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Transmissible gastroenteritis (TGE) virus is one of the major agents causing acute diarrhoea in pigs. The virus causes severe diarrhoea and high mortality in neonatal pigs. A rapid and reliable diagnosis has been required for the control of the disease. In 1963, Harada et al. [3] first isolated the TGE virus which produced a cytopathic change in swine kidney (SK) primary cell cultures and then it became possible to perform the virus titration and serum neutralization test in vitro. Witte [6] established the micro-method for the titration and the serum neutralization test of TGE virus with secondary pig thyroid cells. However, there is still such troublesome as to collect the fresh thyroid tissues and it has certain deficiencies for applying to numerous samples. Such being the case, the authors made an attempt to develop a micro-method with newly established porcine kidney cell line CPK cells. The newly developed method was as effective as the conventional tube method with SK primary cell cultures.

The TO-163 strain of cytopathogenic TGE virus adapted to primary SK cell was employed [4]. The primary SK cells and CPK cell line were used for this experiment. The CPK cell line derived from porcine kidney was originated by one of the authors [2]. Briefly, the cells were passaged about 430 times to establish a strain designated as SK-H. Then they underwent cloning three times. After cloning they were subjected to about 100 passages before they were used for the experiment. The passage of these cells had been conducted by digesting the cells with a mixture of 0.1% trypsin and 0.02% EDTA every 3 or 4 days. The growth medium was Eagle’s MEM containing 10% inactivated bovine serum, 10% tryptose phosphate broth and 1% of 7% NaHCO₃. When the medium was used for microplates, 1.5% of NaHCO₃ was added. Virus titration of TGE virus with a microplate was performed as follows. TGE virus was diluted tenfold serially in test tubes. Each dilution was dispensed into ten wells in 0.05 ml amounts. After that, 0.1 ml of CPK cell suspension, 4~5×10⁵ cells/ml, was added to each well. The microplates were placed in a humidified incubator containing 5% carbon dioxide. After incubation at 37°C for 5 days the culture was examined for the appearance of cytopathic effect (CPE). The appearance of CPE was judged by two methods as described in the previous report [5]. A distinct CPE was observed in the CPK cells infected with TO-163 strain 3 days after incubation, but uninfected cells remained normal. They showed crown like appearance which consisted of group of cells surrounded by ring-forming dense cells (Fig. 1). When observed after fixation and staining, cells were stained in CPE negative or control wells, but not stained at all in CPE positive wells (Fig. 2).

The growth curve experiment of the TO-163 strain of TGE virus on CPK cells was shown in Fig. 3. The virus infective titer of the fluid reached the maximum level at 16 hours after inoculation, and remained at almost the same level over a period from 32 to 56 hours.

Sera collected from 54 pigs in the field were employed for neutralization test. In order to compare with conventional and micro methods, the neutralization test with roller tubes was carried out by the method of Harada et al. [4]. Micro-method was performed according to the procedure described previously [5]. As illustrated in Fig. 4, there was a high correlation between the neutralizing antibody titers obtained by both methods. The coefficient of correlation was r=+0.976 at a probability rate of below 1%.

The neutralization test for TGE virus have generally been performed using the tube method and the plaque-reduction test with SK or swine testicle cells [1, 4]. Witte [6] developed the micro-color test with secondary pig thyroid cells for titration of TGE virus. He pointed out the advantage of the method in the saving of time.
Fig. 1. Cytopathic effect of TO-163 strain in CPK cells. Uninfected control (A), a day after virus infection (B) and three days after virus infection (C). Unstained. ×100.
and labor. The authors' method is highly practicable for titration and neutralization test of TGE virus. It may have further advantages as follows; ease of supply for cells, small quantity of samples and capability of processing numerous samples in short time.
Fig. 4. Correlation of neutralizing antibody titer between swine kidney and CPK cells.

REFERENCES


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豚腎株化細胞を用いた豚伝染性胃腸炎ウイルスのマイクロ中和試験（短報）：駒庭英夫・福所秋雄1)・清水悠
紀和2)（栃木県宇都宮家畜保健衛生所，1)農林水産省家畜衛生試験場）——豚腎株化細胞 CPK 細胞を用いて、マ
イクロプレート法による豚伝染性胃腸炎ウイルス定量中和反応を確立した。この方法により測定した血清中和抗
体価と、従来用いられてきた豚腎初代培養細胞を用いた試験管法により測定された中和抗体価の間には高い相関
（r=+0.976）が認められた。