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.

| Primary and secondary cell cultures | Secondary or sub culturing |

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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 throughout the year from a single kidney collected.
from slaughterhouse.

**REFERENCES**


**Fig. 1**

**H&E stained photomicrograph of normal monolayer of primary lamb kidney cells (100 x)**
Observations of the maintenance of *in vitro* primary and secondary lamb kidney cells

**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)