EVUSHELD: Delivering Bioassay Methods and Critical Reagents Under Highly Accelerated Timelines from Clinical to Commercial phases

CASSS BIOASSAYS 2023

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April 18th 2023

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EVUSHELD

AZD7442: AZD1061(cilgavimab) and AZD8895 (tixagevimab)

- AZD7442 is monoclonal antibodies (mAb) combination therapy comprised of 2 antibodies targeting non-overlapping epitopes on the RBD of the SARS-CoV-2 spike protein.
- The product is a co-pack of the 2 mAb and route of administration is intermuscular injection. Coformulation was investigated but never launched.
- AZ received Emergency Use Approval (EUA) for EVUSHELD in Dec 2021 and was the paused from the market in Jan 2023.
- The target population is immuno-compromised patients and is the only mAb authorized for pre-exposure treatment.
- Five mAbs authorized for EUA for treatment of mild to moderate COVID-19 disease include: **ACTEMRA® (tocilizumab),** Bamlanivimab, Bebtelovimab, REGEN-COV® (casirivimab and imdevimab), Sotrovimab.

https://www.cms.gov/monoclonal, Dippel, Andrew et al. “Developability profiling of a panel of Fc engineered SARS-CoV-2 neutralizing antibodies.” *mAbs* vol. 15,1 (2023)

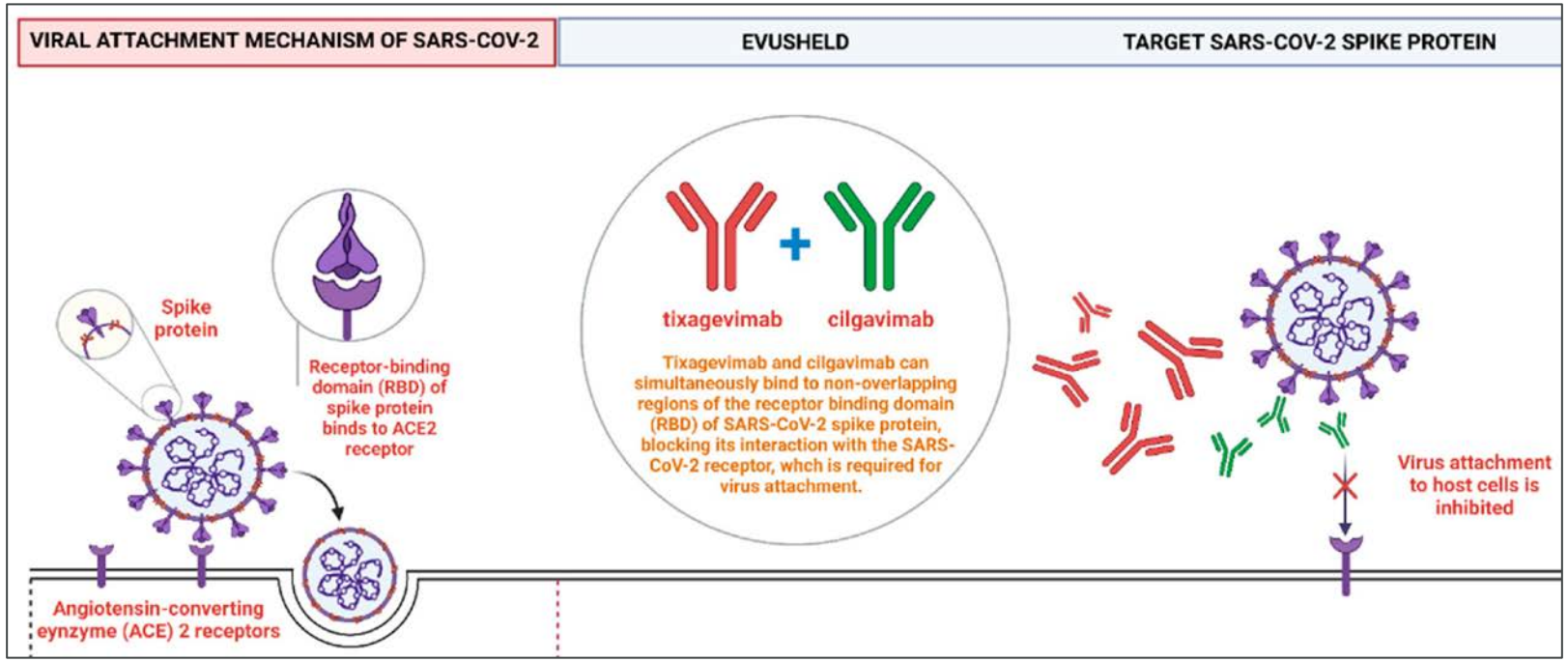
EVUSHELD

MOA: mechanism of action and engineering

MOA: Binding to RBD of SARS-CoV-2 spike protein, blocking viral entry and attachment

Molecular engineering:

- YTE mutation to increase binding affinity to the FcRn protein which increasing product half-life and thus reduces dosing frequency for patients.
- TM (triple mutation) to reduce unwanted Fc effector function, thus increasing safety profile.



Loo YM, et al. Sci Transl Med. 2022 Mar 9;14(635):eabl8124. doi: 10.1126/scitranslmed.abl8124. Epub 2022 Mar 9

Overview of Regulatory Approvals

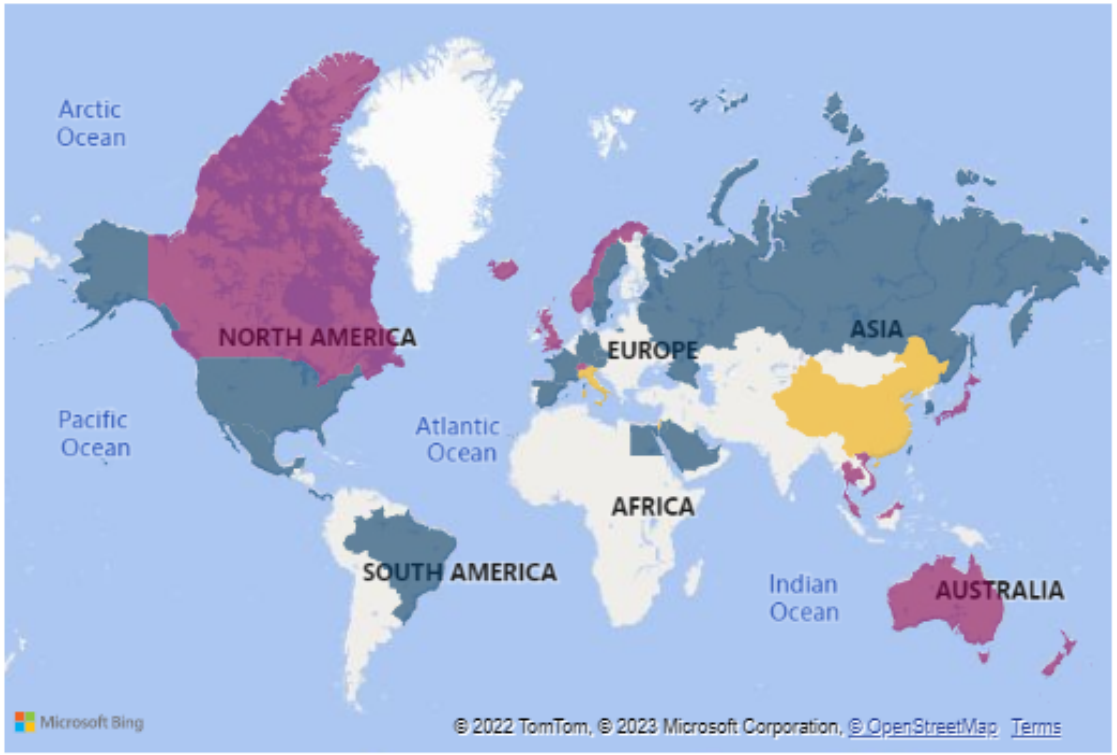
Last Modified: 15 Feb 2023 09:31 AM US ET

EVUSHELD™
tixagevimab, cilgavimab

55	22	29	4	2
Authorizations	Pathway MA	Pathway EUA	Pathway Import	Indications

Market	Area	Group	Sub-Group	Pathway	Status	Indication(s)
Kuwait	INT	MEA	GCC	EUA	Approved	Prophylaxis
Hong Kong	INT	China/HK		MA	Approved	Prophylaxis; Mild to Moderate
Brazil	INT	LATAM	Brazil	EUA	Approved	Prophylaxis; Mild to Moderate
Australia	INT			MA	Approved	Prophylaxis; Mild to Moderate
Saudi Arabia	INT	MEA	GCC	EUA	Approved	Prophylaxis; Mild to Moderate
Russia	INT			EUA	Approved	Prophylaxis; Mild to Moderate
UK	EU-CAN	EU5		MA	Approved	Prophylaxis; Mild to Moderate
Panama	INT	LATAM	CAMCAR	EUA	Approved	Prophylaxis; Mild to Moderate
Taiwan	INT	Asia Area		EUA	Approved	Prophylaxis; Mild to Moderate
Thailand	INT	Asia Area		MA	Approved	Prophylaxis; Mild to Moderate
Vietnam	INT	Asia Area		MA	Approved	Prophylaxis
UAE	INT	MEA	GCC	EUA	Approved	Prophylaxis; Mild to Moderate
Canada	EU-CAN			MA	Approved	Prophylaxis; Mild to Moderate
Costa Rica	INT	LATAM	CAMCAR	EUA	Approved	Prophylaxis; Mild to Moderate
EU (EMA)	EU-CAN			MA	Approved	Prophylaxis; Mild to Moderate
Iceland	EU-CAN	Nordics		MA	Approved	Prophylaxis; Mild to Moderate
Norway	EU-CAN	Nordics		MA	Approved	Prophylaxis; Mild to Moderate
Switzerland	EU-CAN	WESE		MA	Approved	Prophylaxis
Japan	INT			MA	Approved	Prophylaxis; Mild to Moderate
Italy	EU-CAN	EU5		Import	Approved	Prophylaxis; Mild to Moderate
Singapore	INT	Asia Area		EUA	Approved	Prophylaxis
New Zealand	INT	AU/NZ		MA	Approved	Prophylaxis
South Korea	INT	Asia Area		EUA	Approved	Prophylaxis
Mexico	INT	LATAM	Mexico	EUA	Approved	Prophylaxis

Regulatory Pathway ● EUA ● Import ● MA

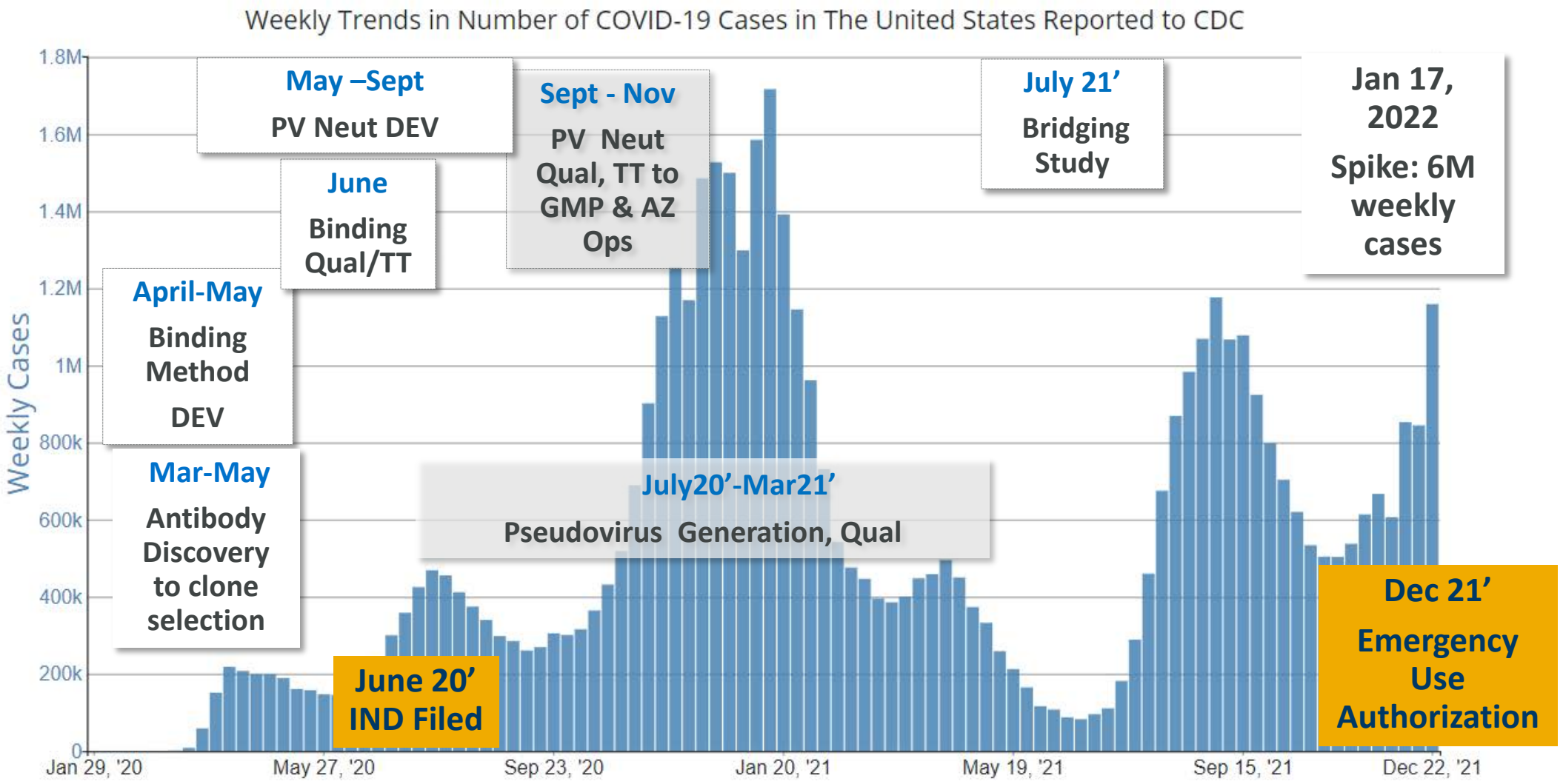


Overall Analytical Strategy

Delivery of full panel of lot release methods in 8-10 weeks.

Analytical Release Panel
Appearance - clarity
Appearance - color
Appearance – particles
Bioburden
Capillary isoelectric focusing (cIEF)
Endotoxin
High performance size exclusion chromatography (HPSEC)
Host cell proteins
Host cell DNA
Non-reducing CE-SDS
Osmolality
pH
Protein A
Polysorbate 80
Sterility
Sub-visible particles
SARS-COV-2 RBD binding DELFIA
Total protein

Accelerated Delivery of Potency Methods



https://covid.cdc.gov/covid-data-tracker/#trends_weeklycases

Bioassay Strategy

Guidance Documents for Anti-viral Products

Pre-pandemic – Dec 2020:

"Guidance for Industry: Anti-viral Products: Conducting and Submitting Virology Studies to the Agency" 71 FR 32351, first published in 2006.

January 2021:

"Guidance for Industry: COVID-19 Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting SARS-CoV-2 Infectivity."

March 2023:

"Guidance for Industry: Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens."

Regulatory Guidance released from FDA, in January 2021

COVID 19: Potency Assay considerations for monoclonal antibodies and other therapeutic proteins targeting SARS-CoV-2 infectivity

A. Methods

Although a binding assay is generally sufficient to serve as a potency assay at the IND stage of development, because it demonstrates binding between the mAb or therapeutic protein and its target, a binding assay assesses only one aspect of the potency of a product; therefore, sponsors should subsequently develop methods that more comprehensively monitor the proposed mechanism(s) of action of the products. These methods should be incorporated into drug substance and drug product release testing and stability protocols. Potency assays should be described, justified, qualified, and validated to support a BLA.

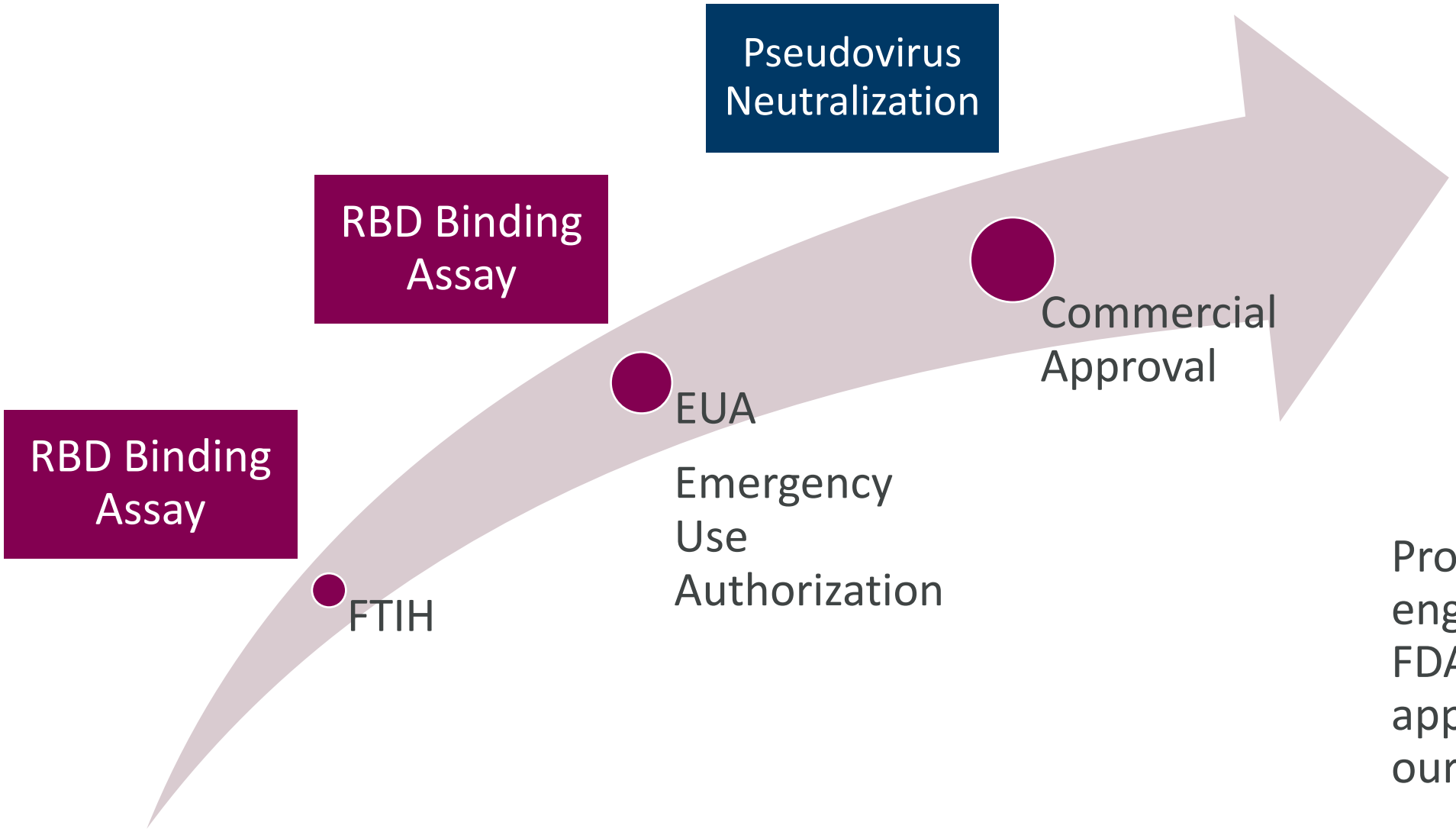
2. Viral Neutralization Assays

In comparison to binding assays, in vitro viral neutralization assays more comprehensively confirm a mAb's or therapeutic protein's mechanism of action and potency in blocking viral entry into susceptible cells. Because of the potential importance to evaluating these products, the Agency recommends establishing an in vitro viral neutralization assay early in development. This type of assay can be useful for advancing development, quality control, and characterization of neutralizing mAbs and other products targeting viral entry. The SARS-CoV-2 cellular entry includes four steps:

(1) Binding to the cell surface receptor ACE2
(2) Proteolytic cleavage of the spike (S) protein (by TMPRSS2 and/or Cathepsin L)
(3) Six-helix bundle formation leading to virus-cell fusion
(4) Release of the viral capsid into the cytosol

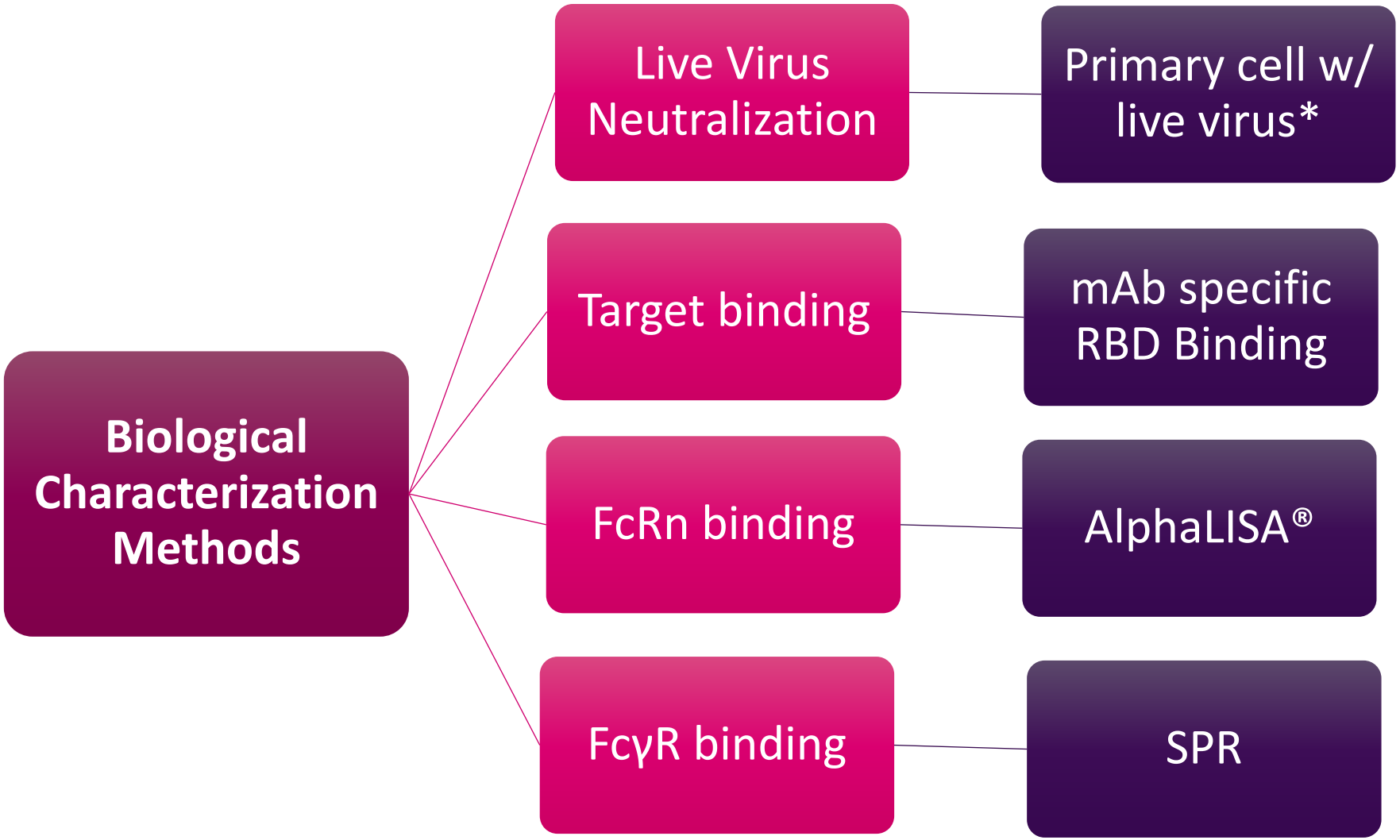
Assays that assess the ability of the protein(s) to inhibit any of these steps are predominantly cell-based assays and typically involve the use of wild-type (wt) virus, pseudotyped virus, or pseudotyped virus-like particles (VLP). When considering which method to use, sponsors

Potency Lot Release Strategy



Proactively engaged with FDA for approval of our strategy.

Characterization Strategy

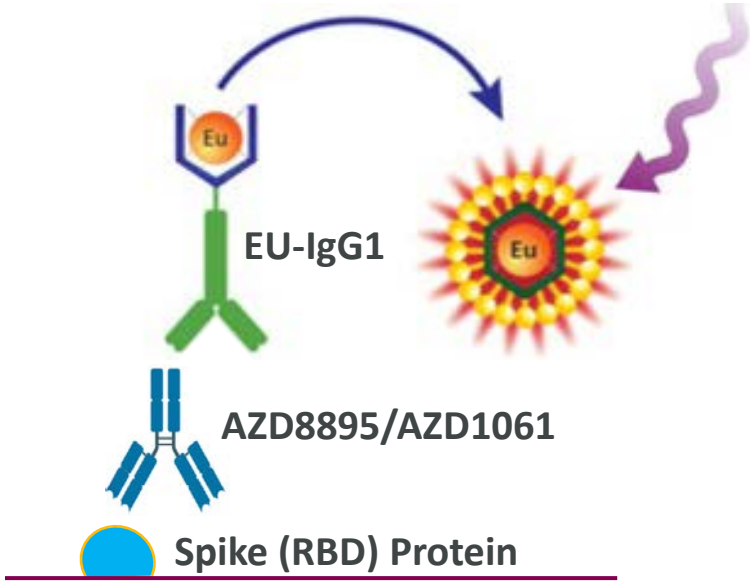


* External lab

Lot Release Binding Assay

Utilized DELFIA® binding method for rapid development

- 1 plate assay design
- Established incubation times
- Critical reagents main point of development
  - o Binding protein (Spike, S1, RBD?)
  - o EU-IgG secondary mAb
  - o Product mAb concentration and dilution interval

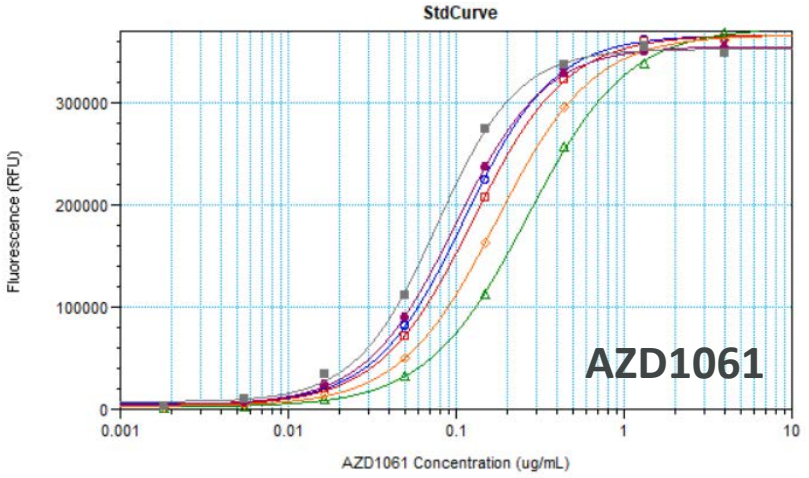


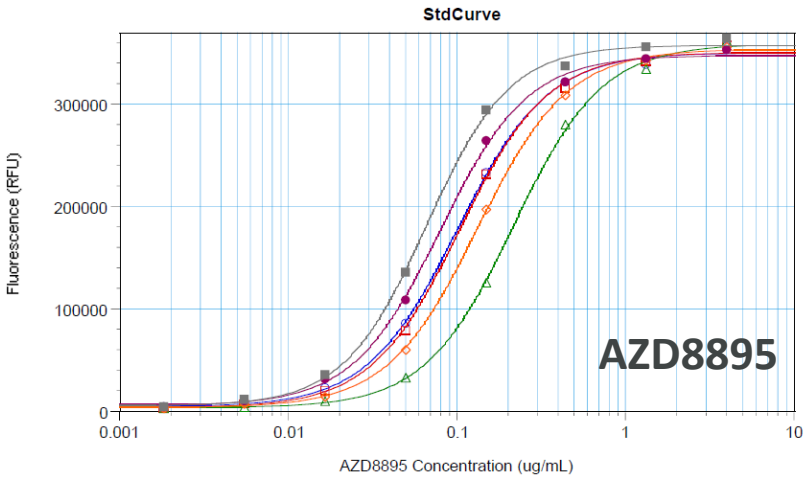
- Quick transfer to GMP lab due to analyst familiarity with DELFIA assay format
- Ability to initiate automation early

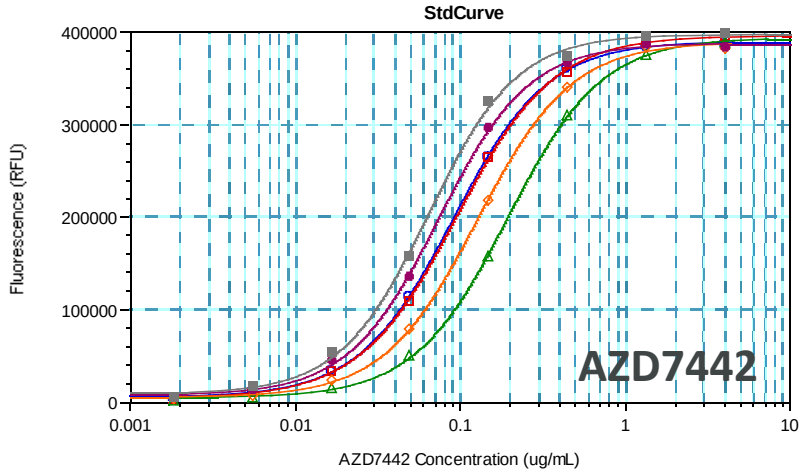
From initial clones to qualification in 2 months

Method Qualification

- Qualification was conducted according to ICH guidelines Q2 R1, R2.
- All assays passed criteria for **linearity, intermediate precision, repeatability, range (50-150%), specificity and stability indicating properties**.
- Performed method co-qualification with GMP testing group.
- Also qualified method on Hamilton Robot for DEV and GMP testing.







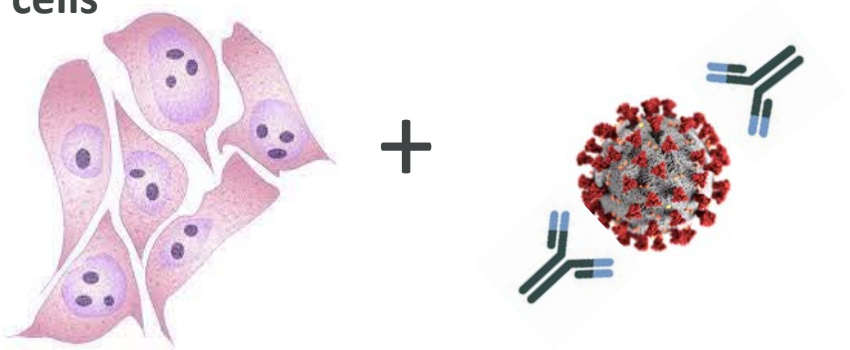
Also tested emerging strains of SARS-CoV-2 for product characterization.

Virus Neutralization Assays

Virus Neutralization Assays

Live Virus Killing Assay – BSL3

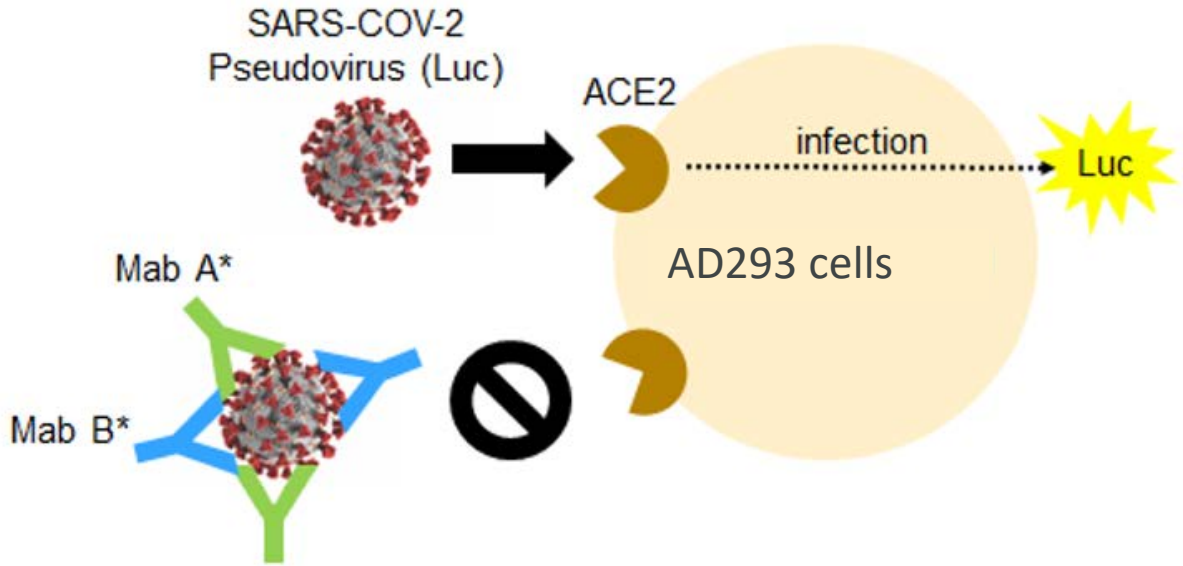
Vero cells



Cells fixed

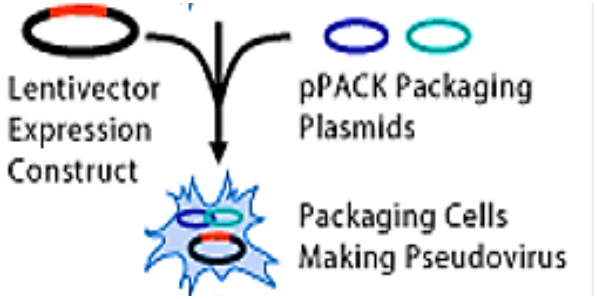
Immunoassay with
Fluorescence readout

Pseudovirus Neutralization



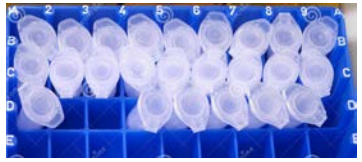
“Making a Pseudovirus small scale <3L”

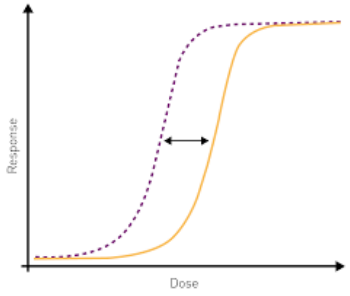
Research process in place.



Harvest

ultracentrifugation





~ 50 Individual use aliquots

- Transfection in 293X cell
- Harvest (spin and filter)
- Concentrate/purify
- Resuspend pellets
- Aliquot
- Test for activity

“But how do we Scale-up?”

Major Hurdles found during Scale-up and need for GMP quality reagents

Major Hurdles for Scale up:

1. Low virus Titer
2. Concentration Step:
   - Large volumes 3L to 10 L
   - Equipment availability
3. Speed of the process (viral activity matters)

Solution:

1. Changed the plasmid ratio
2. Moved from using an ultracentrifuge to floor centrifuge + sucrose cushion
3. Moved purification step from research to development labs


SCIENTIFIC REPORTS

OPEN

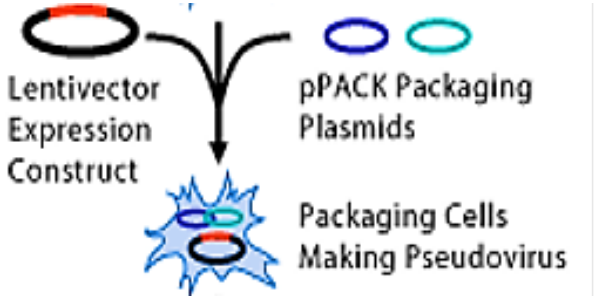
An optimized method for high-titer lentivirus preparations without ultracentrifugation

Received: 31 March 2015
Accepted: 23 August 2015
Published: 08 September 2015

Wei Jiang[1,2,*], Rui Hua[1,2,*], Mengping Wei[1,2,*], Chenhong Li[1], Zilong Qiu[1], Xiaofei Yang[1] & Chen Zhang[1]

Wei Jiang et al. *An optimized method for high titer lentivirus preparations without centrifugation*. 2015. Scientific Reports.

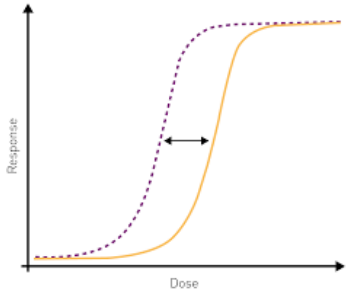
Updated plasmid ratio & Concentration Step!



Harvest

Centrifugation

Individual use aliquots



- Transfection in 293X cell
- Harvest (spin and filter)
- Concentrate/purify
- Resuspend pellets
- Aliquot
- Test for activity

New process production was successful.

“How many scientists does it take to make Pseudovirus?”

Day 1

- 7 liters of harvested virus media
- Add into 24 bottles
- Add sucrose to bottles, balance
- Load 4 centrifuges

Day 2

- Unload 4 centrifuges
- Resuspend 24 pellets, pool together
- Aliquot into 300 pre-labeled vials
- Freeze at -80°C

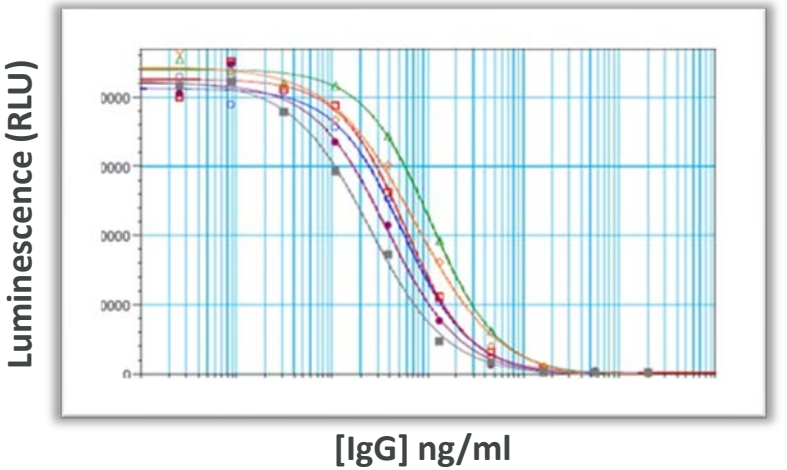
Sequence#	#vials	Dilution in assay
-Lot #5	290	1:50
-Lot #6	320	1:50
-Lot #7	324	1:50

After moving the concentration step to the development lab, we were able to make 3 active lots in sequence.

PV Method Development and Qualification

Development:

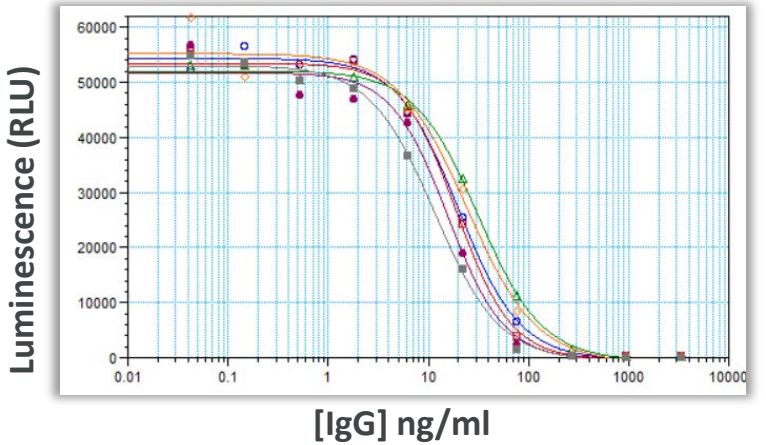
Investigated many parameters: reagent volumes, pre-plating cells, use of dextran, incubation temperature, media components; however, no major changes were made. Found that the activity of the PV was the most impactful component for method performance and variability of the upper asymptote.



Qualification:

Qualification was executed in April 2021 according to ICH guidelines Q2 R1,R2. Assays passed the qualification targets; however, results for **accuracy, intermediate precision, repeatability** were close to the limits of the acceptance criteria.

Methods for AZD1061 and AZD8895 were successfully transferred to the GMP team, commercial team and eventually to CROs in China and South Korea.



Bridging Study: DELFIA binding vs PV Neutralization

Equivalence Across Potency Range:

Potency Levels	Mean % Recovery Target Binding	Mean % Recovery PV Neut	Mean Difference	90% CI of Mean Difference	
50%	100.4	93.1	-7.3	-17.4	2.8
75%	102.1	92.8	-9.4	-19.5	0.8
100%	100.1	92.8	-7.3	-17.4	2.8
125%	101.5	105.0	3.5	-6.6	13.7
150%	105.5	100.9	-4.6	-14.7	5.6

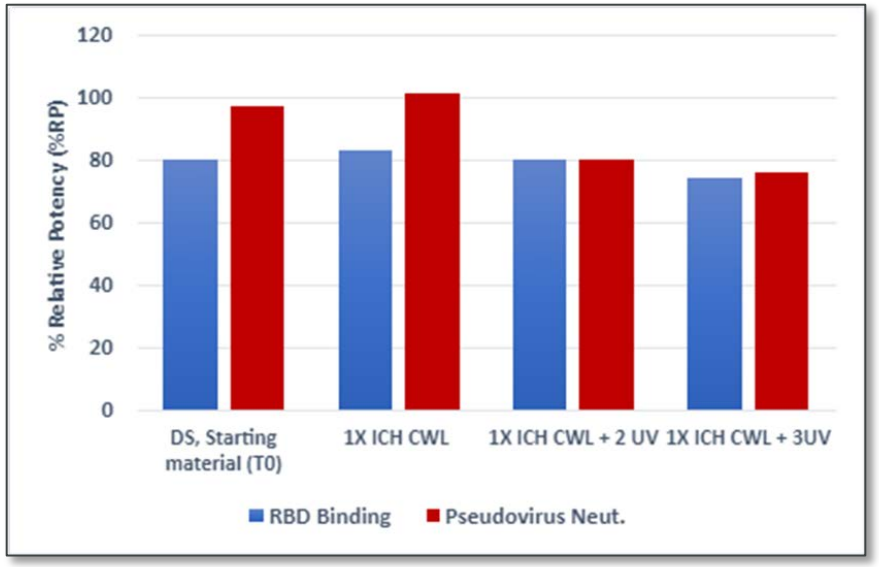
Comparison of DS Release result across multiple lots:

Mean Difference	Estimated Standard Error	90% CI of Mean Difference	
7.5	2.9	2.2	12.7

- 2 x Process 1, 2000L scale @ site 1
- 3 x Process 2, 2000L scale @ Site 1
- 6 x Process 2, 15K scale @ site 2

Comparison of stressed samples and stability samples.

No significant different in %RP between methods for samples stressed with UV or 40°C.



Remaining Hurdles for use of PV Neutralization Method

- Assay variability is higher than desired.
- Production of additional lot of virus requirement significant time resources.
- Significant time and required to meet shipping requirements for sending PV to China and possibly other countries.
- No commercial manufacturer of the PV, therefore not the most viable option for commercial lot release.
- Major re-work required to manufacturer new strains of PV.

What should be our path forward for our next generation of anti-COVID mAbs?

"Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens
Guidance for Industry"

DRAFT GUIDANCE

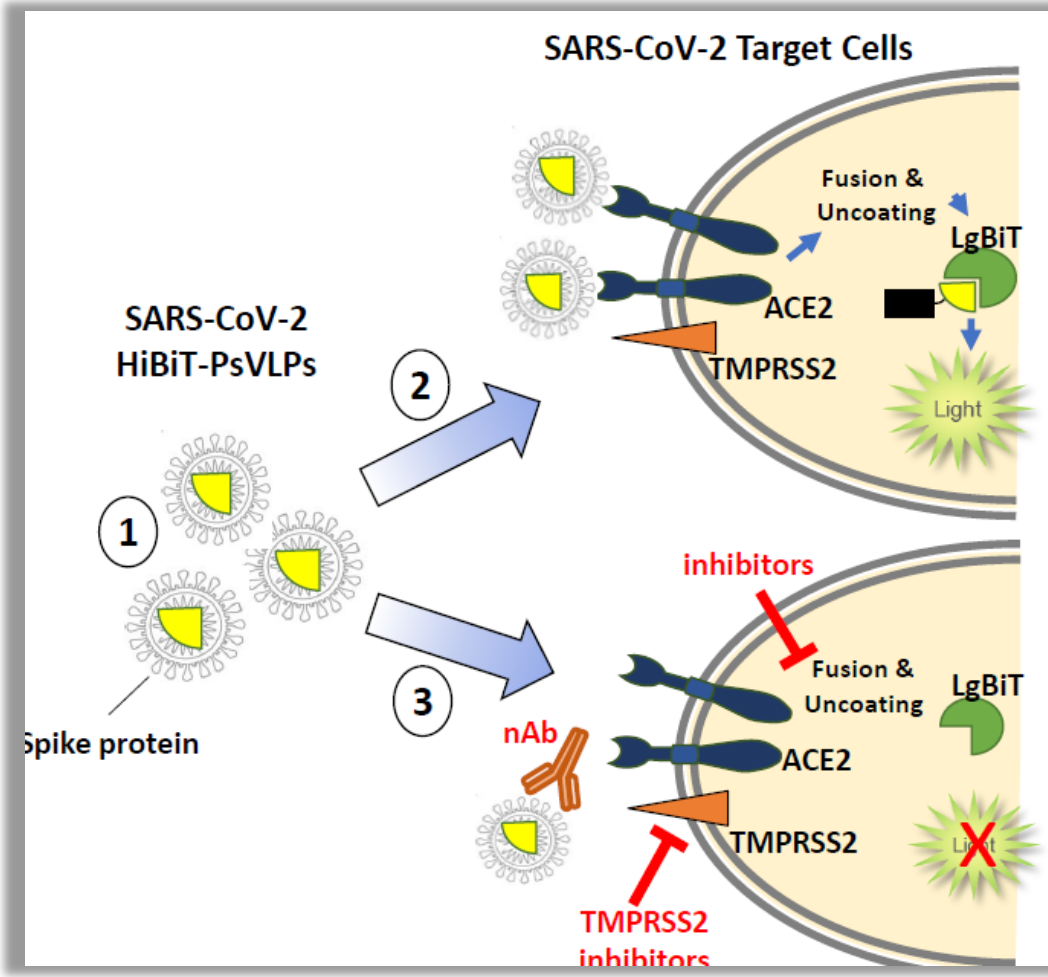
March 2023

2. Viral Neutralization Assays

In comparison to binding assays, in vitro viral neutralization assays more comprehensively confirm a mAb's or therapeutic protein's mechanism of action and potency in blocking infection of susceptible cells. Because of the potential importance to evaluating these products, the Agency recommends establishing an in vitro viral neutralization assay early in development. This type of assay can be useful for advancing development, quality control, and characterization of neutralizing mAbs and other products targeting viral attachment and entry. Given the diversity of mechanisms for viral attachment and entry into host cells, the assay should reflect that virus's mechanisms for attachment and entry.

Assays that assess the ability of the mAbs or other therapeutic proteins to inhibit any of the binding or entry steps are predominantly cell-based assays and typically involve the use of wild-type (wt) virus,[19] pseudotyped virus, or pseudotyped virus-like particles (VLP). When considering which method to use, sponsors should select a method that best monitors the binding/entry step the product is expected to target in the virus replication cycle. Although wt virus neutralization assays are considered the gold standard for in vitro potency assays, alternative methods may be acceptable. For example, a potency assay could be designed to characterize the effect of the product on a specific entry step (e.g., virus-cell fusion). Additionally, accessibility to appropriate BSL laboratories, as well as challenges to qualifying critical reagents and validating the overall assay performance, should be considered in assay selection. For methods using transfected cell lines, sponsors should also address target cell viability and variability. Whichever method is ultimately used, sponsors should observe all provisions of the select agent regulations[20] (if applicable) and other applicable governmental and institutional biosafety and biosecurity provisions.

VLP Assay System from Promega



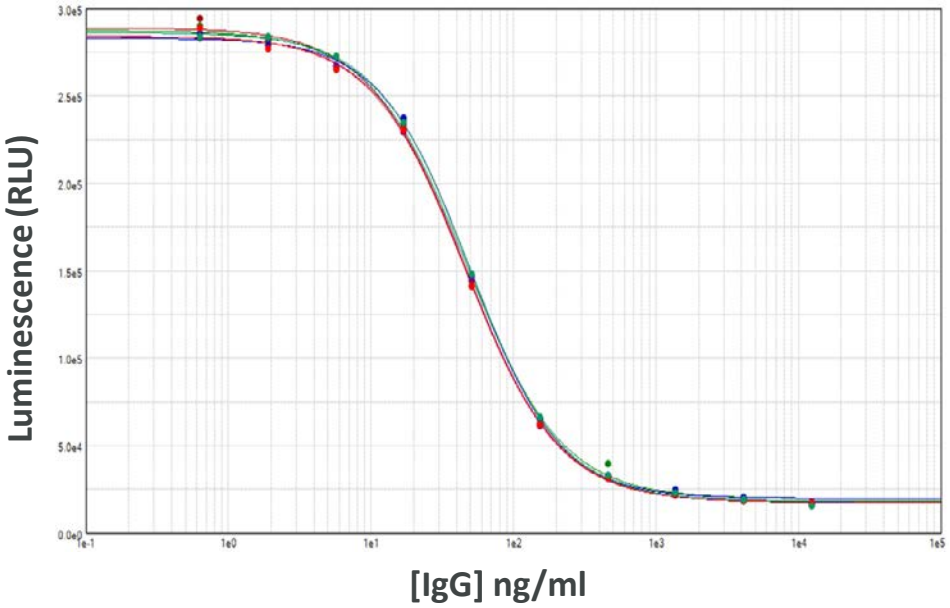
Assay Design:

1. HiBiT-tagged VLPs pseudotyped with SARS-CoV-2 Spike protein are added to SARS-CoV-2 Target Cells
   - HiBiT is packaged inside the PsVLPs
2. In the absence of inhibitors or neutralizing antibodies (nAbs), SARS-CoV-2 HiBiT-PsVLPs bind to target cells via Spike/ACE2 interaction and undergo membrane fusion mediated by cellular proteases. HiBiT is released into target cells and binds to LgBiT to generate a luminescent signal in the presence of substrate.
3. In the presence of inhibitors or nAbs of SARS-CoV-2 entry, the entry/fusion processes of PsVLPs are blocked, thereby preventing HiBiT release. No luminescent signal is produced.

https://www.promega.com/applications/infectious-diseases/viral-research/sars-cov2-therapeutic-vaccine-discovery

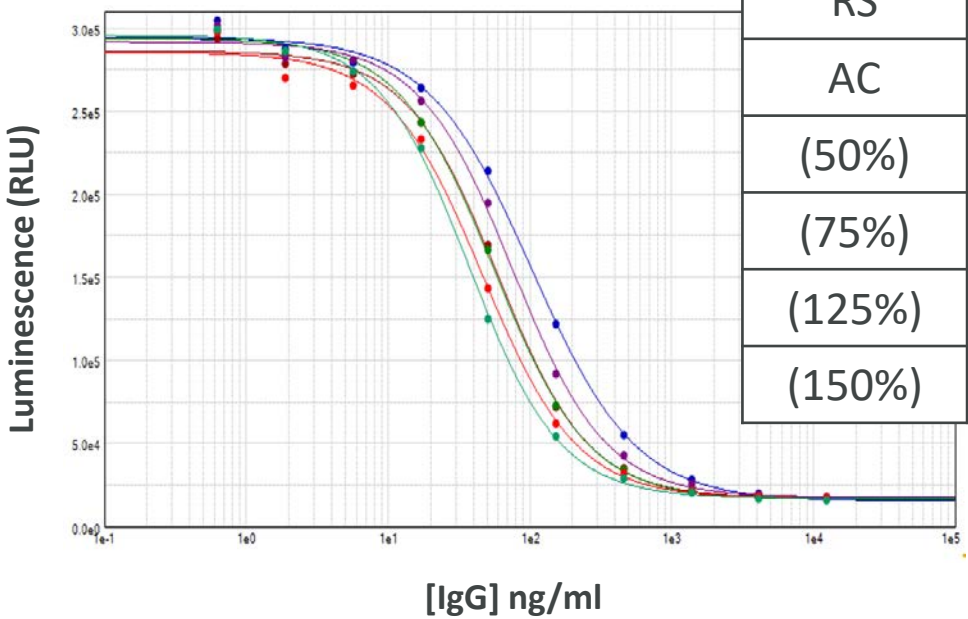
VLP Neutralization Results (omicron strain)

Repeatability



Average accuracy = 95%

Linearity



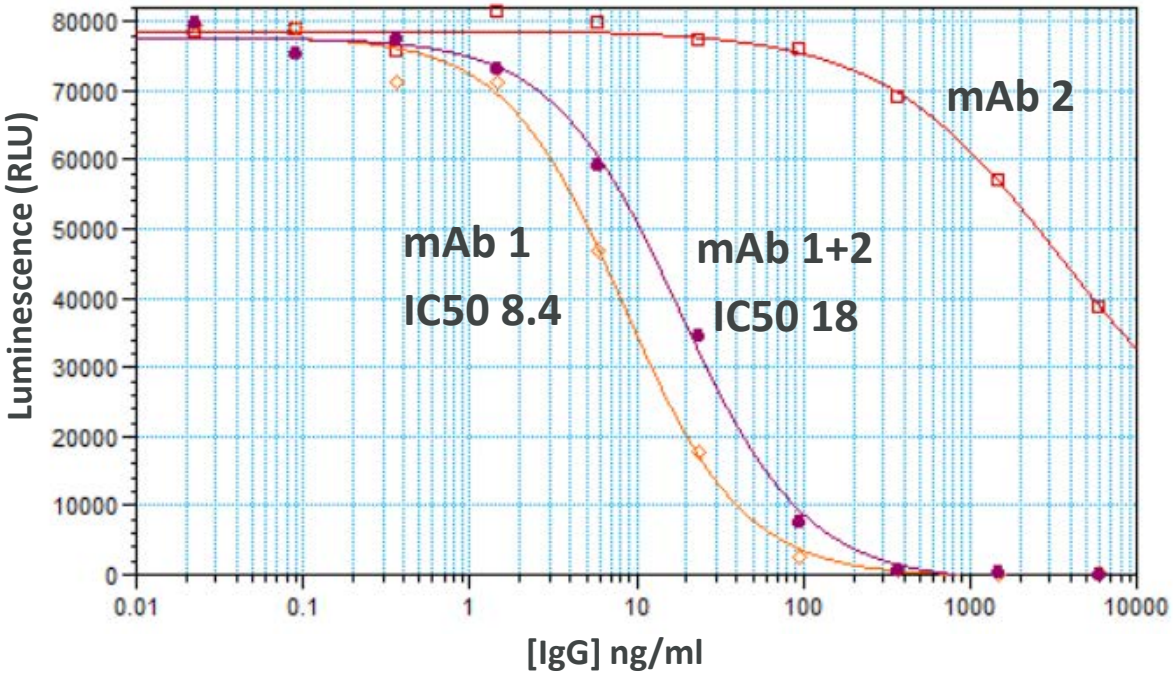
sample	% RP	% accuracy
RS	NA	NA
AC	101	101
(50%)	54	108
(75%)	74	99
(125%)	128	102
(150%)	147	98

Average accuracy = 102%

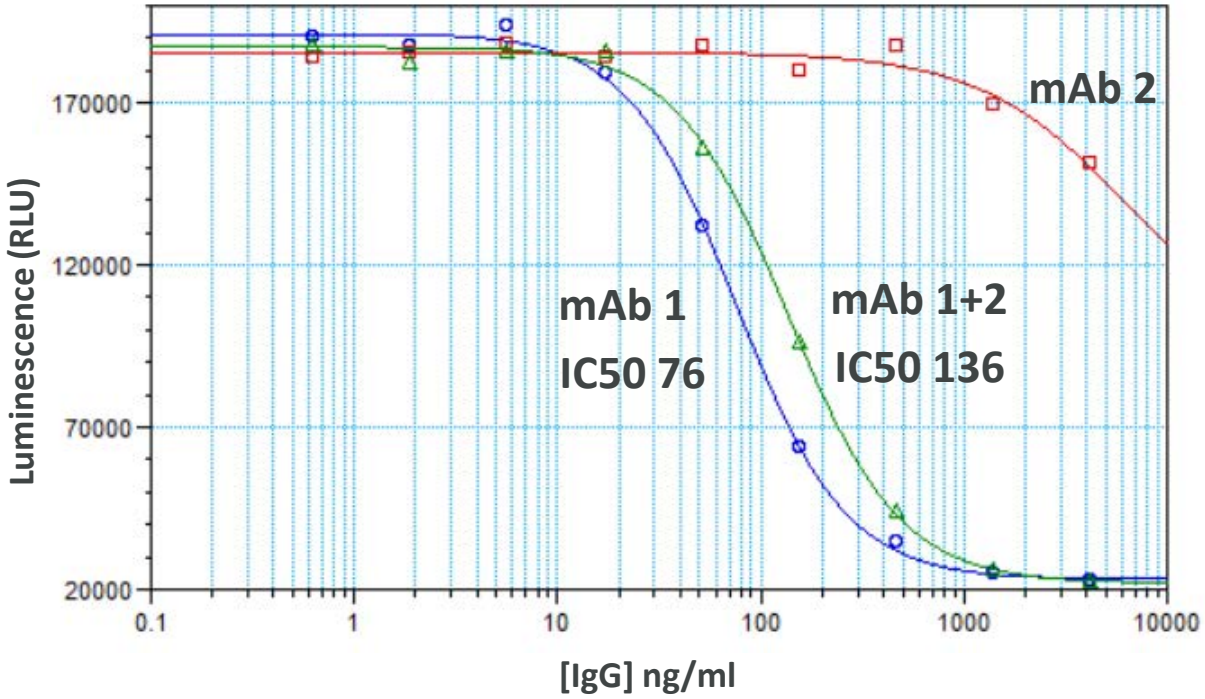
Assay system produces very accurate results with little optimization required.

PV vs VLP Neutralization Assay (omicron strain)

PV Neutralization



VLP Neutralization



Similar shifts in neutralization between antibodies indicates methods are comparable and representing virus neutralization using similar system kinetics.

PV vs VLP neutralization method:

	Pseudovirus method	VLP method
Assay incubation time?	20-22 hrs	4 hrs
Commercial source of CR?	No	yes
Inhouse plasmids needed?	Yes	No
Biosafety concerns for shipping?	Yes	No
New strains can be made rapidly?	Yes, but not in bulk. Scale up can take months of optimization and testing.	Likely yes. Would take 1-2 months for new lots. Multiple strains are available.
Method variability?	Medium	Low
Recommended by FDA as surrogate for live virus?	Yes	yes

VLP method is preferred method for testing of future anti-SARS-CoV-2 IgGs

Critical Reagents purchased/made, vialed & qualified

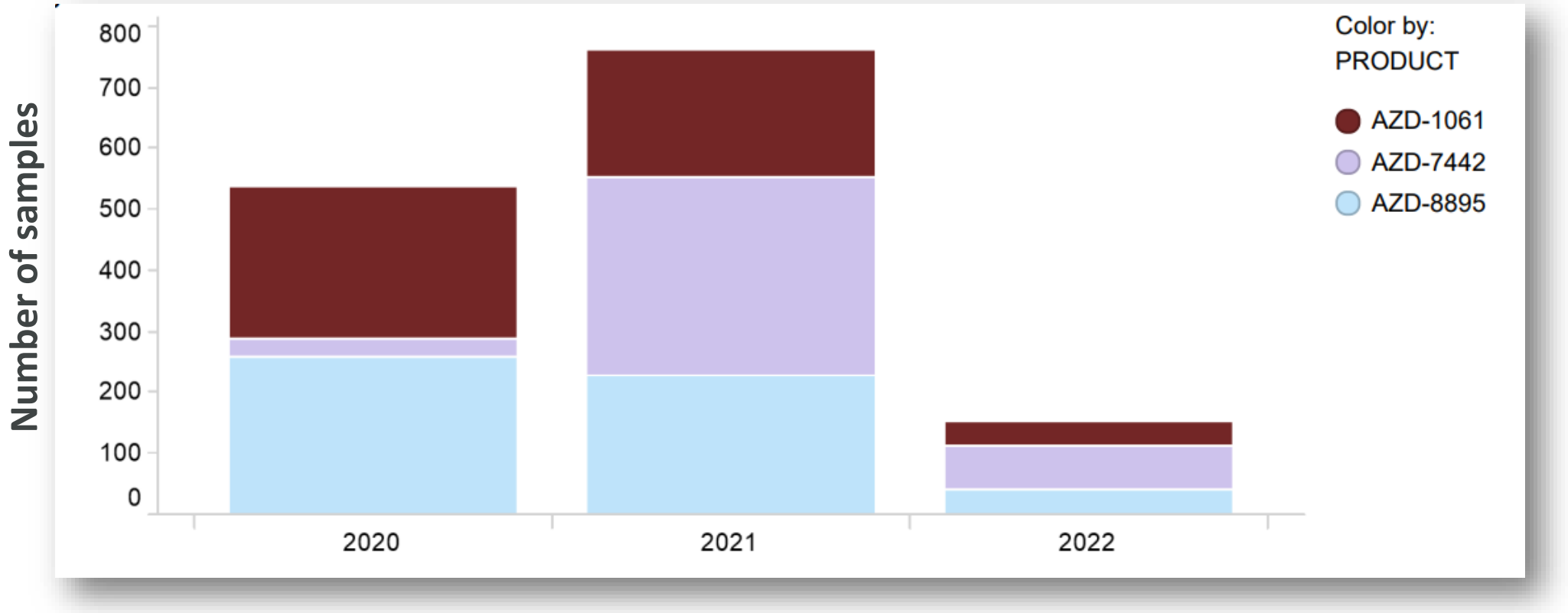
2020-22 for EVUSHELD

Reagent	# of Vials	Source
Wuhan RBD	1600	External
EU-IgG	1600	External
RBD mutant (1061)	500	AZ
RBD mutant (8895)	500	AZ
Pseudovirus Lot#1,2	580	AZ
Pseudovirus Lot#5,6,7	950	AZ
ACE-2 (ARCB) inhouse	390	AZ
ACE-2 (ARCB) contract lab	1000	External

6,120 vials

2000 Samples Tested in18 months

1,400- DEV
600 - GMP



Thank-you!