Animal Rabies Vaccine Production in BHK-21 cells

Introduction

Rabies is a zoonotic disease (a disease that is transmitted from animals to humans), caused by the rabies virus, of the Lyssavirus genus, within the family Rhabdoviridae. Domestic dogs are the most common reservoir of the virus, with more than 99% of human deaths caused by dog-mediated rabies. (Fooks *et al.*,2017)

The virus is transmitted in the saliva of rabid animals and generally enters the body via infiltration of virus-laden saliva from a rabid animal into a wound (e.g. scratches), or by direct exposure of mucosal surfaces to saliva from an infected animal (e.g. bites).(Centers for disease control and prevention, 2019)

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.(Rabies Global Conference, 2015)

Being a 100% vaccine-preventable disease, it is imperative for countries facing this problem of Rabies and humans being affected by it, elimination programs are essential.

In order to do this, a rapid, cost-efficient and large-scale production of Vaccine is essential. Therefore, in this study an evaluation of the bench-top Bioreactor –the CelCradle™ - 500 A was undertaken as a viable option to potentially replace the use of conventional systems such as Roller bottles /Roux bottles for culture and

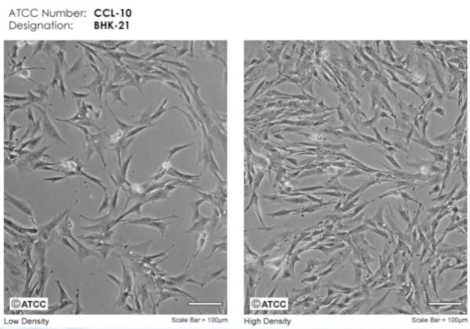

***Figure 1*: Microscopic image of BHK-21 cells at 10x magnification (source: ATCC)**

Aim of the experiment

To compare the effectiveness of producing a Rabies Vaccine (Pasteur strain) in BHK-21 cells growing in bench-top Bioreactor 500 A with existing conventional systems such as Roller bottles and Roux bottles.

Materials and methods

Device	CelCradle™-500A
Cells	BHK-21
Medium	EMEM (Hyclone) + 10% FBS + 1% Penicillin-Streptomycin
Seeding Density	2 x10^8 cells/bottle

Inoculum preparation (roller bottle)

BHK-21 cells were seeded at 10 million per roller bottle and incubated at 37°C for 48h in a walk-in incubator. 200 x10^6Cells from about 4 Roller Bottles were harvested and seeded onto one CelCradle™-500A bottle for further Rabies Virus infection and Culture.

Inoculation and Immobilization

The inoculation of cells into the CelCradle™ was done in the following way:

- 500ml of EMEM media (Hyclone) containing +10% FBS, and 1% Penicillin/streptomycin was pre-warmed in a 37 ̊C water bath
- 2x108 cells harvested from Roller bottles was suspended into 120ml of pre-warmed media and mixed gently. The “Reverse seeding” protocol was followed as detailed on the next page.
- A single CelCradle™-500A bottle was brought into BSC Class II hood and the cell suspension was poured gently into it and swirled gently while taking care to avoid formation of bubbles.

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Reverse seeding

- The bottles were covered with the white cap without filter.
- The bottles were reversed , the contents mixed gently, and placed in a 37 ℃ incubator.
- Gentle mixing was done every 30 min for up to 4 hours.
- After 4 hours of incubation, the bottles were brought into the BSC, the contents gently mixed and ~10 µl of media was sampled for cell counting.
- The suspended cells that were left in the culture media were counted manually using a haemocytometer to determine % of attachment. (In general, if 90% attachment rate is achieved (i.e. less than 10% of cells remain in media) the following parameters are set in the CelCradle™ and cells are allowed to grow overnight. If cell attachment is not optimum, 1 additional hour of cell attachment is required).
- The attachment was found to be 90%. Therefore, the following parameters were set for allowing the cells to grow.

CelCradle™ parameters for culture cultivation

Rising Rate	1 mm/sec
Top holding time	10 sec
Down rate	1 mm/sec
Bottom holding time	30 sec

Virus infection and propagation

After the cells had reached a density of $2.6x10^6$ cells /ml ($1.7x10^9$ cells) per bottle, The Rabies Virus (Pasteur strain, PV 35321, WHO, 1985) was inoculated into the culture for Virus propagation. The Virus Titre was 10^7 LD_{50}/ml and the MOI was 0.5. Incubation conditions were 33°C during and following Virus inoculation and the CelCradle™ parameters were set as in the following table. The harvest was after 96h.

CelCradle™ parameters for viral propagation

Rising Rate	1 mm/sec
Top holding time	30 sec
Down rate	1 mm/sec
Bottom holding time	10 sec

Monitoring Cell-Growth BHK-21 Cell Growth Curve

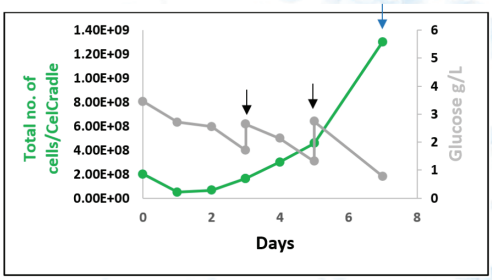
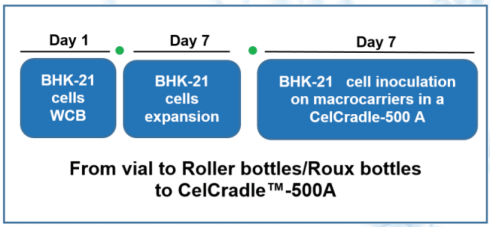
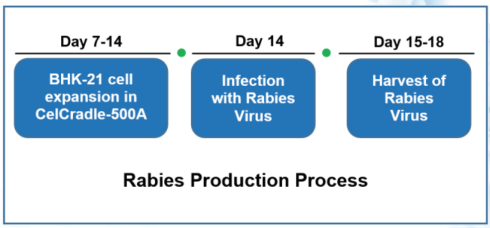


***Figure 2*: BHK-21 cell growth curve and time – blue arrow points to time of infection with rabies virus and black arrows indicate media replacement times**

Timeline of Rabies Virus Infection in BHK-21 Cells





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Virus Inoculation and Harvest Method

Time Length	Action
Day 0	Virus Inoculation - addition of 65ml of virus (10^7 LD_{50}/ml at an MOI of 0.5) + 55ml of media
Day 0	Virus Propagation
Day 1	No harvest; sampling of medium (2ml) for assaying of Virus Titre
Day 2	No harvest; supplementation of Glucose and L- Glutamine; sampling of medium (2ml)
Day 3	Partial Harvest 100ml – sampling of medium(2ml) ; replace with 100ml of fresh medium
Day 4	Harvest 450ml of Virus-sampling of medium (2ml); replace with 50ml of medium
Day 5,6,7	Virus propagation for 2nd harvest
Day 8	Harvest of 500ml of Virus

**Virus titre is carried out with all the samples and pooled together to determine total virus titre*

Comparison of Rabies Vaccine production process in CelCradle™ 500A vs Roller and Roux bottles

	CelCradle ™-500A	Rottle Bottles	Roux Bottles
Media	1000 ml	4550 ml	4550 ml
Fetal Calf Serum	100 ml	455 ml	455 ml
Glassware	-	26	13
Filtration	-	Y	Y
Washing	-	Y	Y
Packaging	-	Y	Y
Sterilization	-	Y	Y
Virus Titre	10^{6-7} LD_{50}/ml	10^6 LD_{50}/ml	10^6 LD_{50}/ml

Measuring Rabies Virus Titre

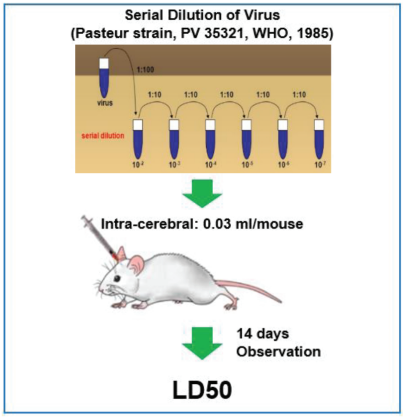

***Figure 3*: Graphic Representation of Virus Titration Method**

Conclusion

Cell propagation in macrocarriers has resulted in BHK-21 cell concentrations with 1.3 x10^7 cells per CelCradle™ bottle, with virus titres of 10^{6-7} LD_{50}/ml. Overall, 1 CelCradle™ bottle yields BHK-21 cell densities equal to that obtained in 26 and 13 Roller and Roux bottles respectively. It was found to be more effective than a monolayer system on a roller bottle for the production of Virus and hence could potentially be an efficient solution for the large scale production of Animal Rabies Virus. The use of Tide motion Bioreactors in large Scale production of Animal Rabies Virus could be very cost-effective in terms of OPEX and CAPEX.

References

1. *Fooks AR, Cliquet F, Finke S, Freuling C, Hemachudha T, Mani RS, Muller T, Nadin-Davis S, Picard-Meyer E,Wilde H and Banyard AC. Rabies. Nature Reviews Disease primers; 2017;3,17091.*

2. *Reports of the Rabies Global Conference. Global elimination of Dog-mediated Human Rabies. 2015*

3. *Centers for Disease Control and Prevention. How is Rabies transmitted? 2019*

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

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