

OBSERVATIONS ON THE MAINTENANCE OF *IN VITRO* PRIMARY AND SECONDARY LAMB KIDNEY CELLS

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ABSTRACT

Studies were conducted on propagation, maintenance, storage and revival of primary and secondary lamb kidney cells. It was observed that the primary lamb kidney cells stored in vapor phase of liquid nitrogen could be successfully revived up to 6 months of study period and from each revival, the cells could be sub cultured for 10 serial passages at the split ratio of 1:3. The secondary lamb kidney cells monolayer-produced characteristic CPE when infected with PPRV, BTV and sheep poxvirus. It was also observed that primary cultures could be successfully made from the bits of cortical tissues stored in storage medium at -70°C even after one month.

Key words: lamb kidney, primary, secondary cultures, revival, viruses

In vitro lamb kidney culture is used for isolation of peste des petits ruminants (PPR), sheep and goat pox (SGP) and Blue tongue (BT) viruses (Taylor and Abegunde, 1979; OIE manual, 1996). Vero cells and BHK₂₁ cells were routinely used for isolation of PPR virus (Nanda *et al.*, 1996) and SGPV and BT virus respectively (Kitching *et al.*, 1986; Ramesh Babu *et al.*, 1992). However, primary and /or secondary lamb kidney cells were reported to be more sensitive than the cell lines mentioned. Attempts were made to evolve continuous supply of primary and secondary lamb kidney cells and the observations made during the last six months are presented.

MATERIALS AND METHODS

Source of lamb kidney

Whole lamb kidney with capsule and fascia was collected in sterile phosphate buffered saline, pH 7.6 containing antibiotics from Perambur slaughterhouse at Chennai during slaughtering from a lamb.

were made from this kidney as per the methods suggested in FAO, Animal Production and Health paper 118 (1994). The fascia and capsule were removed and the kidney was washed thrice in PBS with antibiotics. Cortex tissues were removed and minced to small bits and washed thrice in PBS with antibiotics. Then the cortex tissue was trypsinised (0.25 % trypsin in PBS with antibiotics). The first trypsinisation collection after 20 minutes was discarded. Subsequent trypsinisations were allowed for 30 min at 37°C. The cells were harvested with 10 ml of fetal calf serum (FCS) and stored at 4°C. Three such collections were made and the cells were pelleted at 1000 rpm for 20 min. at 4°C. Then the cells were reconstituted to a final volume at the ratio of 0.5 ml of pelleted cells in 250 ml of growth medium (GMEM with 10 % FCS and antibiotics). Antibiotics for PBS and GMEM were same (Penicillin 100 IU, Streptomycin 100 ig, Kanamycin 25 IU/ml). The cell suspension was sieved through sterile SS mesh No.400 and seeded into Roux flasks, 25 cm² flasks and tubes with flying cover slips at the rate of 100 ml, 5 ml and 2 ml respectively and incubated at 37°C.

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This was conducted at the split ratio of 1:3 and 10 secondary cultures were attempted.

Storage of primary cells

These were stored as tissue bits in storage medium (GMEM with 20 % FCS) in 30 ml vials at -70°C (Scientemp, USA).

Storage of monolayer cells

Cells from monolayer were trypsinised with Trypsin-EDTA suspension and then harvested in 10 ml of storage medium (GMEM + 10 % FCS + 10 % sterile glycerol) and stored in vapor phase of LN₂.

Revival of the cells

Primary cells

Tissue bits were once washed in PBS and subjected to trypsinisation. From these, all the methods followed for primary culture was carried out up to seeding into 25 cm² tissue culture flasks.

Stored cells

The vials were quickly thawed at 37°C and reconstituted at the rate of 1 vial of stored cells into 5 ml of growth medium and incubated at 37°C at monthly intervals.

Virus susceptibility

Viruses like PPRV (Arasur 87/1- first isolate of India from Tamil Nadu reported by this institute), BTV 23 (Isolate from Tamil Nadu isolated by this institute and Sheep and Goat Pox virus(SGPV)-RPT vaccine strain (From Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu) were used to infect 5th and 10th passage secondary lamb kidney cells.

RESULTS AND DISCUSSION

The primary cell monolayer was complete between 4th and 5th day after seeding (Fig.1). No morphological changes were observed in the secondary cells up to 10th passage. The cells were successfully revived up to 6 months (up to the period of observation). The tissue bits stored in storage medium when used for primary culture after 34 days of storage at -70°C, formed monolayer. All the above cultures supported growth of PPRV, BTV and SGPV with respective cytopathic effect (Fig.2 - 4).

Lamb kidney cells were reported to be more sensitive in isolation of field virus than Vero and / or BHK₂₁ cell lines. Cell lines are preferred in most diagnostic laboratories over primary or secondary cell culture due to their ease of maintenance and storage. Not many reports are available on the methods of propagation and storage of primary cells for continuous supply of primary or secondary cells. A report by Parthiban *et al.* (2005) showed that chicken embryo fibroblast cells could be stored up to 15 days at 4°C in storage medium (EMEM with 30 % FCS) with a successful revival and used for propagation of infectious bursal disease virus which could produce CPE in 48-72 h post infection. Hence, attempts were made to check up the reliability of primary and secondary cells. It is evident from the observation made from this study that the tissue bits stored at -70°C in storage medium could be successfully made into primary cells after one month. This method could salvage the tissue bits collected from the slaughterhouse every time primary culture is intended. Similarly repeated revival of cells up to 6 months (study period), 10 times sub culturing at the ratio of 1:3 for each culture and the formation of monolayer between 2nd and 3rd day post seeding were possible. The susceptibility of cells for PPRV, BTV 23 and SGPV RPT strain viruses were checked for up to 10 subcultures. Results of this study indicate that a more sensitive host system can be made available through out the year from a single kidney collected

from slaughterhouse.

REFERENCES

FAO Animal Production and Health Paper 118 (1994). Quality control testing of rinderpest cell culture vaccine. Standard operating procedures. p 1-18.

Kitching, R.P., Hammond, J.M. and Taylor, W.P. (1986). A single vaccine for the control of capripox infection in sheep and goats. *Res. Vet. Sci.* 42: 53-60.

Nanda, Y.P., Chatterjee, A., Purohit, A.K., Diallo, A., Innui, K., Sharma, R.N., Libeau, G., Thevasagayam, J.A., Bruning, A., Kitching, R.P., Anderson, J., Barrett, T. and Taylor, W.P. (1996). The isolation of peste des petits ruminants virus from North India. *Vet.*

Microbiol. 51: 207-216.

OIE manual (1996). Manual of standards for vaccines and diagnostics. Chapter 2.1.10. Sheep pox and Goat pox. P 119-127.

Parthiban, M., Manoharan, S., Wilson Aruni, A., Daniel Joy Chandran, N. and Koteeswaran, A. (2005). A simplified method for maintenance of chicken embryo fibroblast culture. *Asian J. Microbiol. Biotechnol. Env. Sci.* 7(2): 251-253.

Ramesh Babu, N.G., Byregowda, B.M., Bragitha, A.J. and Gopal, T. (1992). Isolation and characterization of blue tongue virus from sheep. *Indian Vet. J.* 69: 1071-1074.

Taylor, W.P. and Abegunde, A. (1979). The isolation of peste des petits ruminants virus from Nigerian sheep and goats. *Res. Vet. Sci.* 26:

Fig. 1

H&E stained photomicrograph of normal monolayer of primary lamb kidney cells (100 x)

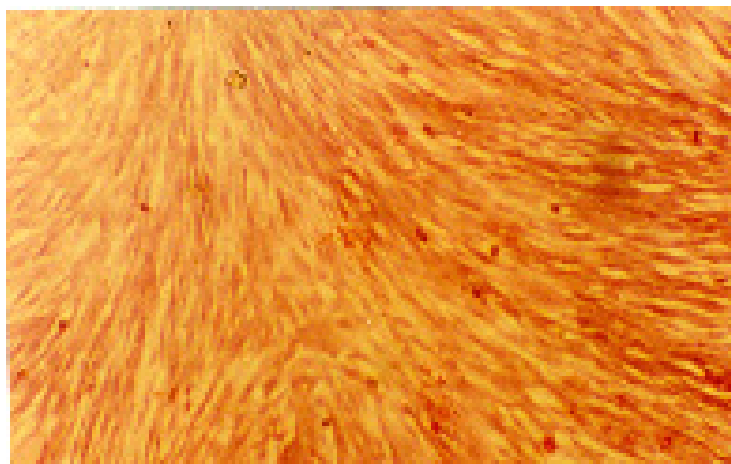


Fig. 2

Phase contrast photomicrograph of lamb kidney cells infected with PPR virus -72 h post infection (400 x)

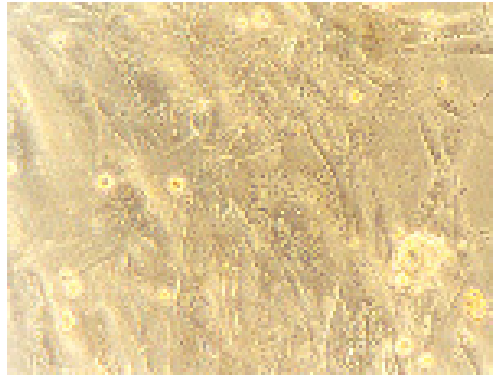


Fig. 3

H&E stained photomicrograph of lamb kidney cells infected with BTV 23 – 72 h post-infection

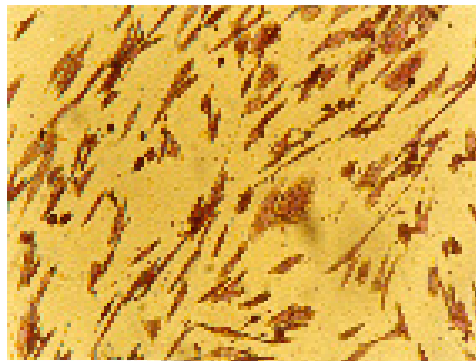


Fig. 4

H&E stained photomicrograph of lamb kidney cells infected with sheep and goat pox virus – 72 h post infection (400 x)

