

Tide Motion® : An Adherent and Scalable Platform for Animal and Human Vaccine Production

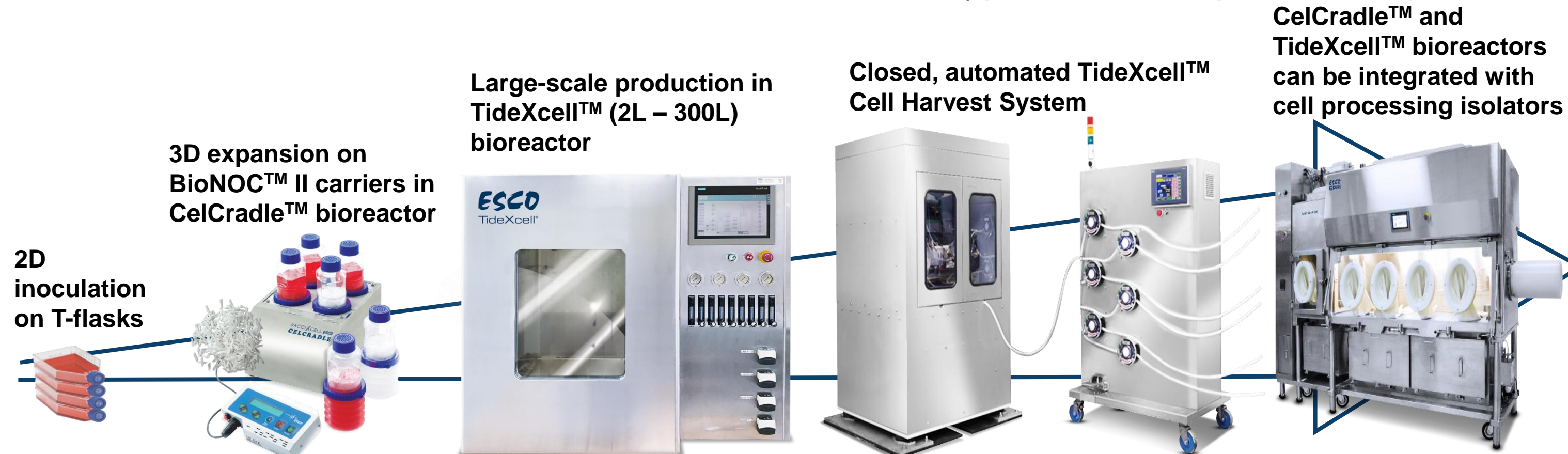
Nandini Prabhakar, Micaela Goh Yue Lyn, Arnel Sicam, and Xiangliang Lin



Introduction

Vaccination against bacterial and viral diseases is integral to prevent communicable diseases worldwide. Timely production and deployment of vaccines is required to deal with epidemic, endemic, and pandemic outbreaks of such diseases.

Esco Aster focuses on high-quality biomanufacturing of vaccines, biologics, and cell-therapy products. We demonstrate that the Tide Motion® manufacturing platform, modularly integrated with Esco Cell Processing Isolator, helps to localize vaccine production making it more affordable – in terms of CAPEX & OPEX – for developing countries by ensuring a smooth bioprocessing workflow. Illustrated below is an example of how a smooth and seamless workflow is ensured in a typical vaccine production process.



Tide Motion® Platform + Cell Processing Isolator

Animal Vaccine Production in Tide Motion® Bioreactors

We focus herein, on Animal Vaccines for diseases that plague developing countries and particularly on the cultivation of cell substrates and viruses in them for the following diseases.

Swine Vaccines

Infectious diseases pose constraints and challenges to swine production, and outbreaks of swine diseases are increasing in occurrence with globalization of the swine industry. It is imperative to address the need for a rapid turnaround time for swine vaccines, particularly **Fig. 1A** : classic swine fever (Hog Cholera), **Fig. 1B** : Porcine Epidemic Virus (PEDV), and **Fig. 1C** : Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in TideXcell™ and/or CelCradle™-500A. Viral titers are presented and the rabbit pyrogen method or the TCID50 (CPE) method was used for virus titration. **Fig. 1D** represents the equivalence to virus cultivation in roller bottles or T-flasks.

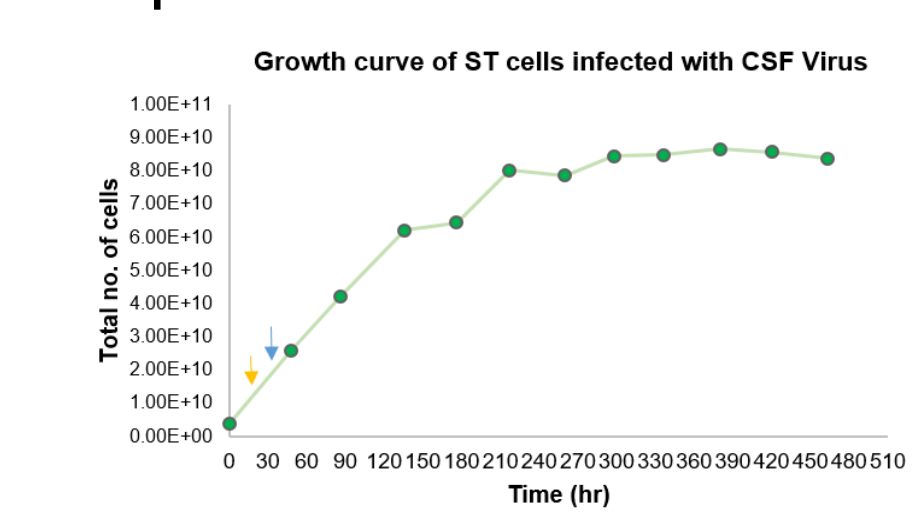


Fig. 1A Hog Cholera

Virus harvest (hr)	Virus harvest volume (L)	Virus titer (represented as virus-dilution fold)
86	8	200
136	9	700
176	9	700
217	9	700
260	9	700
298	8.5	700
336	8.5	400
380	8.5	<200
420	8.5	<200
463	8.5	<200

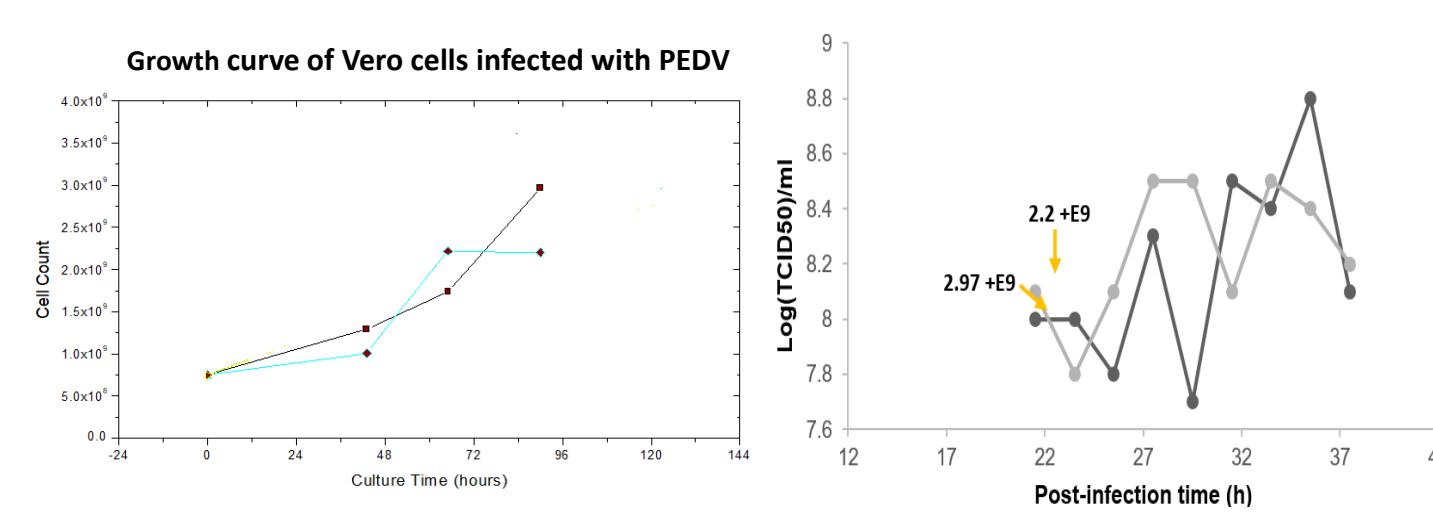


Fig. 1B PEDV

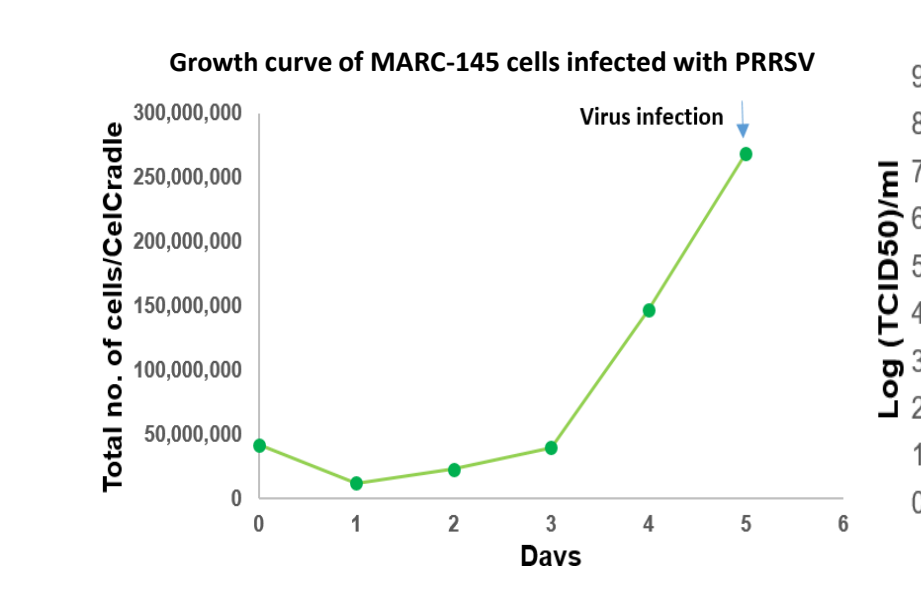
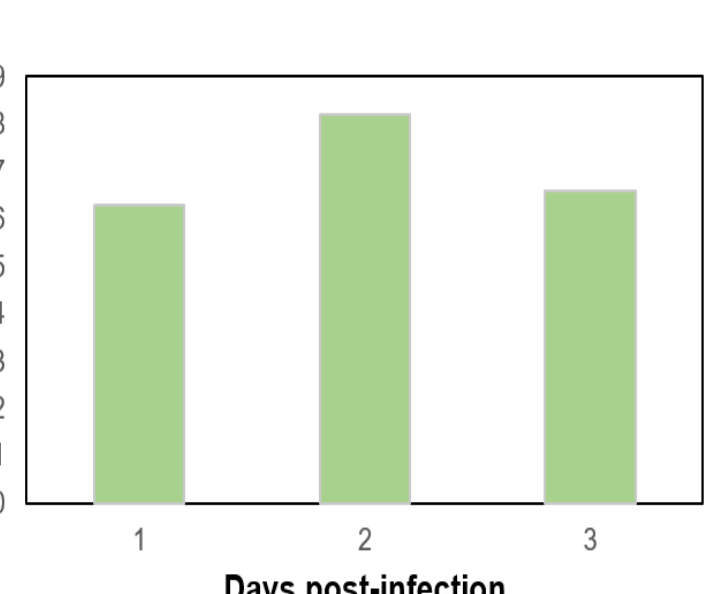


Fig. 1C PRRSV



Vaccine	2D Culture system (No. of units)	Tide Motion® Bioreactor (No. of units)
Hog cholera	Roller bottles (2500cm ²) → 123	TideXcell™-002 (1)
PEDV	T150-flasks → 285	CelCradle™-500A (1)
PRRSV	T175-flasks → 132	CelCradle™-500A (1)

Fig. 1D EQUIVALENCE

Rabies Vaccine

Canine-mediated human rabies disproportionately affects poor rural communities, particularly children, with the majority (80%) of human deaths occurring in rural areas where awareness and access to appropriate post-exposure prophylaxis is limited or non-existent. Being a 100% vaccine-preventable disease, it is of prime importance for developing countries to implement a rabies eradication program. Simplified biomanufacturing processes, utilizing TideXcell™ bioreactor in high-density cell cultures, can be implemented to produce these vaccines locally rather than relying on imports. **Fig. 2A**: BHK-21 cells were cultivated in a CelCradle™-500A; the timeline of virus production. **Fig. 2B**: the growth curve, infection time of BHK-21 cells, and medium replenishment. **Fig. 2C**: a comparison with conventional 2D systems.

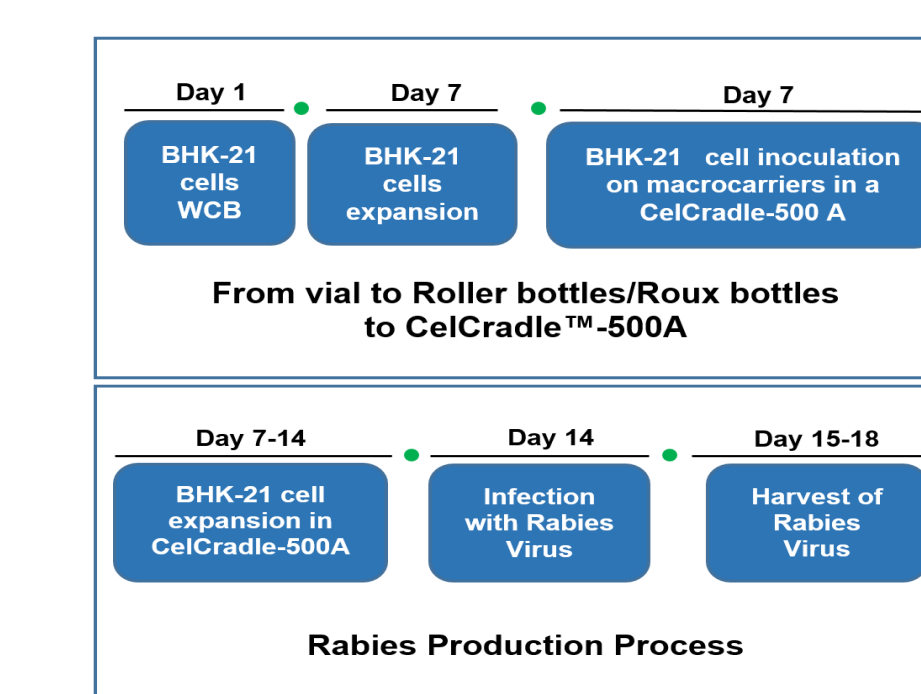


Fig. 2A

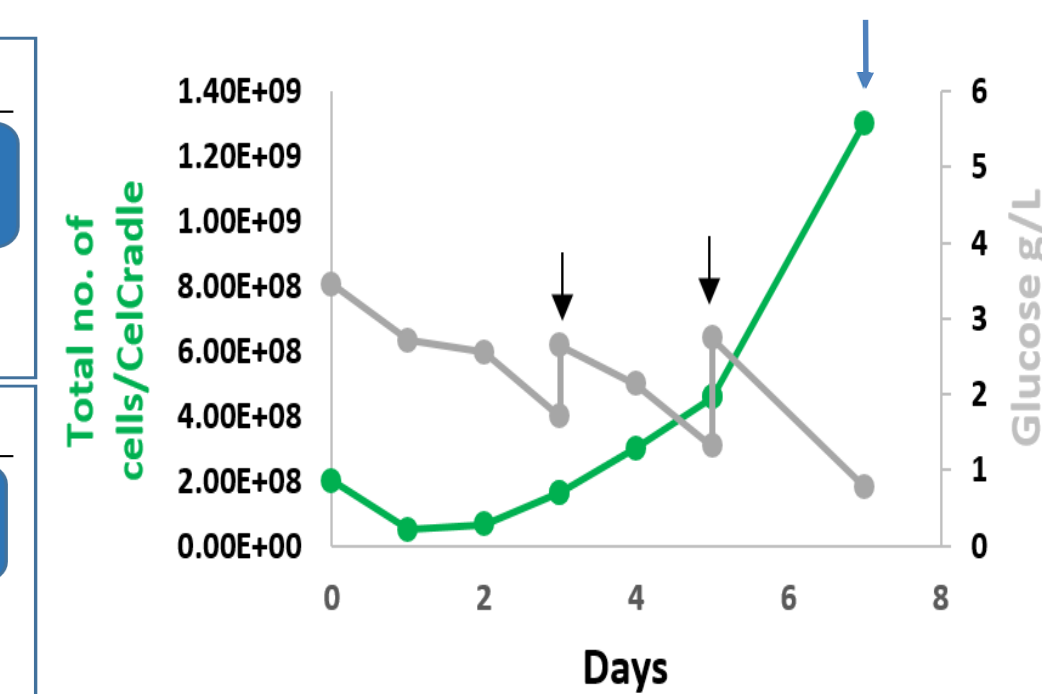


Fig. 2B

	CelCradle™-500A	Roller Bottles	Roux Bottles
Media	1000 ml	4550 ml	4550 ml
Fetal Calf Serum	100 ml	455 ml	455 ml
Culture system	1	26	13
Filtration	-	Y	Y
Washing	-	Y	Y
Packaging	-	Y	Y
Sterilization	-	Y	Y
Virus Titer	10 ^{6.7} LD ₅₀ /ml	10 ⁶ LD ₅₀ /ml	10 ⁶ LD ₅₀ /ml

Fig. 2C EQUIVALENCE

Peste des Petits Ruminants

Peste des Petits Ruminants (PPR) is an important contagious viral disease of goats and sheep often associated with high morbidity and mortality. Current disease control measures include isolation and disinfection of the contaminated environment and administration of a live attenuated vaccine. PPR virus was produced in a Vero cell seed train(**Fig. 3**); equivalence and virus titers obtained in comparison with roller bottle cultures.

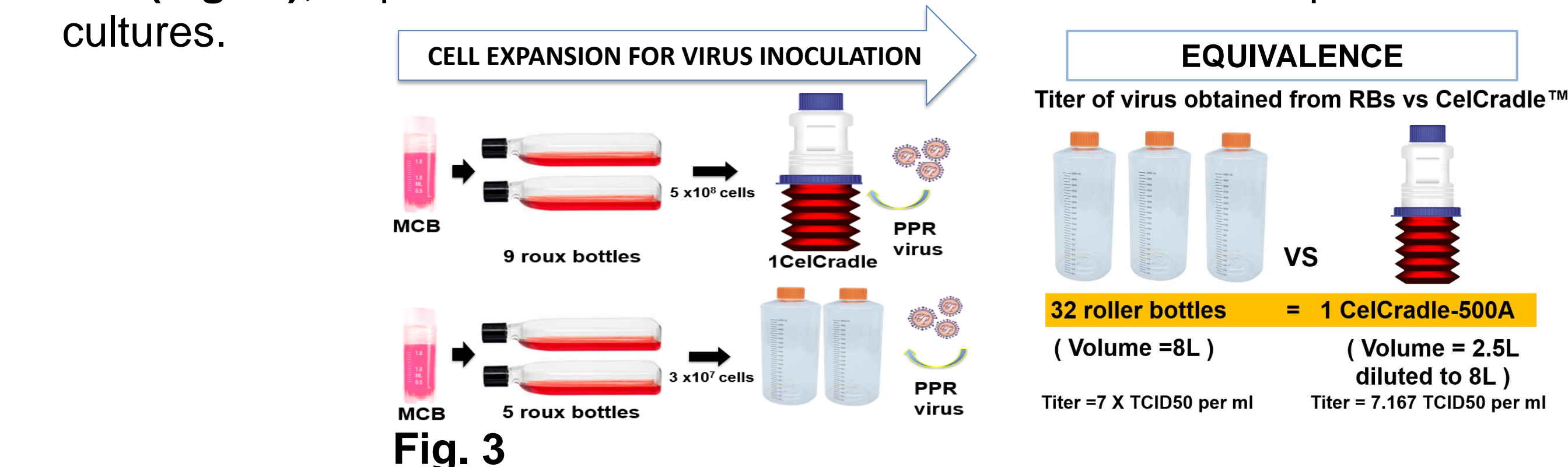


Fig. 3

Human Vaccine Production in Tide Motion® Bioreactors

The importance of human vaccines cannot be over-emphasized in terms of its impact on human health and lives. Transmission of diseases from animals to humans and reassortment of viruses poses a significant threat globally. Annual vaccination is an important strategy to prevent influenza infection during pandemic outbreaks. Timely vaccine production and deployment is essential. We describe herein the production of selected human vaccines in Tide Motion® Bioreactors.

Influenza A

Fig. 4A Vero cells were grown in serum-free media to high densities and infected with influenza A virus. The red cross denotes the time of infection. **Fig. 4B** and **Fig. 4C** represent virus titers obtained by FFU assay and comparison with conventional cell factory cultures.

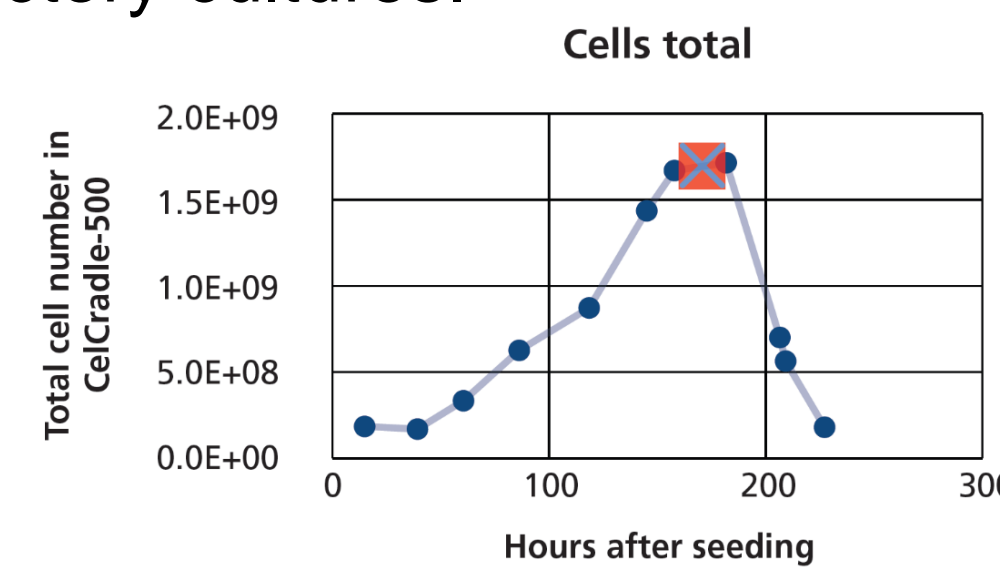


Fig. 4A

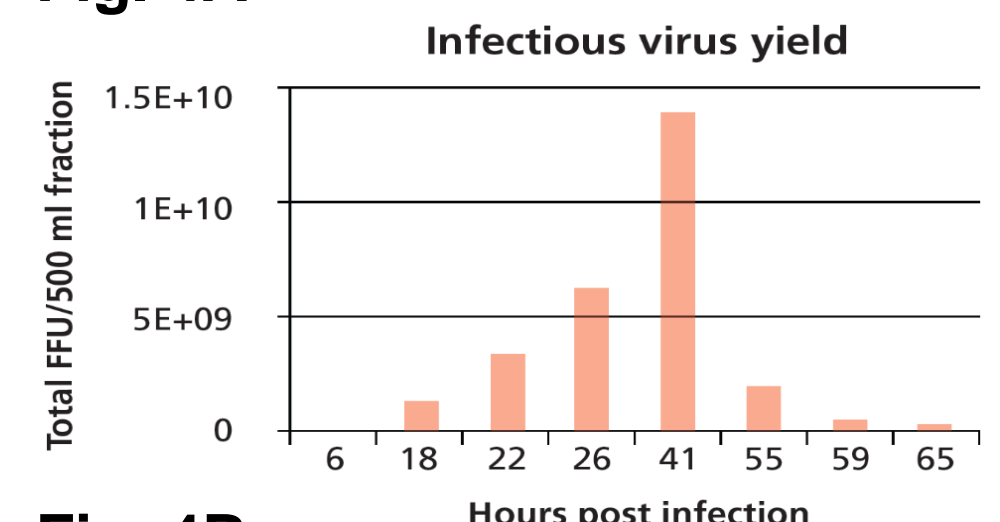


Fig. 4B

	2D cell factories CF 10	3D BioNOC™ II carriers
Cell morphology	Monolayer	Densely populated carriers
Cell density	0.7 million per ml	3.2 million per ml
Surface area	1.5L	0.5L
Working volume	0.632 m ²	1.58 m ²
To obtain an equivalent cell density	1.6 x CF10	1 x 500mL CelCradle™

Fig. 4C EQUIVALENCE

H5N1 and H1N1

Avian influenza (H5N1) also known as bird flu, is a disease of wild birds and domesticated poultry and several avian influenza strains have been known to cause illness which is deadly in humans. The virus could potentially mutate in humans and result in a pandemic or widespread outbreak of epic proportions in humans. Swine flu (H1N1) is a subtype of influenza A virus, which causes upper and lower respiratory tract infections in the host it infects. Swine influenza viruses can potentially cause infections in humans if antigenic characteristics of the virus change through reassortment.

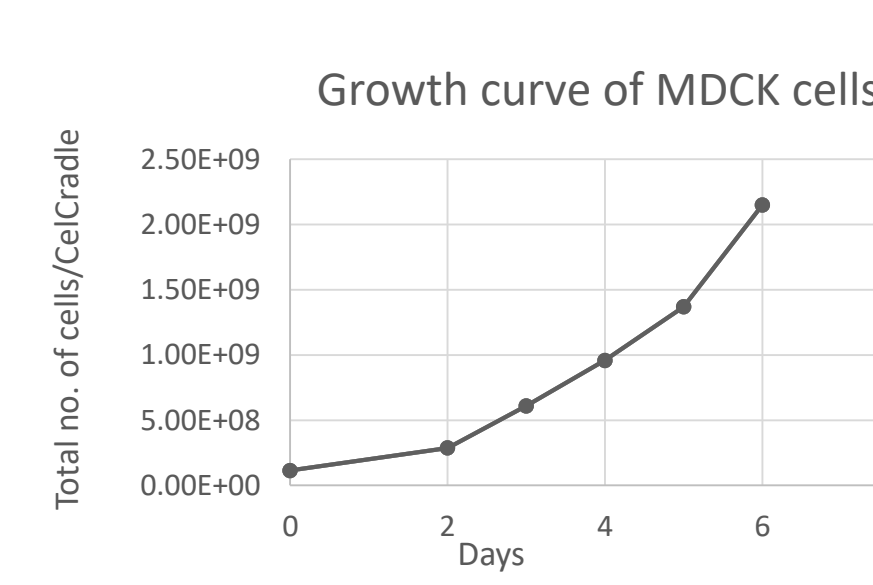


Fig. 5A

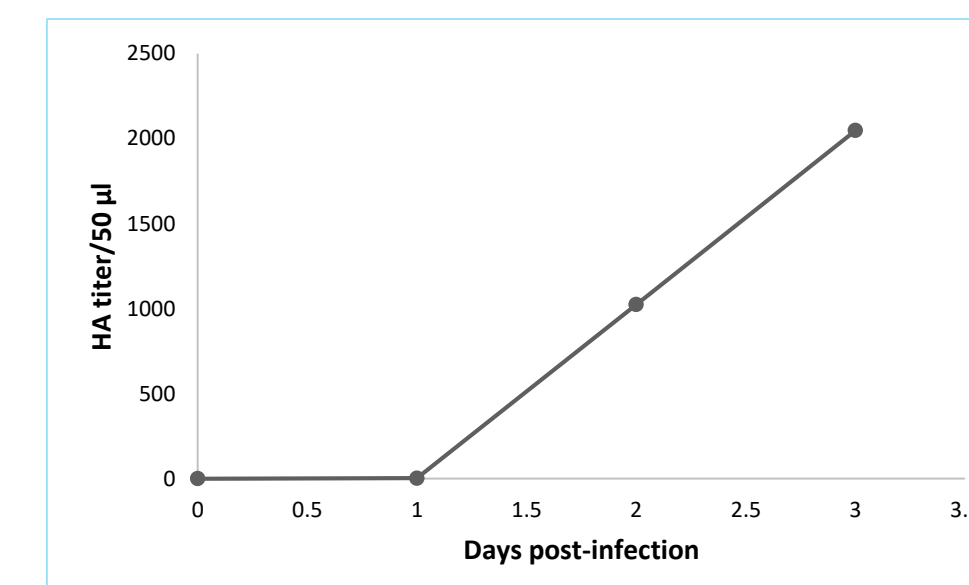


Fig. 5B H5N1

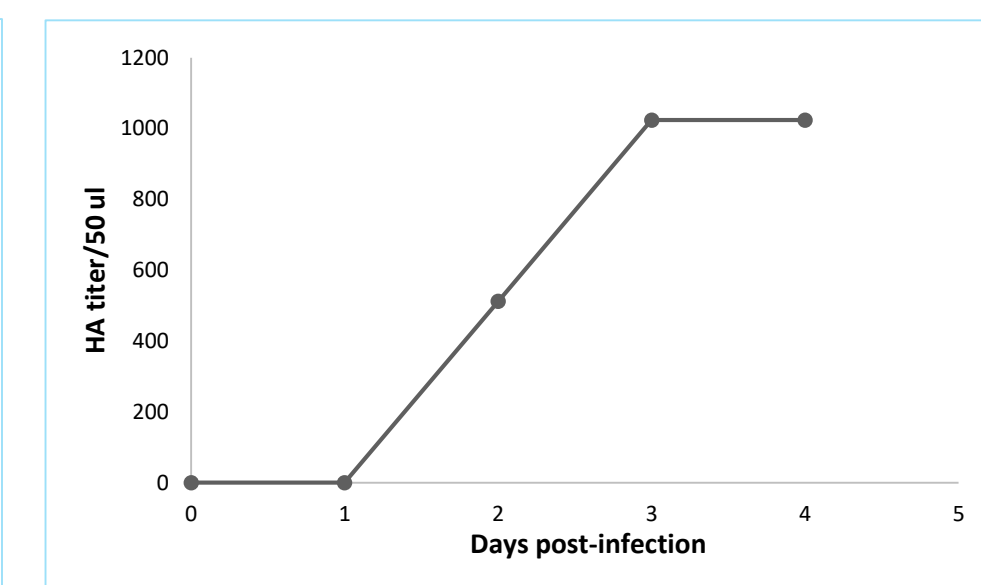


Fig. 5C H1N1

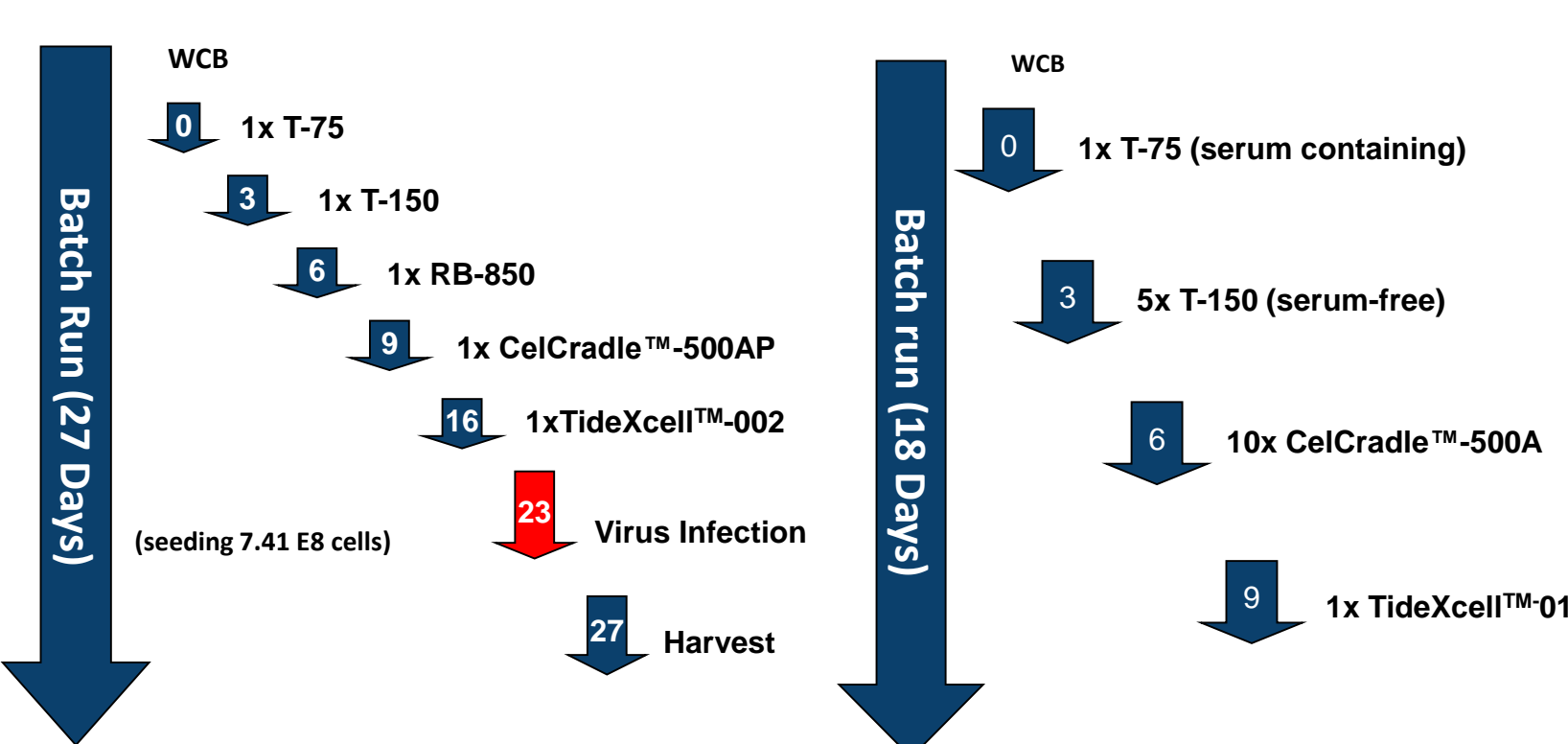


Fig. 5D TideXcell™-002

Fig. 5E TideXcell™-010

Vaccine	Roller Bottle (850cm ² , 200ml)	CelCradle™ (500ml)	TideXcell™ (2 L/10 L)
H5N1 (HA titer)	256	2048	1024
H1N1 (HA titer)	128	512	512-1024

Fig. 5F TITER

Vaccine	Roller Bottle (850cm ² , 2 L)	TideXcell™ (10 L)
H5N1	1000-2000	1
H1N1	1000	1

Fig. 5G EQUIVALENCE

Fig. 5 Cell substrates for producing the vaccines indicated were cultivated in the CelCradle™-A and/or TideXcell™-002/010 bioreactors. **Fig. 5A**, **Fig. 5B**, and **Fig. 5C** represent the growth curve of MDCK cells (in the CelCradle™-500A), which is the cell substrate for cultivating H5N1 and H1N1 viruses, and the virus titers obtained in production scale TideXcell™ 2 L and/or 10 L packed-bed volumes, respectively. **Fig. 5D** and **Fig. 5E** represent the seed train and timeline for producing the H5N1 and H1N1 viruses in TideXcell™ 2 L and 10 L packed-bed volumes. **Fig. 5F** represents comparison of titers, and **Fig. 5G** the equivalence to virus cultivation in roller bottles.

Japanese Encephalitis Virus

JEV, a flavivirus, is the main cause of viral encephalitis in many countries in Asia with an estimated 68,000 clinical cases every year. **Fig. 6A**: High-density batch cultivation of Vero cells and infection with JEV in the CelCradle™ system; **Fig. 6B**: the virus was titrated by the plaque assay method. Viral titer obtained was significantly higher than in T-175 flasks.

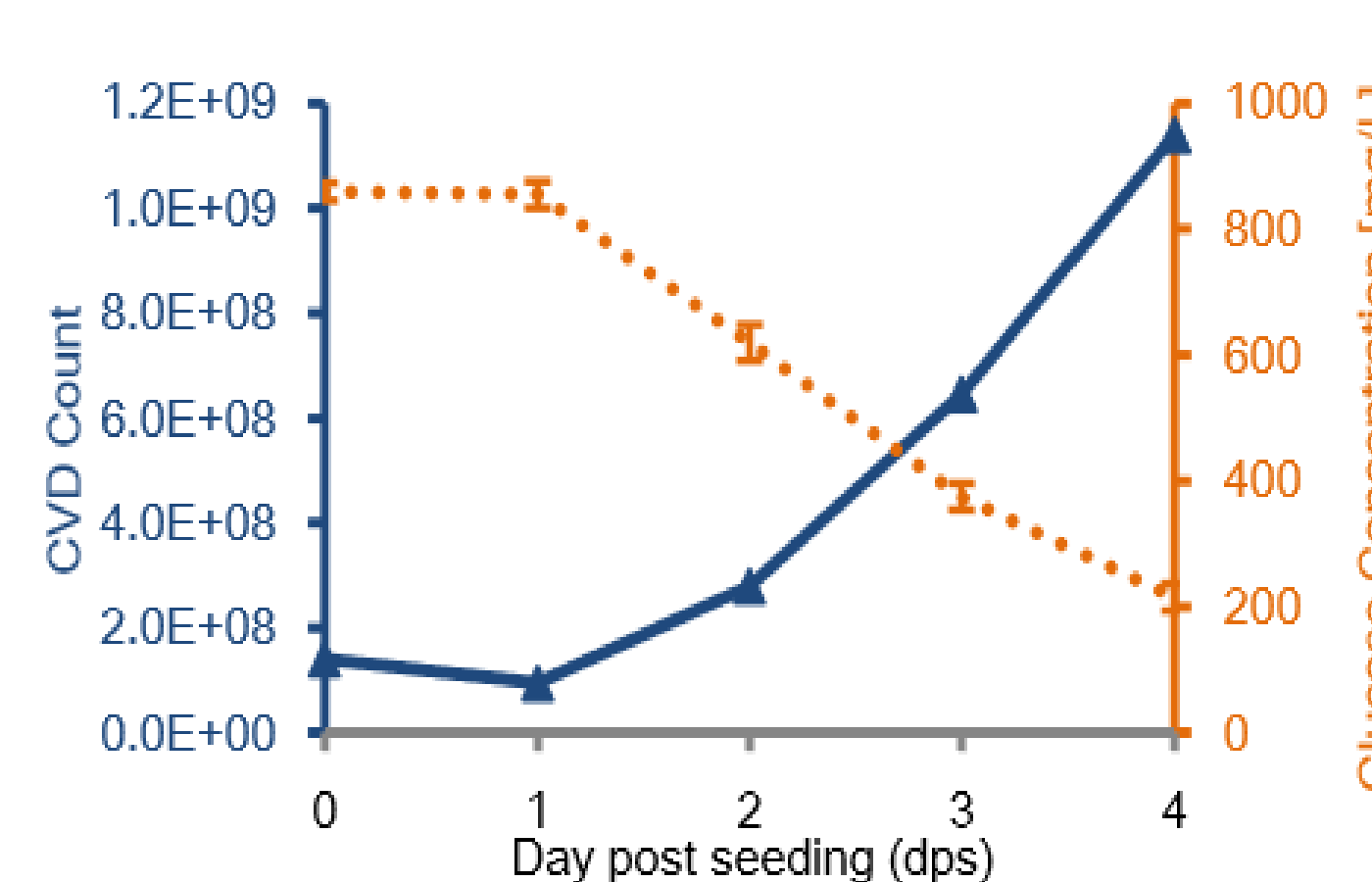


Fig. 6A

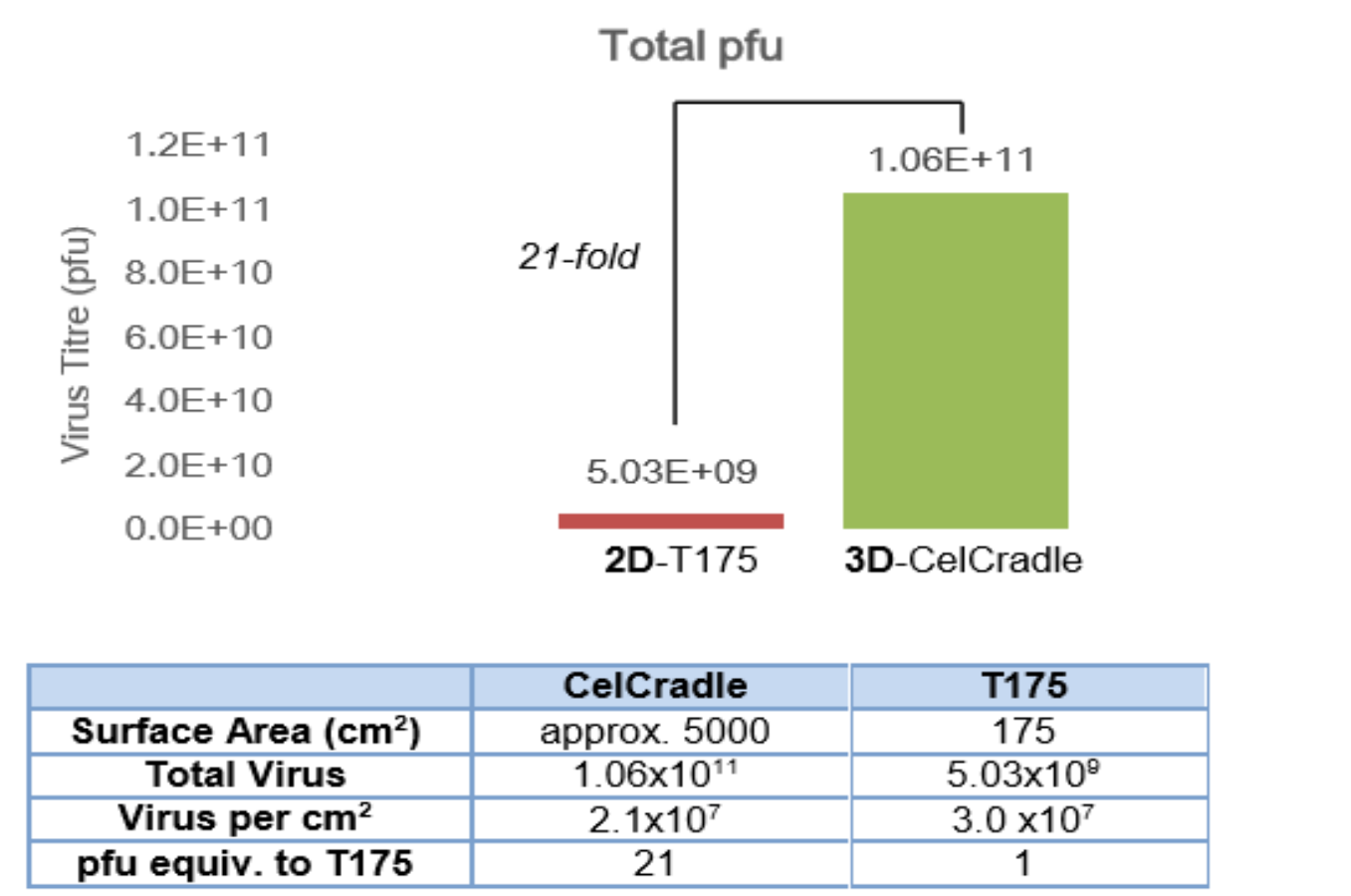


Fig. 6B EQUIVALENCE

	CelCradle	T175
Surface Area (cm ²)	approx. 5000	175
Total Virus	1.06x10 ¹¹	5.03x10 ⁹
Virus per cm ²	2.1x10 ⁷	3.0x10 ⁷
pfu equiv. to T175	21	1

Viruses in the Pipeline

- Marek's Virus
- Hepatitis C
- Yellow Fever

Conclusion

Vaccine manufacturing processes should offer a broad portfolio as well as faster reaction times to deal with pandemics and epidemics. Factors to be taken into consideration are cultivation systems and scale-up strategies. Tide Motion® bioreactors offer an excellent option to replace conventional culture systems in terms of economy, ease of production, higher process control, and high viral titers while ensuring product quality.