

## CULTIVATION OF BUFFALO POX VIRUS IN PUP KIDNEY CELL CULTURE

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Buffalo pox virus (BPV) has been reported to grow in RK-13 cells (1), chicken embryo CE fibroblasts (2) and HeLa cells (3). The present paper deals with the cultivation of buffalo pox virus in primary pup kidney cells, a new cell system for its cultivation.

Primary pup kidney cell culture were grown to confluency in tissue culture bottles or in Leighton tubes according to the method of Plowright and Ferris (4) and infected with buffalo pox virus strain BP<sub>4</sub>. After development of cytopathic effect (CPE), the virus was harvested by freezing and thawing and used for further successive passages. Coverslip cultures were infected with 0.2 ml of virus at its fifth passage and stained with May Grünwald's-Giemsa stain at 12, 24, 48, 72 and 96 hr post-inoculation (p.i.). The plaque morphology was studied in liquid overlay system after staining with 10% gentian violet. The infectivity titre was assayed in CAM of developing CE and the fifty per cent egg infective dose was calculated. In addition, 1 ml of virus suspension obtained from the fifth passage was inoculated in each of the five pups by intradermal route. The pups were observed for 10 days for the development of lesions.

Buffalo pox virus produced characteristic CPE in pup kidney cells since 40 hr p.i. in the first passage involving a few cells. The time of CPE appearance decreased in two successive passages and in third passage the CPE was observed as early as 18 hr p.i. The CPE in all the passages was characterized by rounding of infected cells, multinucleated giant cell formation, clumping of cells and cytoplasmic vacuolation. At 36 and 48 hr these changes were more marked and eosinophilic intracytoplasmic inclusion bodies were occasionally detected. At 72 hr p.i., degenerative changes including fragmentation of nuclei were seen in addition to the above changes. The plaques appeared as clear, unstained, circular areas ranging between 1.5–2.0 mm diameter with serrated margins. The infectivity titre of BPV was found to be 2.5 log EID<sub>50</sub>/0.2 ml in the first passage. With increased passage level, the titre increased to 6.5 log EID<sub>50</sub>/0.2 ml at 4th passage (Table). The pups were found to be refractory to infection and develop no lesions up to 10 days.

Passage No.	Titre (log <sub>10</sub> /0.2 ml)
1	2.5
2	3.75
3	4.75
4	6.5
5	6.5

## References

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