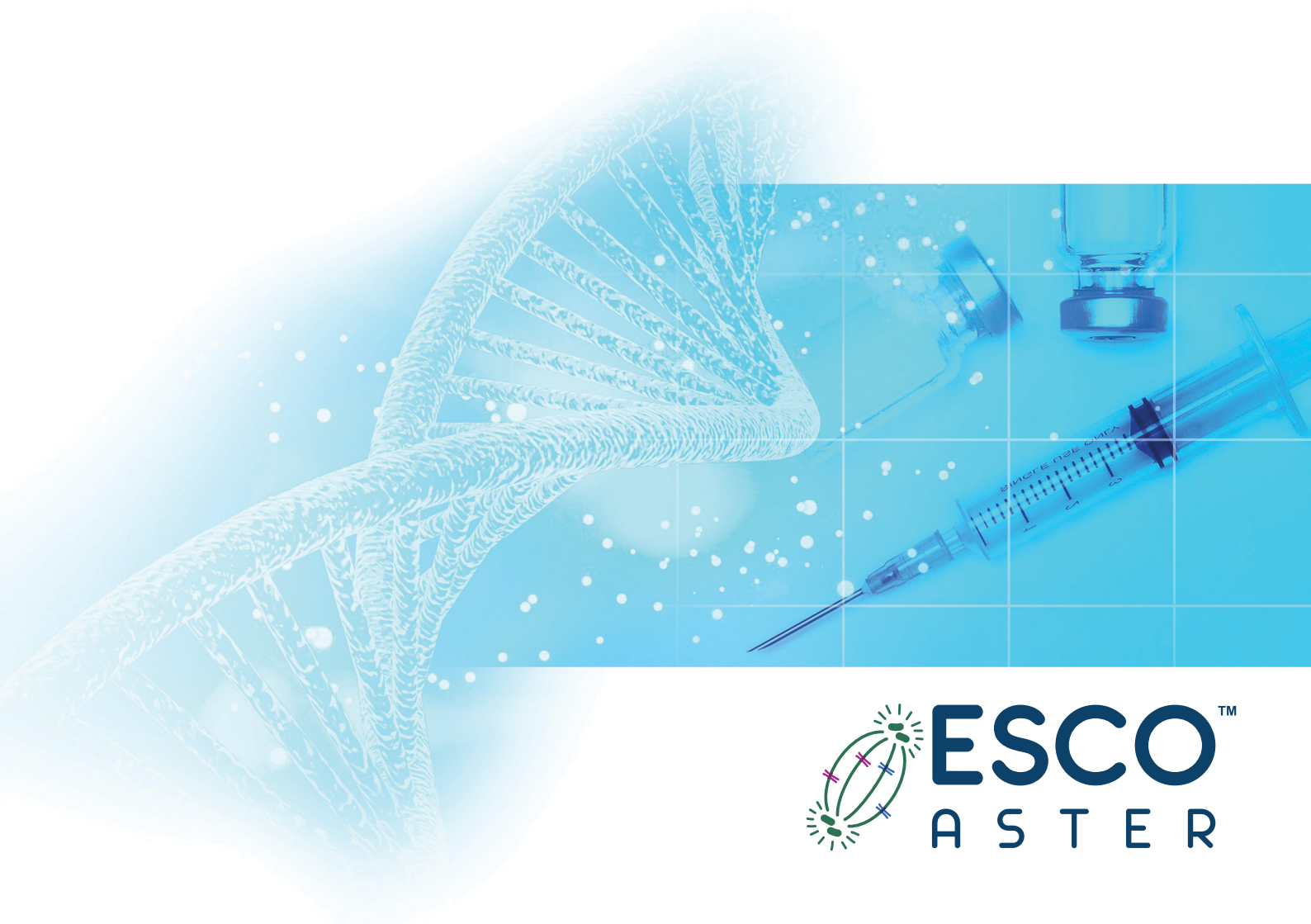
Application Note

Veterinary Vaccine Production: Optimised Upstream Bioprocessing Performance Using TideMotion™ Bioreactor

Background and **Introduction**

The global animal vaccine market is estimated to surpass USD 9 Billion by 2025 according to Global Market Insights Inc. due a reduction in annual meat production in established countries which in turn is a result of the prevailing livestock diseases. The maintenance of livestock, amidst various food- borne zoonotic diseases, poses a serious challenge to the meat production economies. A case in point is China having recently reported an outbreak of the deadly African swine fever in large pig farms. The increase in prevalence of food - borne zoonotic diseases catalyses the worldwide demand of veterinary vaccines as the whole meat production-supply chain tends to be disrupted, resulting in an abrupt culling of "infected" animals.

With an aim of ensuring a reliability of vaccine supply and immunisation, we herein introduce the means of achieving high-cell density working cell bank culture (HD WCB) to support scalable production of Veterinary vaccines using TideMotion™ Bioreactors.



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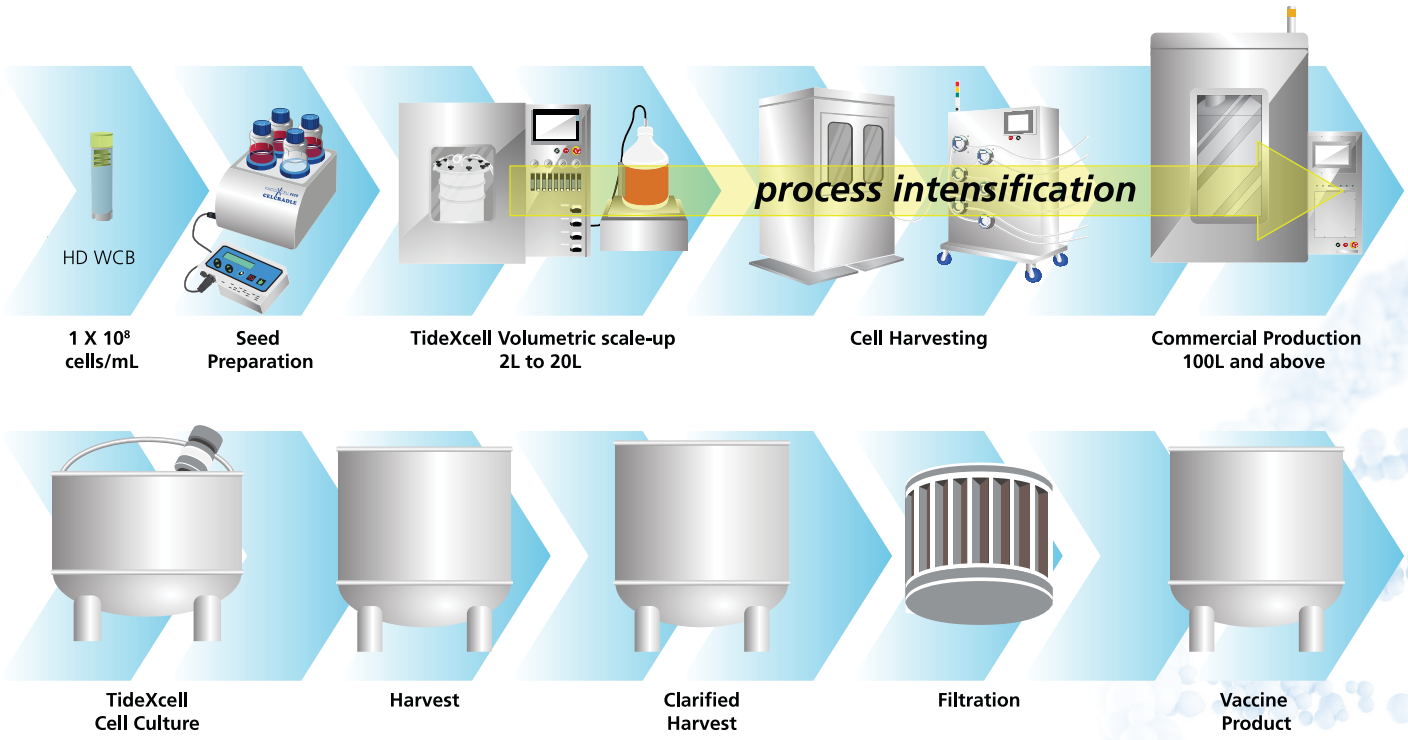
Improved **Seed Train** as **Scale-up Strategy**

Use of TideMotion™ Bioreactors, provides process simplification as follows. The seed train is initiated from a HD WCB of 1 X 10^8 cells, propagated into static culture systems and thence cultivated in single-use CelCradle 500A TideMotion™ bioreactors. Subsequently, a process intensification can be further used to optimise CelCradle 500A to CelCradle 500AP process as an improved seed train before proceeding on to culturing in production scale TideXcell 2L to 20L TideMotion™ bioreactors. Due to high cell-densities, higher volumetric productivities can be achieved leading to higher space-time-yields (S-T-Y).



Upstream Considerations

1. The initial cell seeding density (number of cells/ BioNOC II carriers) enabling a high cell density and maximum cells-to-carrier distribution has been optimised. For any given 3D carrier or scaffold, the initial seeding density is a critical factor in determining the maximum achievable cell density and spatial distribution of cells within the carriers. This has a bearing on the homogeneity of cells within the packed-bed matrix.

2. It is advised to determine the best attachment rate. In general, at least 90% of cell attachment on the carriers is observed after three hours of incubation adopting the 'reverse seeding' technique using both CelCradle 500A and 500AP TideMotion™ bioreactors.

3. A preliminary experiment is recommended using the TideMotion™ principle in a small-scale BioNOC II experiment which employs a slow "rocking" motion simulation before proceeding with actual implementation of process in the bioreactors. This is akin to small biomass testing to obtain viable cell numbers at the end of cell growth phase and can form a basis for the experimental design when one proceeds to using the bioreactors. Refer to Testing BioNOC II Protocol *(http://www.escoaster.com/white-paper-and-protocols/)*.

4. It is critical to maintain the pH of the culture medium between 7.2 to 7.4 to ensure maximum attachment efficiency of cells on the BioNOC II carriers. If a batch culture is performed, it is recommended to monitor the pH and glucose levels twice a day.

5. Monitoring of cell-growth, on a daily basis, is best done by live-cell counting using a haemocytometer or cell counter to obtain the number of viable cells/ ml in the packed-bed volume.

6 VERO-SFM / BHK-21 / MARC-145 adherent cells are commonly used in production of animal vaccines. As culture conditions tend to be variable for different cell lines, it is recommended to perform proof-of concept experiments to obtain the growth curve, glucose, glutamine uptake and lactate and ammonia production rates in the cultures. This will provide a guideline to determine:

a. Optimal infection time with reference to cell density.

b. An indication for supplementation of glucose in the culture medium (when glucose concentrations are depleted to below 1.0g/L, please perform partial or total replacement of medium. In general, glucose concentration in the medium is an indicator of cell-health.

c. Information about the other metabolites in the medium may be potentially used by researchers to derive mathematical modelling equations for optimising cell cultures for seed trains and cell-banking purposes.

CelCradle TideMotion™

In a first setting, we suggest the CelCradle 500A batch cultivation for cell growth and expansion. Additional set of Celcradle 500AP perfusion experiments can be further carried out.

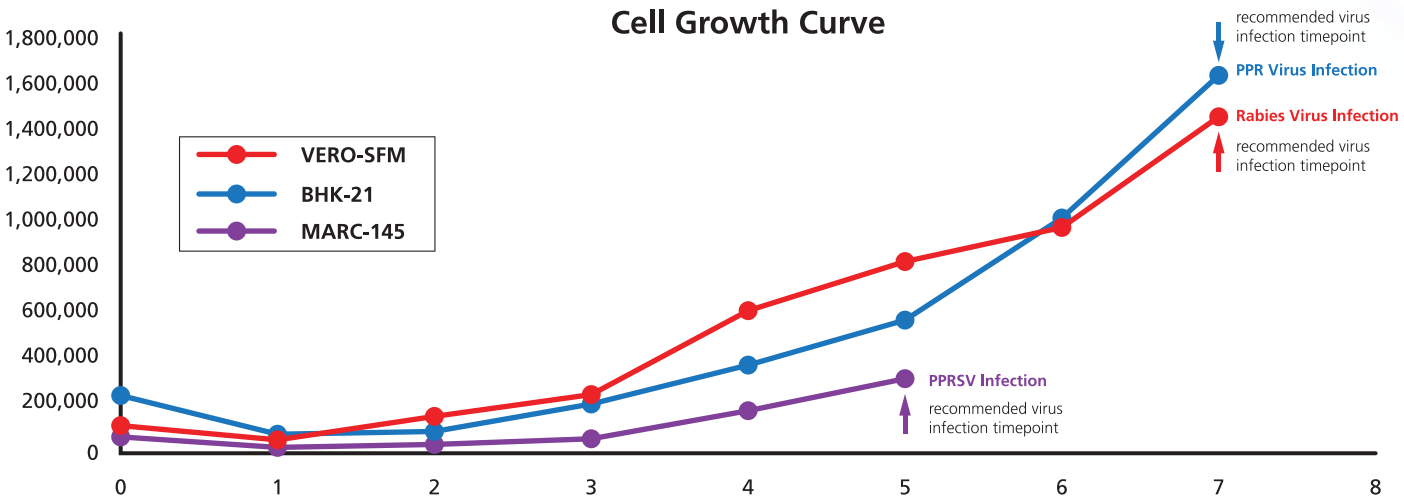
Device	Cell Line(s)	Media	HD WCB
CelCradle-500A (batch culture)	VERO-SFM BHK-21 MARC-145	Prepare 500 ml for CelCradle-500A bottle using DMEM containing10%FBS and NaHCO3 + Glutamine + 1% antibiotics/ MEM with 10%FCS + Glutamine as applicable to the cells being cultured	$1x10^8$ cells/mL

CelCradle- 500A (Volume in ml)	Parameters
500 (batch culture)	**Day 0 to Day 4** Uprate: 1 mm/sec Uphold: 0 mins Downrate: 1 mm/sec Downhold: 0 sec
CelCradle- 500AP (Volume in ml)	**Parameters**
2,200 (perfusion culture)	**Day 0 to Day 4** Uprate: 1 mm/sec Uphold: 0 mins Downrate: 1 mm/sec Downhold: 0 sec **Pump 1** Volume: 1999 mL Cycles/Day: 24 Schedule: 1111111

Cells – Carriers Seeding Index

A Cells – Carriers Seeding Index to fill the knowledge gap in relation to the best seeding density in CelCradle 500A batch cultivations is presented below:

BATCH	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
VERO-SFM	101,000	64,000	183,000	263,000	600,000	802,000	952,000	1,495,000
BHK-21	235,294	67,500	76,470	196,428	361,445	558,536	989,000	1,600,000
MARC-145	49,000	14,200	26,666	46,800	173,000	316,666	-	-



Overall, we highlight the value of achieving high cell-densities in providing an optimum and stable environment for packed-bed matrix as an efficient cultivation technique for seed train and/ or high cell-density banking and production of vaccines. Stable gas exchange, metabolites, interfacing with the TideMotion™ Bioreactors, are achieved since the cells are immobilised onto the carrier matrix. This potential of the packed-bed matrix translates to a sustain optimal growth conditions as cell density increases. This strategy can be adopted in larger-scale production processes as using multiple packed-bed bioreactors.

In general, cells for producing animal vaccines can be grown in serum containing media to achieve high cell densities and high virus titters. Shown above is a comparison with Vero cells that were grown in SFM.

MOIs

The multiplicity of infection (MOI) is influenced by the cell density and the virus concentration in the supernatant. During virus propagation, cell growth, virus infection, production of virus-like particles, and potentially cytopathic effects (CPE) occur simultaneously within the packed-bed matrix.

For optimum infection, we recommend reserving 10% of the original volume of infection as inoculum for your subsequent cycles of infection runs. For a 500 mL packed-bed CelCradle, this is 50 mL. For example, infection of cells during their steady-state growth phase with inoculum volume of 10% culture-volume resulted in increased overall yield and lower production costs. Such optimizations are useful for scaling up production.

Downstream Considerations

Animal vaccines in general, do not require much further processing before administration as compared to human vaccines. The impurity profile of the TideXcell generated material can be combined in the downstream purification train. At the industrial level, the strategies used for downstream should be a concentrated stock which is convenient for distribution and handling. Should additional concentration be required, precipitation by reagents such as Polyethylene glycol (PEG), Tangential Flow Filtration (TFF) using hollow fibres or flat -sheet cassette membranes may be examined. Additional purification steps, e.g. chromatography, will depend on the impurities to be removed and the extent of purity required.

The following characteristics of the viruses being discussed here should be noted when designing a downstream purification train.

Rabies	60x180 nm rods	Lipid enveloped
Peste des petits ruminants (PPR)	400 to 500 nm particles	No lipid envelope
Porcine Reproduction and Respiratory Syndrome Virus (PRRSV)	50 to 65 nm particles	Lipid enveloped

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