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INFECTIVITY ASSAY OF BOVINE ROTAVIRUS: EVALUATION OF PLAQUE AND END-POINT METHODS IN COMPARISON WITH IMMUNOFLUORESCENT CELL ASSAY

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Summary. — Three different methods, namely plaque assay, immunofluorescent cell (IFC) count and end-point dilution ($TCID_{50}$) were evaluated for quantitative infectivity assay of the cell culture adapted UK strain of bovine rotavirus in secondary calf kidney (CK) cells and BGM cell line. Plaque and IFC count techniques were found equally efficient for infectivity titration of bovine rotavirus. Addition of trypsin into maintenance medium enhanced the sensitivity of the $TCID_{50}$ method. Both CK and BGM cells served as efficient assay cells for infectivity assay of bovine rotavirus by IFC count and $TCID_{50}$ methods, whereas, for plaque assay, only CK cells were found suitable.

Key words: bovine rotavirus, infectivity, plaque assay, immunofluorescent cell count, end-point dilution method

Introduction

Although sophisticated detection methods exist for rotavirus, there is no simple and accurate quantitative technique to measure the virus infectivity. In the absence of other reliable methods, immunofluorescent cell (IFC) count procedure (Spendlove, 1967) was adapted to assay the infectivity of neonatal calf diarrhoea virus (Barnett *et al.*, 1975). An improved plaque assay method for infectivity assay of bovine rotavirus had been described earlier (Butchaiah and Lund, 1983). Plaque assay and IFC count procedures were compared and found satisfactory for infectivity assay of reoviruses (McClain *et al.*, 1967). No such comparative study was carried out for infectivity assay of rotaviruses. In the present study, the improved plaque and end-point dilution ($TCID_{50}$) methods were evaluated in comparison with IFC counting technique for routine quantitative infectivity assay of bovine rotavirus.

Materials and Methods

Secondary calf kidney (CK) cells and the BGM cells, a continuous cell line derived from African green monkey kidney (Barron *et al.*, 1970) obtained from the State Serum Institute,

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Copenhagen were used. The cells were grown in Eagle's medium supplemented with 10% new born calf serum. The UK strain of bovine rotavirus from the United Kingdom previously adapted to cell culture (Woode *et al.*, 1974) used in this study was kindly made available by A. Meyling of the State Veterinary Serum Laboratory of Copenhagen. The virus was treated with trypsin and grown in CK and BGM cell monolayers in Eagle's medium containing 0.5% bovine serum albumin (BSA) instead of calf serum and 2 μg trypsin per ml. The virus was harvested at 36 to 48 hr after inoculation, frozen and thawed three times, clarified by low speed centrifugation and stored at −80 °C in aliquots for use in this study.

All virus samples were treated with trypsin (1 : 250; Difco) at 10 μg/ml concentration for 30 min at 37 °C prior to assay for infectivity by the following three different methods.

The IFC count procedure as described by Barnett *et al.* (1975) was followed with slight modifications. Cells grown on circular cover slips were infected with 2-fold dilutions of the virus and stained with fluorescein-conjugated goat anti-bovine rotavirus immunoglobulin and the fluorescing cells were counted. The titres were expressed as FC/ml.

The improved plaque assay method used was the same as described earlier for bovine rotavirus (Butchaiah and Lund, 1983). In this method, both trypsin (2 μg/ml) and diethylaminoethyl (DEAE) — dextran (100 μg/ml) were added in the overlay medium for enhanced plaque formation. The titres were expressed as PFU/ml.

An improved end-point dilution method (Butchaiah, 1985) was used in which the test tube cultures were inoculated with serial 10-fold dilutions of the virus. After one hour absorption at 37 °C, the excess inoculum was removed and medium containing 2 μg trypsin per ml and 0.5% BSA was added to the cultures and incubated for 3 days at 37 °C. In the conventional end-point method, trypsin was not included in the medium added to the cultures after virus inoculation. Hence, in this standard method, the cytopathogenic effect (CPE) in infected monolayers could not be easily recognized particularly with higher virus dilutions. Whereas, in the improved method used here, because of the presence of trypsin in the medium, the CPE could be clearly observed in infected monolayers by direct microscopic examination. The titres ($TCID_{50}$/ml) were calculated using the Reed and Muench formula (1938).

Results

Table 1 summarises the average results of 3 replicate infectivity assays of bovine rotavirus by IFC count, plaque and end-point dilution methods using CK and BGM cells. Maximum titres were obtained by IFC count method irrespective of the cells used for assay. The titres in CK and BGM cells did not vary much. Although small indistinct plaques developed in BGM cells, best results were obtained in CK cells which supported the development of distinct large plaques. The CK cells gave 12—16-fold higher plaque titres than BGM cells. The titres obtained by plaque and IFC counting techniques in CK cell system differed by a factor of only 2. The type of cells used for assay did not affect much the $TCID_{50}$ titres. These titres were 2—5-old lower than those obtained by the other two methods with CK cells. Whereas, with BGM cells, the $TCID_{50}$ titres were 4-fold higher than plaque titres and 2—5-fold lower than IFC count titres.

Quantitative differences were observed when average plaque and $TCID_{50}$ titres were compared with maximum titres obtained by parallel IFC count in 3 replicate tests (Table 2). The plaque assay was most efficient in CK cells. The efficiency of plaque assay compared with IFC count was much higher in CK cells when compared with BGM cells. Whereas, the efficiency of end-point assay when compared with IFC counting was low irrespective of the type of cells used for the assay.

Table 1. The results of infectivity assay of bovine rotavirus in calf kidney and BGM cells by different methods

Stock virus grown in	Assay in	Method of infectivity assay					
		Immunofluorescent cell count (FC/ml)		Plaque assay (PFU/ml)		End-point assay ($TCID_{50}$/ml)	
		Range	Mean	Range	Mean	Range	Mean
Calf Kidney (CK) cells	CK cells	1.5×10^8 to 3.2×10^8	2.0×10^8	9.7×10^7 to 2.2×10^8	1.1×10^8	3.7×10^7 to 4.9×10^7	4.0×10^7
	BGM cells	1.2×10^8 to 2.1×10^8	1.6×10^8	5.6×10^6 to 7.8×10^6	6.9×10^6	2.6×10^7 to 3.7×10^7	3.0×10^7
BGM cells	CK cells	9.2×10^7 to 2.3×10^8	1.0×10^8	5.6×10^6 to 6.7×10^6	6.0×10^7	2.9×10^7 to 4.1×10^7	3.0×10^7
	BGM cells	5.4×10^7 to 6.7×10^7	6.0×10^7	4.1×10^6 to 6.3×10^6	5.0×10^6	1.8×10^7 to 2.9×10^7	2.2×10^7

Table 2. Efficiency of plaque and end-point methods in comparison with immunofluorescent cell count assay for infectivity titration of bovine rotavirus

Assay cells	Maximum immunofluorescent cell count titre (FC/ml)	Efficiency of plaque assay[1]	Efficiency of end-point assay[2]
Calf kidney cells	3.2×10^8	0.34	0.13
BGM cells	2.0×10^8	0.03	0.15

[1] Ratio of average plaque titre in indicated assay cells/corresponding maximum immunofluorescent cell count titre.

[2] Ratio of average titre obtained by end-point test in indicated cells/corresponding maximum immunofluorescent cell count titre.

Discussion

It is clear from the present data that plaque and IFC counting techniques measure infectivity of bovine rotavirus in CK cells with comparable efficiency. BGM cells although susceptible to bovine rotavirus were unsuitable for plaque assay. Whereas, these cells in addition to other reported cells could serve as efficient cells for quantitative infectivity assay of bovine rotavirus by IFC count and end-point dilution methods.

The IFC count method is more rapid with economy of time and materials and could be applied to rotaviruses which do not plaque readily. But the inherent defect of this method is that infected cells and associated virus are killed by the procedures required for their immunofluorescent identification which precludes the isolation of viral clones. The plaque assay is very accurate but about 5 days are required and titres depend very much on the susceptibility of assay cell system and the additives in the overlay medium. Plaque technique, unlike IFC count could be useful in providing the basic experimental tool for genetic investigation of viruses.

By inclusion of trypsin in the maintenance medium in end-point assay, the CPE could be clearly observed within 3 days of incubation. Although the efficiency of this method was comparatively low and lacks accuracy of direct counts, this method is simple and could be adapted for routine infectivity assay of bovine rotavirus in laboratories where facilities for other methods do not exist.

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