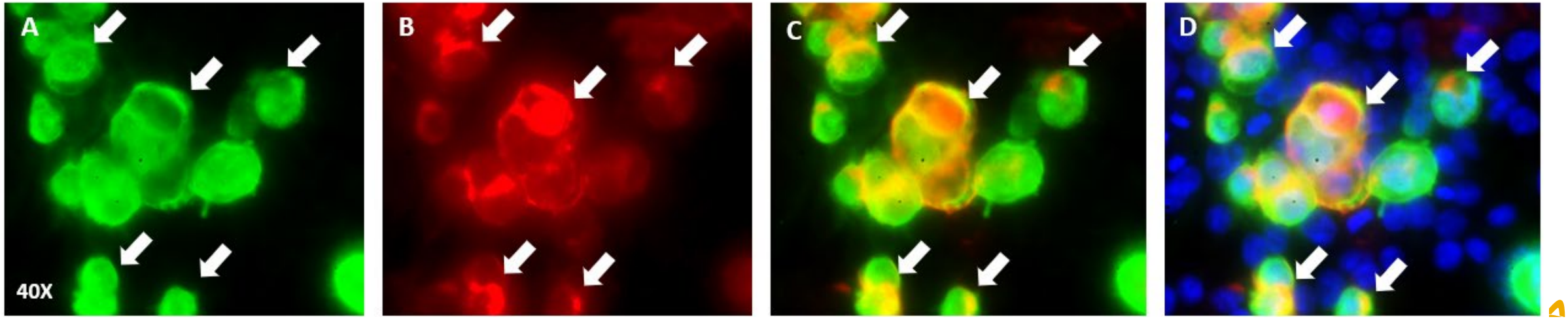
Co-Expression of Hexon/S-protein

Colocalization shows that Hexon expression is a good surrogate for Spike expression

- Double immunohistochemical staining was performed 48 hrs after AZD1222 infection in HEK293 cells to confirm the **colocalization** of Hexon protein and S protein.



Mouse anti Hexon | Rabbit anti S protein | Overlay | Overlay-DAPI



Mouse anti Hexon | Rabbit anti S protein | Overlay | Overlay-DAPI

Qualification

Qualification Parameter	Qualification Target	Qualification Result (24-well manual)	Qualification Result (96-well automated count)
Linearity	$R^2 \geq 0.970$ for linear regression plot Slope of linear regression is between 0.8 and 1.2.	$R^2 = 0.999$ Slope = 1.0	$R^2 = 0.999$ Slope = 1.0
Precision	Repeatability: Within run %CV is ≤15%. Intermediate Precision: Overall %CV is ≤25%.	Repeatability: %CV = 9% Intermediate Precision: Overall %CV = 12%	Repeatability: %CV = 9% Intermediate Precision: Overall %CV = 12%
Accuracy	Mean accuracy is between 75-125% at each potency titer level.	The mean accuracy at each potency titer level is 91-107%.	The mean accuracy at each potency titer level is 90-107%.
	95% confidence interval for overall accuracy is between 75-125%.	The 95% confidence interval for overall accuracy is 96-102%	The 95% confidence interval for overall accuracy is 93-99%

Transgene expression quantification in infected cells

Day 0

Seed HeLa cells → 96 well plate, 24 wells per sample

Infect with ChAdOx1 nCoV-19 → Virus samples dilution based on ifu/ml

Day 2

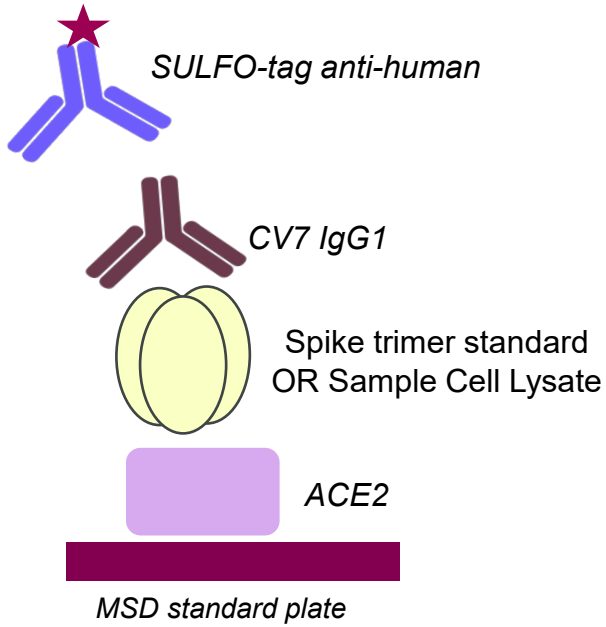
Lyse cells

Quantify total protein → For system and sample suitability

Run immunoassay for Spike (MSD)

ng spike protein/ml of cell lysate

Schematic for MSD



- Uses in-house recombinant CHO-expressed Spike trimer as Standard
- Amount of Spike in cell lysates interpolated from the Standard curve
- Biological and technical replicates used to assess variability

MSD – Meso Scale Discovery - proprietary plates & reader

VAX1222 Transgene Expression "Qualitative"

Fit for purpose Characterisation assay developed to quantitate transgene expression within a month

- Critical reagents are a large challenge: recombinant spike, ACE-2, mAbs

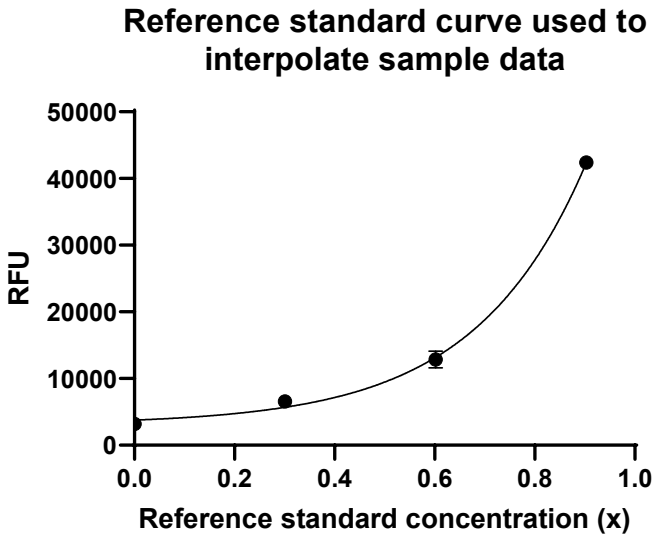
>100 lots successfully tested

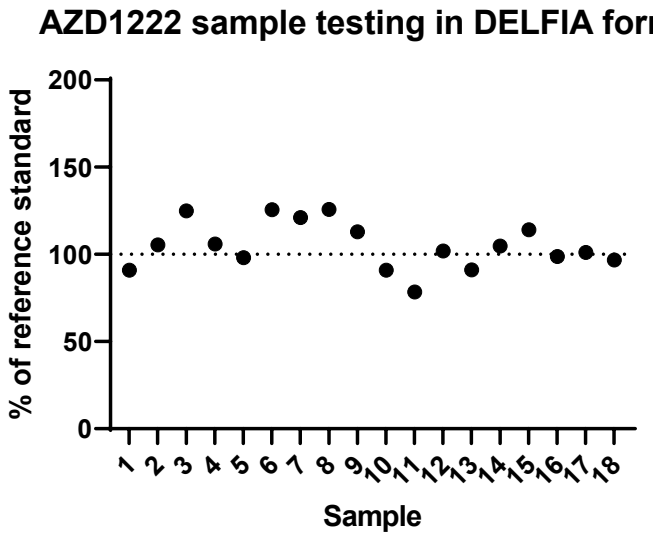
Interpolation from standard curve and sample testing at a single concentration made the assay semi-quantitative

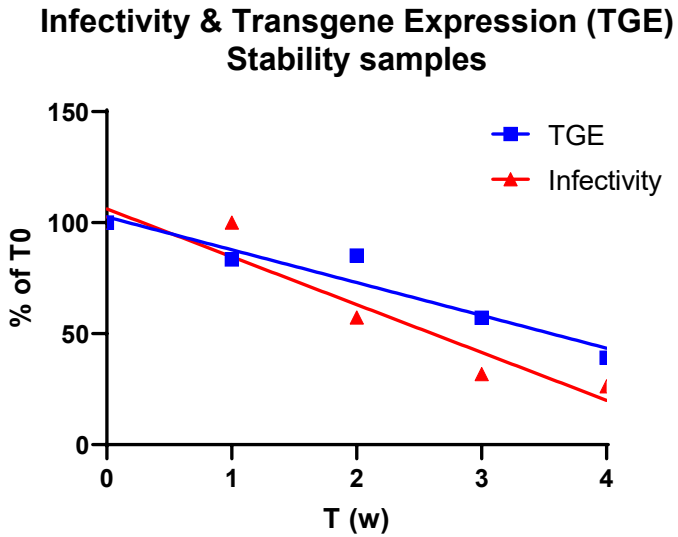
Regulators requested to include TGE as release assay in a qualitative format for AZD1222 (Wuhan) and a quantitative format for characterisation of future vaccine variants.

Developing QC assay to meet Regulatory requirements

- QC assay developed in-line with Regulatory requirements
- Characterisation assay adapted to a QC suitable format and then further optimised from qualitative (for AZD1222) to a quantitative format (for variant characterisation)
- Reporting samples as % TGE of corresponding virus reference standard material to test independent lots
- Assay is stability indicating and trends with infectivity.







AZD1222 Transgene Expression "Semi-Quantitative"

Release assay developed in a transferable format for CROs to support Drug Substance lot release

QC assay uses corresponding reference standard to report %RP, like a typical Bioassay

QC assay developed from qualitative to quantitative format

Biosafety

- Biosafety testing strategy for AZD1222

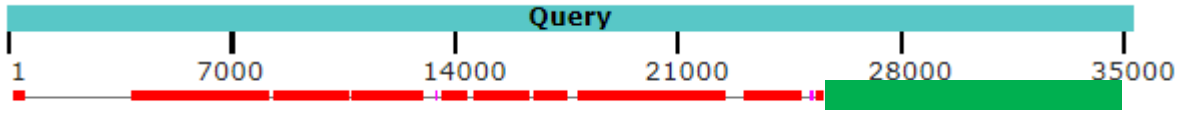
1. Removal of animal testing and introduced selected human panel of viruses by Nucleic Acid Technologies (NAT)
2. Procedure and regulatory compliance assessment for outside testing laboratories for
    1. *In vitro* adventitious agent testing
    2. NAT assays for human virus panel
    3. Rapid replication competent adenovirus (RCA) assay
3. Science-based risk-assessment saved drug substance (DS) batches (~60 million doses)

4. Streamlined biosafety testing strategy for future variant COVID vaccines
    1. No control cell cultivation and testing
    2. Removal of animal testing
    3. Removal of reverse transcriptase assay

Cross-Contamination Method

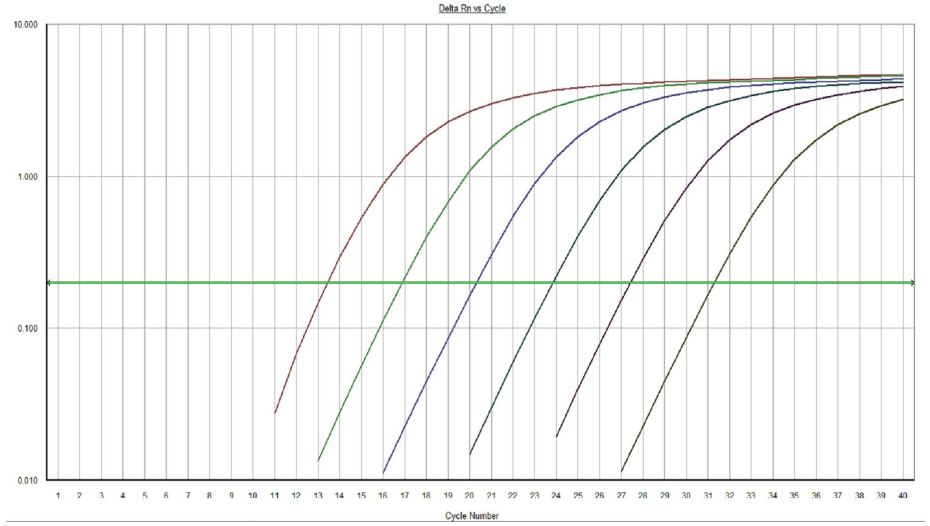
- As part of process development and transfer of the AZD1222 production process (p4), contamination of bioreactors with AZD1222 were observed:
  - Control cell bioreactors (intended to be AZD1222 free)
  - Pre-infection of production bioreactors prior to inoculation
  - Cross contamination of 2nd Client Vaccine bioreactor with AZD1222
- Due to these contamination events and potential for future contamination events, several analytical methods were developed to detect contamination and/or surveil surfaces and media to prevent contamination
  - Detection of 2nd Client's vaccine in AZD1222 Drug Substance
  - Detection of AZD1222 in un-inoculated bioreactors
  - Detection of AZD1222 on swabbed surfaces to monitor cleaning
  - Detection of aerosolized AZD1222
  - Detection of AZD1222 in ChAdOx1 variant bioreactors (and vice versa)

Detection of Client 2 Vaccine in AZD1222 Drug Substance – Method Design

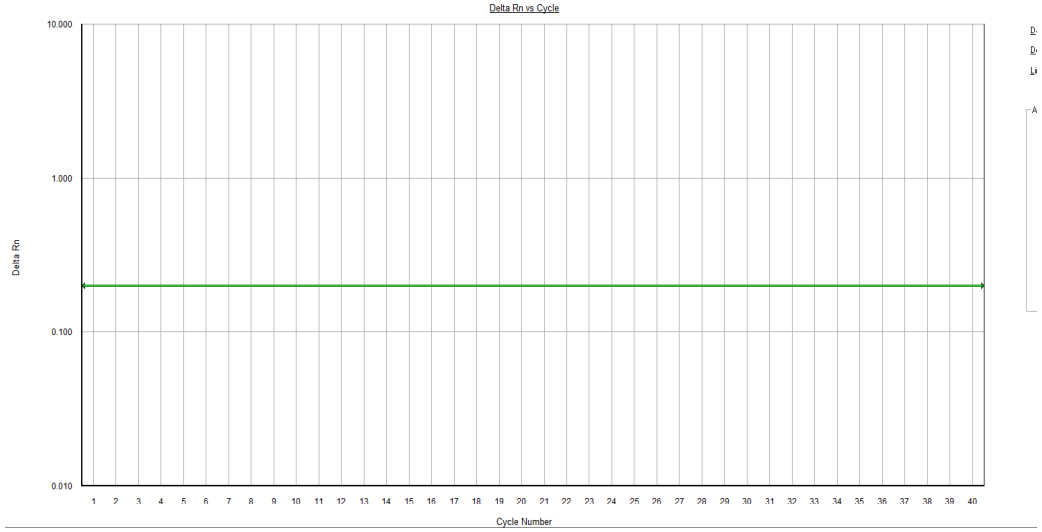


Method was validated according to the ICH Q2R1 as a limit assay by spiking known amounts of Client 2 Vaccine into AZD1222 DS
Method can detect 0.00005% Client 2 vaccine in AZD1222 DS

- DNA sequences from AZD1222 and Client 2 vaccine were aligned and a PCR specific to the Client 2 vaccine was designed (area in green represent sequences different between vaccines and target of the PCR assay)
- Client 2 vaccine material was obtained for use as a method standard and evaluated in the method showing robust and linear detection. Likewise, no detection was observed of AZD1222, or Client 2 vaccine in AZD1222



Client 2 Vaccine 1e10-1e5 Viral Particles



AZD1222 1e10-1e5 Viral Particles

Occurrence of Thrombosis and Thrombocytopenia Syndrome (TTS) and Proposed Mechanisms

- Real world data suggested extremely rare events of TTS following AZD1222 vaccination, primarily after the first dose*
- This triggered a burst of causal hypotheses, mostly in the form of scientific preprints
- Two notable hypotheses included a role for host cell protein (HCP), and binding of platelet factor 4 (PF4) to ChAdOx hexon trimers
- Analytical Sciences tackled both of these issues
  - Exploration of HCP quantitation on control system, resulting in improved method
  - Developing a PF4/ChAdOx binding assay which supports modeling work

*Bhuyan P, Medin J, Gomes de Silva H, et al. Lancet. 2021;398(10300):577–8.

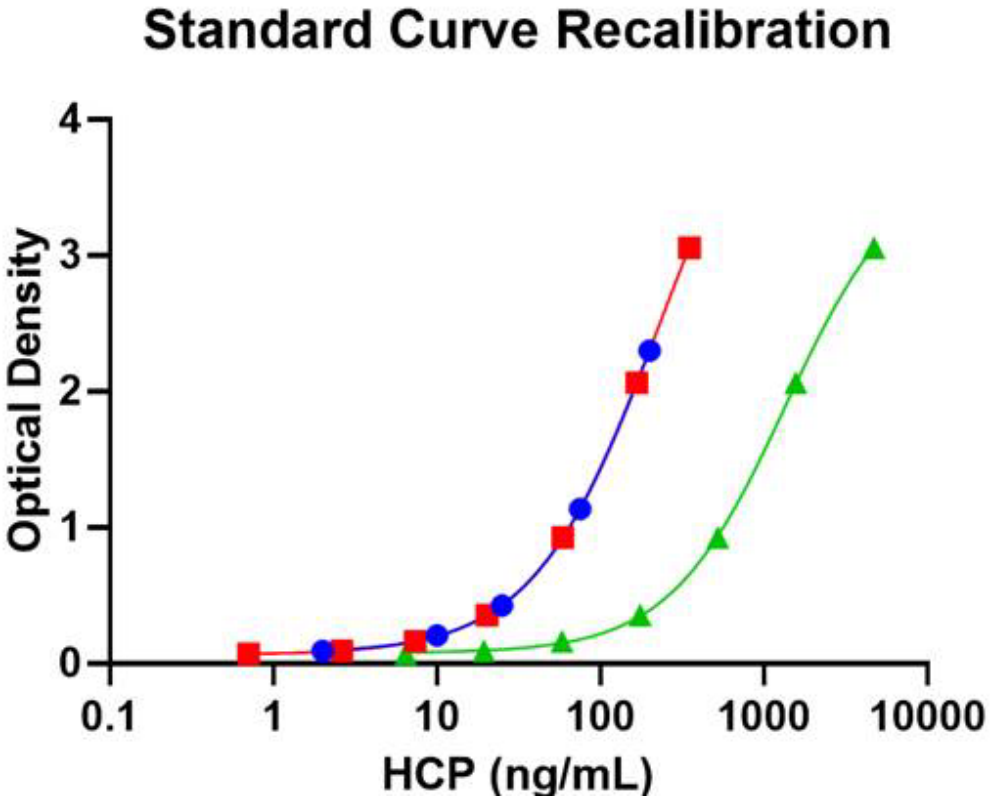
Host Cell Protein (HCP) Assay

- In order to meet the highly accelerated timeline of the AZD1222 project, an off-the-shelf HCP ELISA (HEK-293 cell line) was tested, qualified, transferred to Commercial QC labs and validated
- Orthogonal characterization tests were executed (gels, LC-MS) to identify abundant proteins
- In silico immunogenicity analysis was performed by EpiVax
- Hypothesis of role of HCP in induction of TTS was published by Prof. Greinacher and colleagues*
- Work with AstraZeneca pharmacovigilance group in UK and EU to look for any correlations between HCP levels and SAE, in particular TTS, on a per lot basis: no correlation seen
- Public posting of HCP levels by non-quantitative methods (MS, SDS-PAGE) accelerated investigation and redevelopment of existing HCP method

*Greinacher, A, et al. Blood, 2021, **138** p 2256-2268

Cygnus HEK293 HCP Method Development

- New process-specific HCP standard was created and quantified by total protein assay
- Included as a replacement for the Cygnus kit standard in redeveloped, qualified method



Red shows Cygnus kit standard

Blue shows AZ standard overlayed onto kit standard concentrations.

This show identical method parameters (fit, signal to noise, etc.)

Green curve shows the more accurate concentration generated by the new method

Reflects an increase in reportable HCP relative to previous method

HCP ELISA Update Plan

Extensive clinical surveillance in the UK and EU showed no correlation between TTS and HCP levels was observed.

- The existing method was updated
  - Use of new Cygnus lot of polyclonal anti-HCP
  - Inclusion of new process-specific standard and curve dilutions
- Aliquots of this process-specific standard provided for use at all sites
- Method was qualified
- Method transferred to Commercial Operations for validation and further transfer to commercial test sites
- Method bridging study executed
  - Previously released lots selected and tested to establish a new specification criteria
- Regulatory package presented to EMA
  - Due diligence in method improvement
  - Improvements with regard to accuracy
  - Justification of the new specification
  - New method and specification accepted

Role of Platelet Factor 4 (PF4) binding ChAdOx

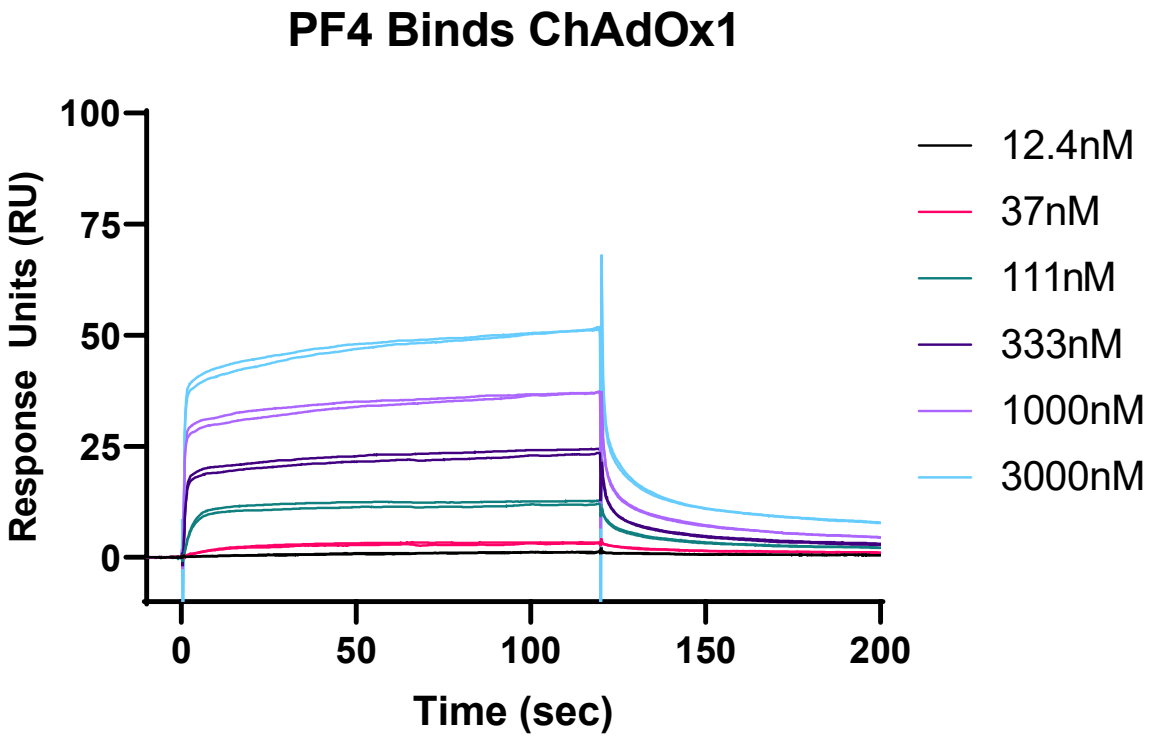
- Complexes of PF4/anti-PF4 bound to heparin are known to cause the related syndrome of Heparin-induced thrombosis with thrombocytopenia (HIT)*
- Binding of PF4 to heparin is charge-driven
  - PF4 has a net positive domain while heparin has a repetitive negative charge
- The surface of ChAdOx was modeled and found to have pockets of net negative charges at the hexon hyper-variable domain trimer interfaces†
- Analytical Sciences developed a surface plasmon resonance (SPR) binding method to support the modelling and vector modification

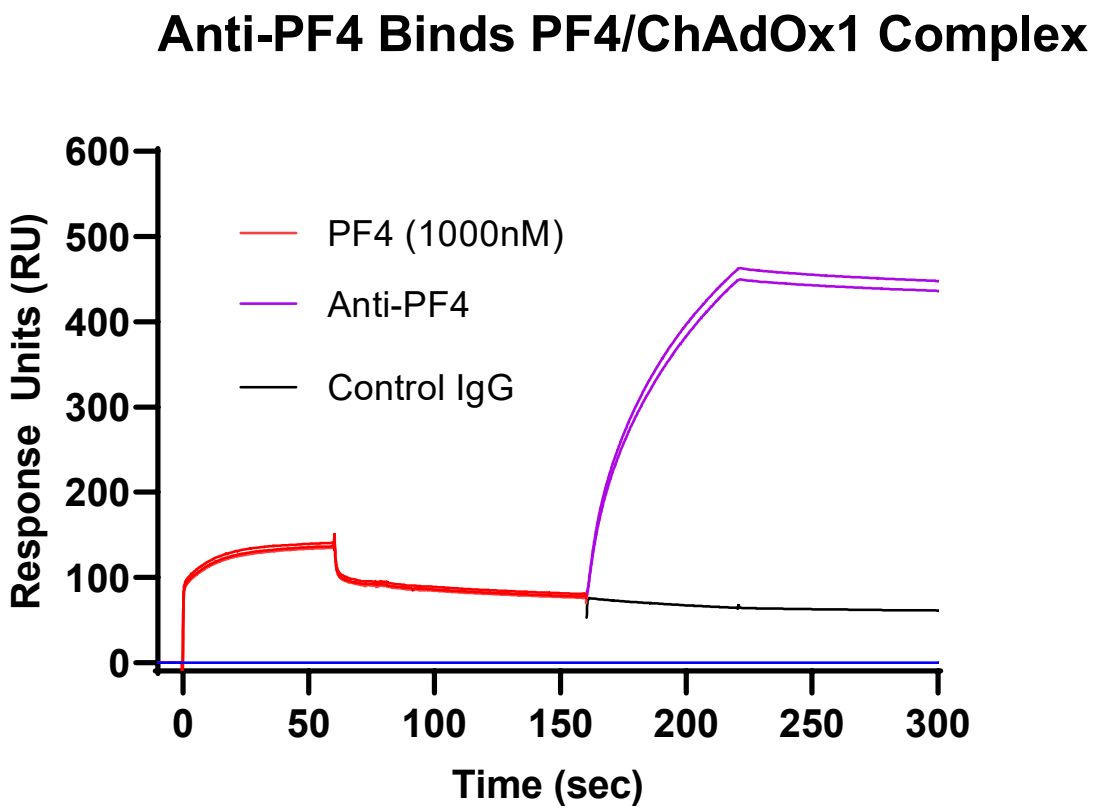
*Nguyen, T, Medvedev, N, Delcea, M, Greinacher, A. Nature communication, 2017; DOI: 10.1038/ncomms14945

†Baker AT, Boyd RJ, Sarkar D, et al. Sci Adv 2021; 7: eabl8213.

PF4 – ChAdOx1 binding assays using SPR

- Developed SPR method to measure the PF4-ChAdOx1 interaction.
- Anti-PF4 antibodies bind to the PF4-ChAdOx1 complex
  - Confirms stability of PF4 complex

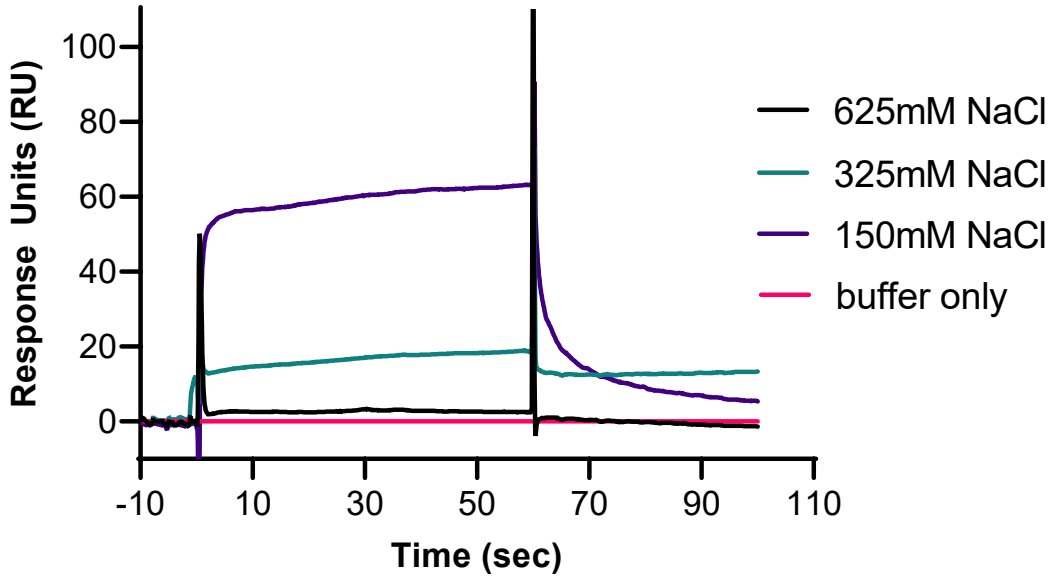


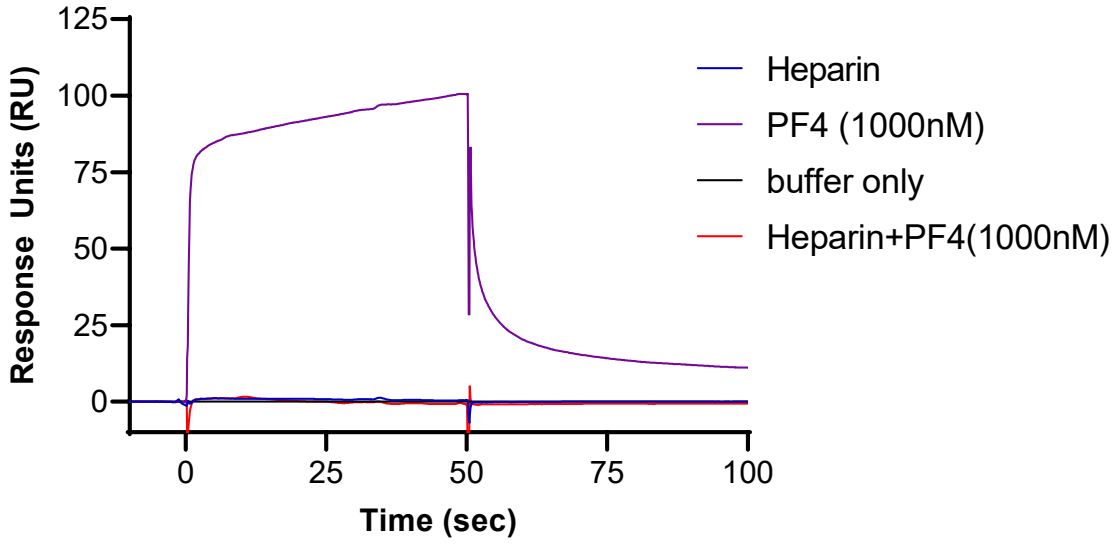


 Baker AT, Boyd RJ, Sarkar D, et al. Sci Adv 2021; 7: eabl8213.

PF4 – ChAdOx1 binding assays using SPR

- Interaction disrupted by increasing salt concentrations
  - Consistent with charge-charge interaction
- Heparin inhibits the PF4 binding to ChAdOx1





Baker AT, Boyd RJ, Sarkar D, et al. Sci Adv 2021; 7: eabl8213.

Conclusions

- Strong commitment of Oxford University and AstraZeneca to meet pandemic needs of global population resulted in successful implementation of AZD1222 at “warp speed”
- Analytical challenges met with science-based control system including extensive physico-chemical assays, biosafety, bioassays and impurities
- Demonstrating analytical comparability across a large, global manufacturing network was challenging, but statistical approach allowed for active identification and correction of site-specific differences
- Warp speed work plan required new ways of working: analytical methods optimized, qualified, transferred and validated in record time
- Rare occurrence of thrombosis thrombocytopenia syndrome involved additional analytical work on HCP assay and the development of PF4/ChAdOx binding assay