Esco Aster Tide Motion Vero Platform

Introduction

Vero cells are anchorage - dependent cells that are widely used for Vaccine production and have been derived from the epithelial kidney cells of African Green monkeys. They have many advantages in terms of high viral infectivity and are thus very effective for primary virus isolation. The array of viruses that Vero cells are susceptible to and the various Viral Vaccines that can be produced in these cells are Influenza Rabies, Reovirus and Japanese encephalitis virus besides being used as a cell substrate for amplifying Oncolytic Viruses. (Ammermann *et al*, 2008)

Considering the multitude of advantages of Vero cells, it is desirable to cultivate Vero cells to a high density in order to optimise virus production. The cells are conventionally grown in T-flasks and Roller bottles in 2-D culture. We culture Vero cells to an optimal density using macrocarriers - BioNOC™ II which form the heart of the Bioreactor technology. Scalable and robust bioreactor technologies that can be applied in a large-scale industrial setting are of paramount importance in Vaccine manufacturing among other applications.

Our Vision

Our primary vision is to help non-vaccine producing countries in their endeavor to be self-sufficient in the manufacturing, storage and distribution of Vaccines. For this purpose, we have partnered with a Biotech company Nuvonis (Austria) to establish efficient bioprocessing workflows that would enable the generation of influenza virus using Nuvonis serum-free Vero cell banks. Besides this since Vero is the first CCL to be approved for Vaccine production as a cell substrate , we use it for other Vaccines against emerging Viral diseases and for Oncolytic Virus production

Benefits

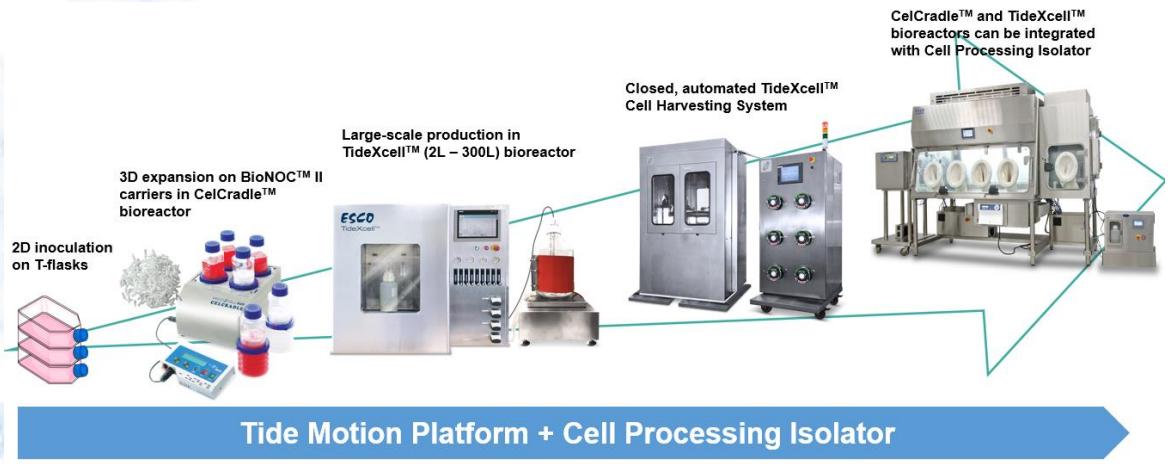
- Fully qualified cGMP cell line
- Available MCB and WCB (adhering to ICH Q5 guidelines)
- Scalable to large volumes
- Excellent ability to support the growth of Virus
- Good track record in technology transfer

Vero Cell Technology

A serum-free Vero cell research cell bank (passage 144-160) is used for all applications. The cell banks –both MCB and WCB banks have been fully characterized including tumorigenicity testing at the end of production level (EOP).These cell banks can be used as growth substrate for a variety of viruses applied in modern vaccine development.

ESCO™ ASTER

Esco Aster's Efficient Bioprocessing Workflow



The vision of Esco Aster is to focus on high-quality biomanufacturing of vaccines, biologics, and cell-therapy products to help non-vaccine producing countries attain self-sufficiency in manufacturing, storing, and distribution of vaccines. The above graphic represents an efficient bioprocessing workflow. Vaccines such as Influenza which are typically grown on Vero cells can be efficiently produced in a bench- top bioreactor and thence in a production scale TideXcell™ A cell harvester is integrated into the above workflow in order to harvest cells for Biomass, cell banking or to obtain intact cells for intarcellular viruses.

In addition, the Tide Motion manufacturing platform plus associated downstream processes modularly can be integrated within an Esco Cell Processing Isolator which is optimum for process intensification for vaccines using live viruses. In these cases the need for complex BSL3/4 facilities can be circumvented. In this way, vaccine production is made more affordable - in terms of CAPEX & OPEX.

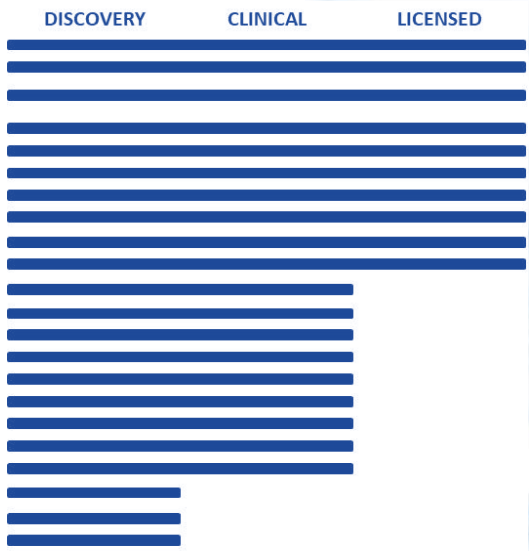
Brief overview of Vaccines produced in Vero cells worldwide

Vero cells are regarded as the workhorse of the Vaccine industry and as such, are a widely used as a cellular substrate for Vaccine Research and Viral Vaccine production.(Osada *et al*, 2014) This cell line provides genetic stability of the hemagglutinin molecule while maintaining the antigenic properties of human-derived viruses, and it has worldwide regulatory acceptance. Besides these obvious advantages, it is the first continuous cell line CCL .The following figure is an overview of Vaccines at various stages of development (research, clinical trials or licensed)

An array of vaccines is produced worldwide on Vero cell substrates. The following diagram is representative of the various stages in the development of different Vaccines worldwide over the last 2 decades.

ESCO™ ASTER

VIRUS	VACCINE
Influenza	H5N1
Japanese Encephalitis	Live recombinant vaccine (IMOJEV®, JE-CV®, ChimeriVax-JE®)
Japanese Encephalitis	Inactivated Vero cell-derived vaccine (JEEV®, SA 14-14-2 strain, attenuated, IXIARO® and JESPECT®)
Poliovirus	IPV
Poliovirus	sIPV
Poliovirus	OPV
Rabies	PVRV, Verorab
Rotavirus	RIX4414
Rotavirus	RV5
Smallpox	ACAM2000 (Dryvax)
Dengue	VDV1, VDV2
Enterovirus	EV71
Influenza	Trivalent Seasonal Influenza Vaccine
Rabies	CPRV
Rabies	WHO Candidate 7th International Standard
Ross River Virus	RRV Vaccine
SARS-CoV	SARS-CoV
Tetanus and Diphtheria, and Polio	TdcP-IPV
Zika	Inactivated whole ZIKV antigen
HIV	HIV vaccine
Influenza	H7
Measles	

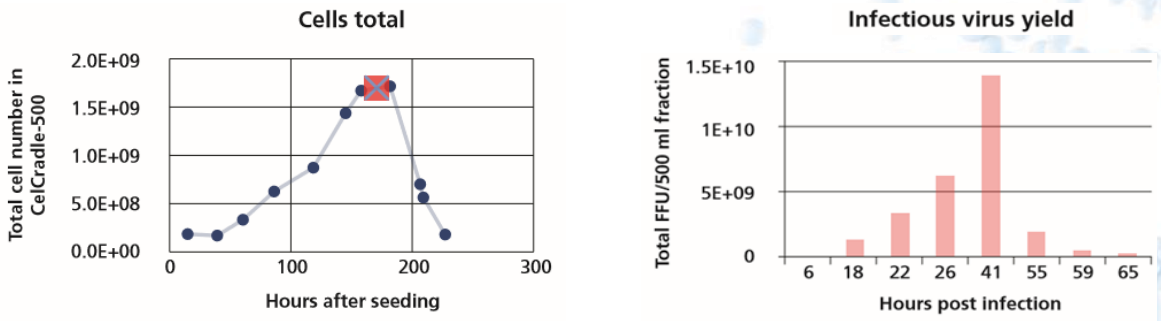


Model Vaccines produced in Vero cells – Influenza A and Japanese Encephalitis virus (JEV) at Esco Aster

Influenza A

A serum-free Vero cell research cell bank (passage 144-160) was used. Serum Free Media was supplemented with L-glutamine before use. A recombinant influenza A model virus was used for this proof-of-concept study. The infectious titre was determined by a Fluorescent Focus Assay

Influenza A Virus Production



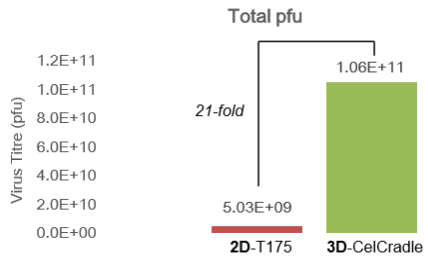
Vero cell growth in serum-free OptiPro SFM medium. The red square indicates the time of infection at 161 hours after seeding (left panel). Infectious virus titre in log FFU/ml (right panel).

	2D Culture Cell Factories CF10	3D BioNOC™ II Carriers
Cell Morphology	Mono/bilayer	Densely populated carriers
Cell Density	0.7 million per ml	3.2 million per ml
Working Volume	1.5L	0.5L
Surface Area	6.320cm^2	15.000cm^2
To obtain 1.6^9 cells	1.6 x CF10	1 x 500ml CelCradle

Comparison between 2D culture systems and the 3D system of the Tide motion Bioreactors demonstrates the significant increase in cell –yield from the latter.

ESCO™ ASTER

Japanese Encephalitis Virus (JEV)



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

Virus propagation using the 3D culture system in a CelCradle is equivalent to that produced in 21 flasks of the T175 flasks and is significantly higher in titre.

The CelCradle™-500AP was used for the efficient culture of Vero cells and production of JEV vaccine. The overall virus yield for one bottle was $1.06x10^{11}$ pfu (plaque assay);as shown below, one CC-500AP bottle has a similar footprint to a T175 flask but produces virus with a 21-fold higher productivity. Note that these high titres represent unoptimized culture conditions and subsequent experiments to determine the best operating parameters for Vero cell cultivation and JEV production will likely increase the yield even further.

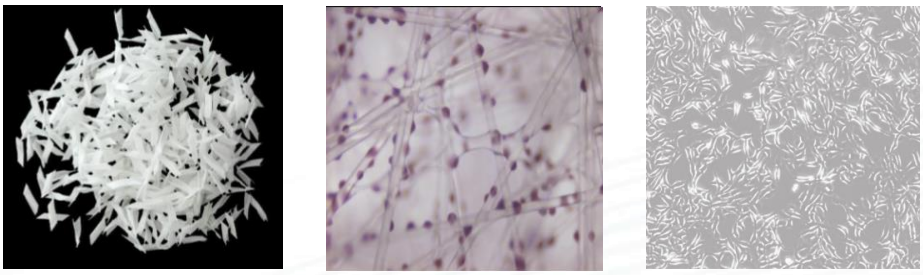
Specialized Medium for Growth of Vero Cells

Plus™ VERO Serum-Free medium (SFM) is a component-defined cell culture medium, formulated without any human or animal-derived components. Plus™ VERO SFM is designed to support the serum-free growth of the Vero cell line of interest in the areas of virology, virus production, and biotechnology. pH, osmolality of the medium are measured and tested for the absence of bacterial fungal and endotoxin contaminants.

The additional features of this medium are:

- Very low protein concentration ~ 5 µg/mL
- No proteins or peptides of animal or human origin
- No complexes such as plant hydrolysate, yeast extract.
- Ease of downstream product purification
- Reduced risk of viral contamination
- Better lot-to-lot consistency
- Equivalent cell growth and virus titers vs. serum-supplemented media

The Heart of Esco Aster's Technology – BioNOC™ II macrocarriers



Vero cell growth supported by BioNOC™ II macrocarriers which provide a large surface area for growth (L)– BioNOC II macrocarriers (R) stained cells on BioNOC TM II (ER) Vero cells under 4x magnification

ESCO™ ASTER

Vero Cell Culture in Tide Motion Bioreactors

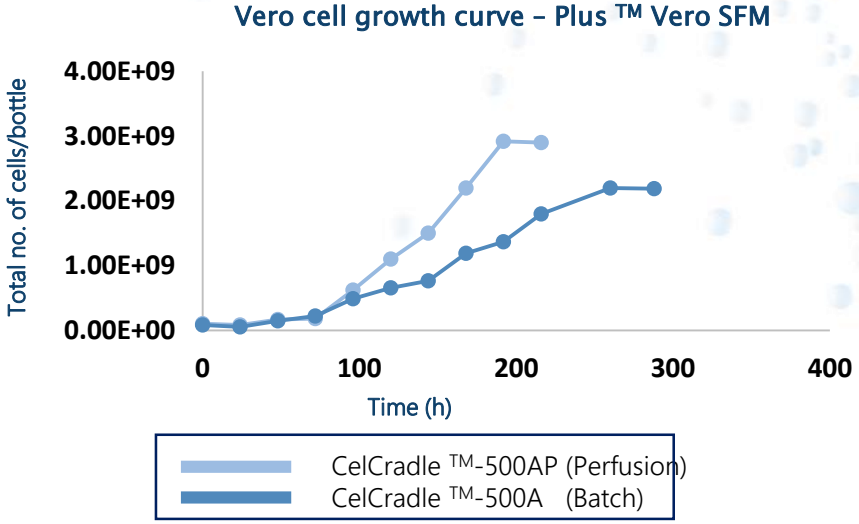
Vero cells have been cultured to high densities in matrices that mimic a 3-D *in vivo* environment. Cells are grown to typically high densities -2.9 x 10^9 to 3.25 x 10^9 using a batch /perfusion mode of culture in either Serum-free or serum-containing medium as represented in (A) or (B)using bench-top bioreactors-the CelCradle™ of 500ml scale. Microscopically stained cells are represented in (C).

Vero cells were also cultivated in a pilot-scale TideXcell ™ -002 Bioreactor that is fully automated (1L matrix volume in 5L single-use matrix vessel) and at the end of the culture period, cells were harvested using the fully automated Tissue culture cell harvester system (TCCHS). The figures D(I) and (II) represent the cell growth curve and Glucose consumption of Vero cells cultivated in the TideXcell ™ -002 in serum-containing medium. The pH of the culture in the TideXcell™ - 002 and CO2% are represented in D(III) . The pH was maintained within acceptable parameters (7.0-7.4) due to the large volume of media and constant monitoring and adjusting of CO2%. D(IV) represents microscopic images of cells grown on BioNOC™ II carriers which from the matrix of the TideXcell ™ -002

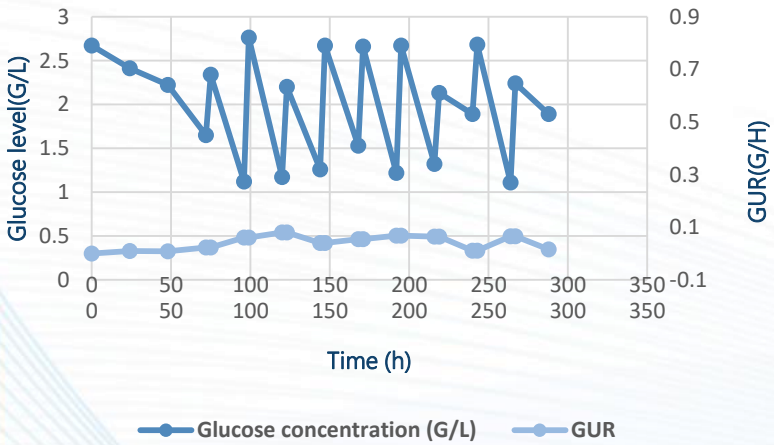
Linear scalability

The surface area and cell numbers achievable for Vero cell growth in different volume bioreactors is as represented in (E). As represented , the technology, therefore, is very robust for linear scalability. and thus saves time, labour and resources for process development.

A (I)

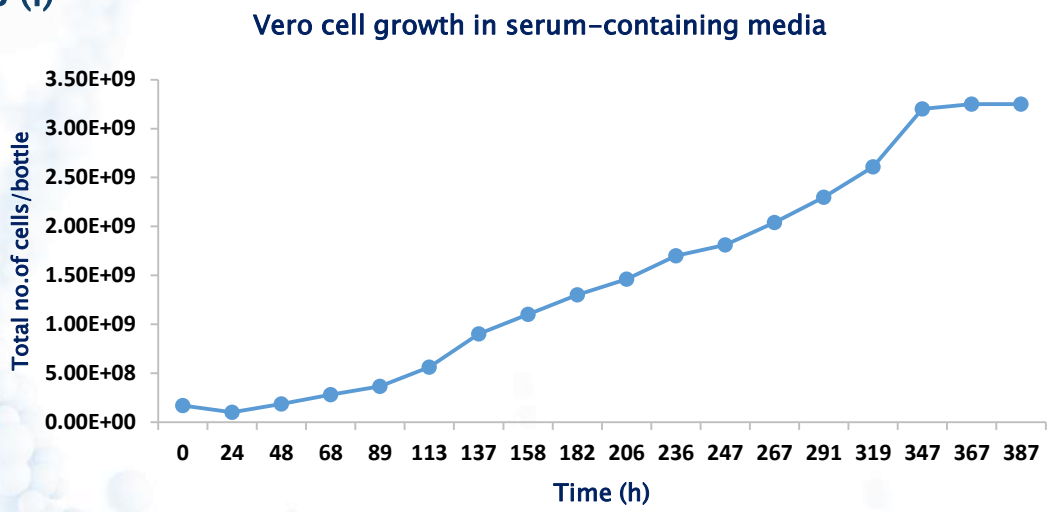


A (II)

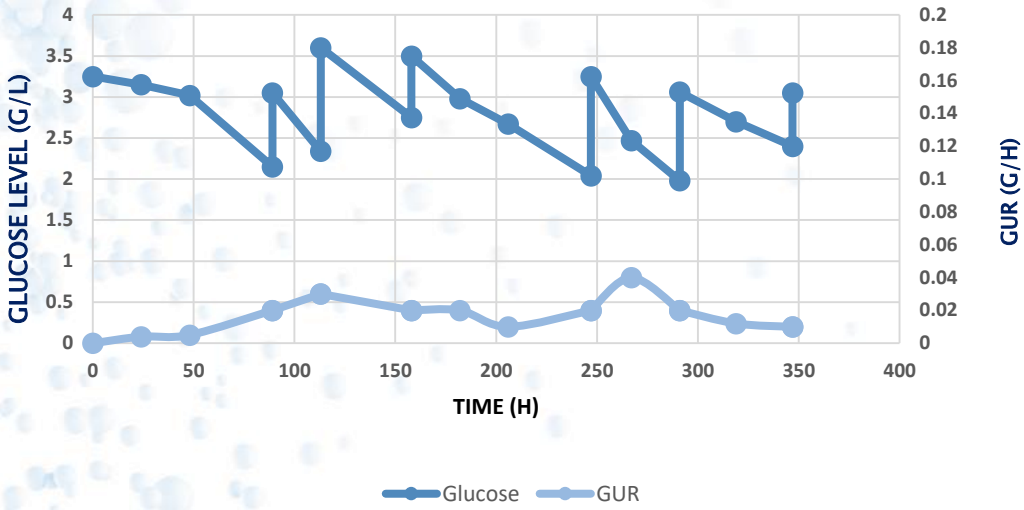


ESCO ASTER™

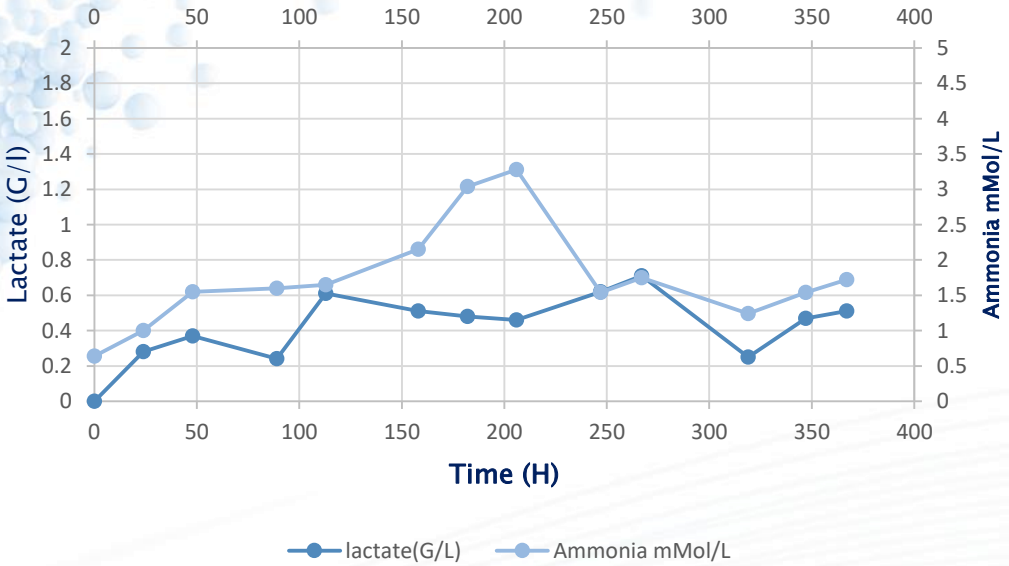
B (I)



B (II)

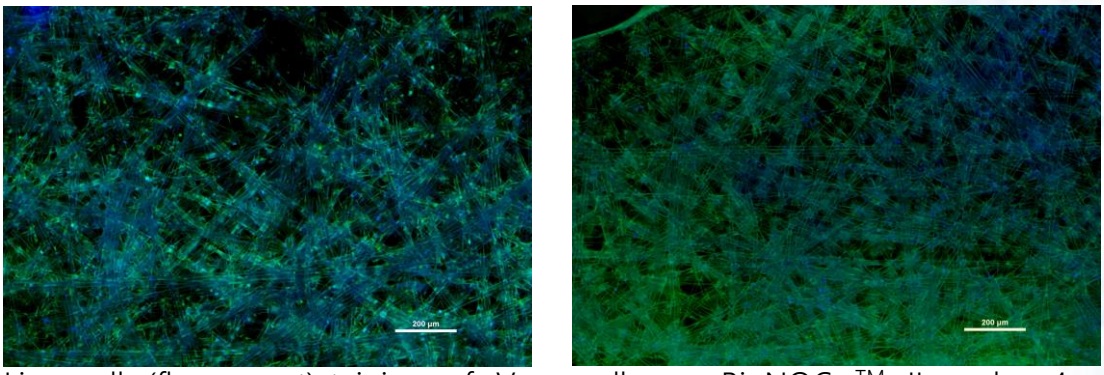


B (III)



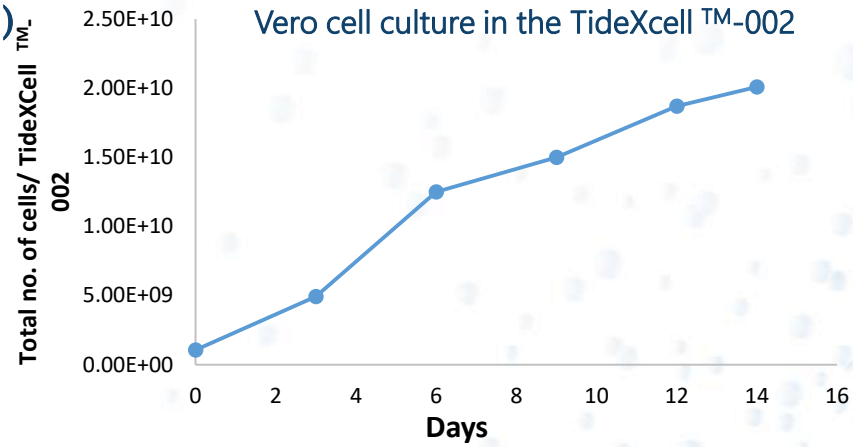
The figures A (i) and (ii) represent the cell growth curve and Glucose consumption of Vero cells in serum-free culture medium and B(i)-(iii) represent the cell growth curve ,Glucose consumption and Lactate and Ammonia metabolite concentrations of Vero cells grown in serum-containing culture medium.

C

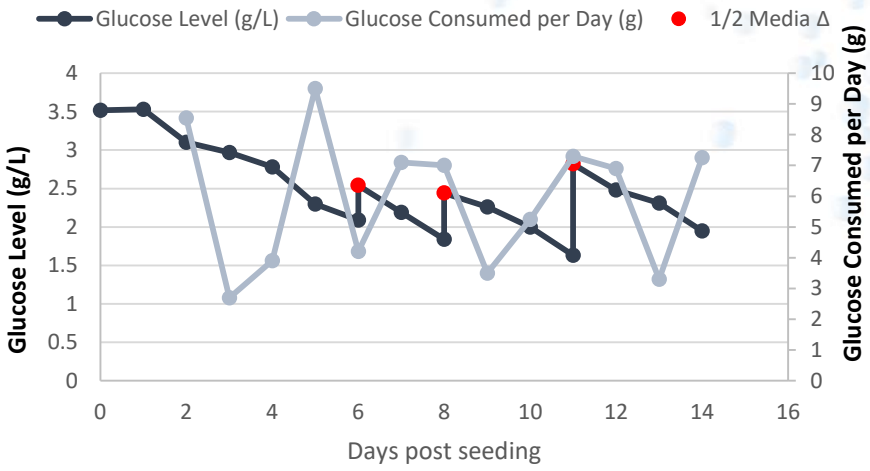


Live cell (fluorescent)staining of Vero cells on BioNOC ™ II under 4x magnification. Calcein green staining of cytoplasm and Hoechst 33342 (blue) staining of the nucleus. The panel on the left represents cells in early culture and the right panel represents cells in late culture.

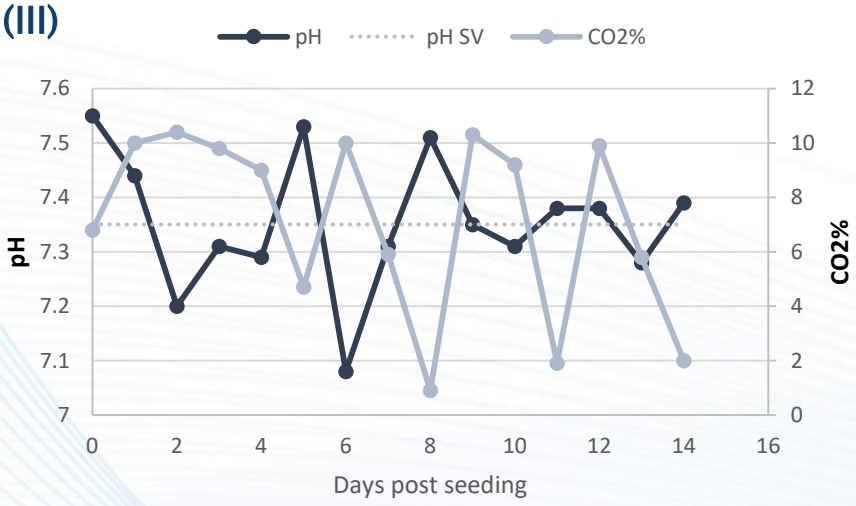
D (I)



D (II)

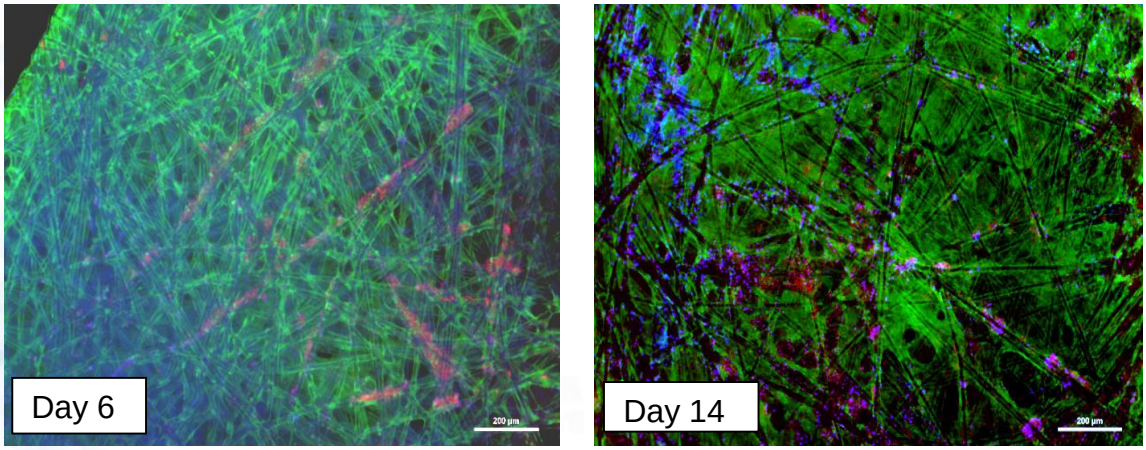


D (III)



ESCO ASTER ™

D (IV)



Live cell (fluorescent)staining of Vero cells on BioNOC ™ II under 4x magnification. Calcein green staining of cytoplasm, Hoechst 33342 (blue) staining of the nucleus and Propidium Iodide stain for dead cells was done.The panel on the left represents cells in early culture and the right panel represents cells in late culture.

Fold-expansion and harvesting

The fold expansion of Vero cells from attachment on BioNOC™ II in a *1L packed bed) to harvest using an automated TideXcell Cell Harvest System (TCCHS) was 19-fold and the efficiency of harvest was 93.6%.

E. Surface Area for Cell Growth and total cell number in Different Bioreactors*

Based on cell growth in SFM *

Model	Fixed Bed Volume (L)	Culture Surface Area (m^2)	Total Cell number
CelCradle	0.1	1.59	2.9x10e9
TideXcell-002	2	3.17	5.8x10e10
TideXcell-020	20	31.78	5.8x10e11
TideXcell-100	100	1585.93	2.9x10e12

Based on cell growth in serum-containing media*

Model	Fixed Bed Volume (L)	Culture Surface Area (m^2)	Total Cell number
CelCradle	0.1	22.75	3.25x10e9
TideXcell-002	2	45.5	7.5x10e10
TideXcell-020	20	455	7.5x10e11
TideXcell-100	100	2275	3.25x10e12

* Surface area and cell density are specific to Vero cells grown in optimized culture conditions and varies with different cell types

Cell density may vary depending on source of Vero cells, media used , culture conditions and may require optimization

F. Cell Growth in Different Carriers

Based on cell growth in SFM

Model	Cell Density
Microcarriers	2.6x10^6 cells/ml
Macrocarriers	5.8x10^6 cells/ml

Based on cell growth in serum-containing media

Model	Cell Density
Microcarriers	3.3x10^6 cells/ml
Macrocarriers	6.5x10^6 cells/ml

G. Comparison of Vero cell yields in common 2-D culture systems vs CelCradle

2-D System	Total number of cells	Bioequivalency *
T- flasks (175 cm2)	32 x 10e8	101
Hyperstack (6000cm2)	400 x 10e8	8
Roller bottles (850cm2)	225 x10e8	14
Hyperflask (1720cm2)	175x10e8	19

This refers to the number of the respective 2-D systems needed to replace 1 CelCradle ™ in terms of Vero cell yields when cultured in serum-containing media.

Esco Aster's Research and Development Pipeline with Vero Cells

- Vaccine Process Development, Oncolytic Virus therapy
- Contract based process development and consultancy production and purification of viral vaccines
- Transfer of processes and technologies to public and private health companies worldwide

References

1. Ammerman NC, Beier-Sexton M, Azad AF. Growth and Maintenance of Vero Cell Lines. Curr. Protoc. Microbiol. Nov 2008: Appendix–4E.

2. Osada N, Kohara A, Yamaji T, Hirayama N, Kasai F, Sekizuka T, Kuroda M and Hanada K, The Genome Landscape of the African Green Monkey Kidney-Derived Vero Cell Line DNA Research 2014:21, 673–683

More information on www.vaccixcell.com (Bioprocessing tools) or www.escoaster.com (CDMO services)

For enquiries, contact us at:
ESCO ASTER PTE LTD
#03-20, 71 Ayer Rajah Crescent
Singapore 139951
Esco Vaccixcell:
mail@vaccixcell.com
Esco Aster:
mail@escoaster.com