Chapter 6

The Preparation of Chicken Kidney Cell Cultures for Virus Propagation

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Abstract

Chicken kidney (CK) cell cultures have historically proved useful for the assay of a number of viruses including coronaviruses. A technique for the preparation of such cell cultures, using a combination of manual and trypsin disaggregation of kidneys dissected from 2- to 3-week-old birds is described. This technique routinely gives high cell yield together with high viability and the resultant adherent primary cultures can be used for virus growth and plaque formation.

Key word Chicken kidney cell culture, Virus growth, Viral assay

1 Introduction

Techniques for the preparation of monolayer cultures from adult kidney cells suitable for the growth and quantitation of viruses have been available for many years; Dulbecco and Vogt [1] described the preparation of Monkey Kidney cultures in 1953 and Youngner [2] published a modification of the process in 1954. Maassab in 1959 [3] describes the preparation of Chicken Kidney monolayer cultures from 4- to 5-day-old chicks, the cultures being used for studies with some human viruses and Churchill [4] reports the use of chicken kidney tissue cultures derived from 3 to 8 week old chickens in the study of avian viruses including Infectious Bronchitis Virus (IBV). The technique for the production of kidney cell monolayer cultures from young birds, as described here, is adapted from those published by Dulbecco and Vogt [1], and Youngner [2] for monkey kidney cells.

Whilst titration of IBV in CK cells gives lower titers than those obtained in embryonated eggs [5] or tracheal organ cultures [6], the ability of CK cells to support the growth of many strains of IBV is well proven. Following adaptation in embryonated eggs, the Beaudette strain of IBV produced characteristic cytopathic effects

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(CPE) on first passage in CK cells, whilst the Massachusetts strain produced CPE in the second CK passage [4]. CPE consists of syncytia formation which occurs at 6 h post inoculation with the Beaudette strain [7]. The syncytia may contain as many as 20–40 or more nuclei and they quickly round up and detach from the culture surface. Growth curves of IBV in CK cells show a lag phase of 2–4 h and maximum virus yield in 18–20 h [5].

The ability of CK cells to support the growth of IBV has been utilized in wide ranging studies including the assessment of pH stability of a series of IBV strains [8], the identification of the presence of a leader sequence on IBV mRNA A [9], the demonstration that the spike protein of IBV is a determinant of cell tropism [10], the induction of protective immunity with recombinant IBV Beaudette [11] through to the identification of novel zippered ER and associated spherules induced by IBV [12].

2 Materials

2.1 Preparation of Kidney Cells

1. 2–3 Week old chicken(s) from specific pathogen free (SPF) flock killed by cervical dislocation (*see* **Note 1**).
2. 70 % Industrial methylated spirits (IMS).
3. Sterile instruments to include large scissors, small scissors, small forceps, and scalpels.
4. Sterile 150 mm diameter petri dish.
5. Sterile glassware including conical flasks and beakers.
6. Funnel.
7. 150 ml bottles with leakproof lids.
8. Sterile wire mesh (50 mesh × 0.200 mm diameter wire) folded into a filter shape to fit a funnel (*see* **Note 2**).
9. 50 ml centrifuge tubes.
10. Dulbecco's phosphate buffered saline without calcium and magnesium (PBSa).
11. Trypsin: 0.25 % porcine trypsin, glucose 0.1 %, PBSa, sterile filtered.
12. Ethylenediaminetetraacetic acid (EDTA): 0.2 mg/ml EDTA, PBSa, autoclaved at 115 °C for 20 min.
13. New born bovine serum (NBBS), heat-inactivated at 56 °C for 30 min (*see* **Note 3**).

2.2 Culture of Kidney Cells

1. Incubator set at 37 °C and 5 % CO_2.
2. Tissue culture grade flasks or plates.
3. Sterile 50 ml syringes.
4. Haemocytometer.

5. Trypan blue.
6. Inverted Microscope suitable for observing cell cultures.
7. Swinnex 25, reusable, syringe driven polypropylene filter unit fitted with metal gauze, 50 mesh × 0.200 mm dia wire (Swinnex Filter 1) (*see* **Notes 2** and **4**).
8. Swinnex 25, reusable, syringe driven polypropylene filter unit fitted with metal gauze 100 mesh × 0.100 mm dia wire (Swinnex Filter 2) (*see* **Notes 2** and **4**).
9. Eagles Minimum Essential Medium (EMEM) with Earle's salts, 2 mM L-glutamine and 2.2 g/L sodium bicarbonate.
10. Tryptose phosphate broth (TPB): 29.5 g/L dry broth in tissue culture grade water, autoclaved at 115 °C for 20 min.
11. HEPES buffer: 1 M HEPES (free acid) and tissue culture grade water, autoclaved at 115 °C for 20 min (HEPES).
12. Penicillin and Streptomycin at 100,000 U of each per ml (P & S).
13. Growth medium: EMEM, 10 % NBBS, 2.95 g/L TPB, 10 mM HEPES, 100 U/ml penicillin and 100 U/ml streptomycin.

3 Method

The number of kidney cells obtained from each bird will vary with the age and the strain of the birds used. We have found that the average cell yield from a 2 week old Rhode Island Red bird is approximately 2.0×10^8 cells.

3.1 Preparation of Kidney Cells

1. Aseptically prepare disaggregation mix by addition of 7.5 ml of trypsin to 80 ml of EDTA and warm to 37 °C (Trypsin/EDTA).
2. Add 50–100 ml NBBS to suitable sterile flask (*see* **Note 5**).
3. Spray work area with IMS, protect with clean paper towels and collect sterile instruments and glassware so that they are close to hand.
4. Spray the back of the birds and under the wings with IMS to clean and dampen the feathers and lay the bird, dorsal side uppermost, on the paper towels.
5. Insert the blade of a large, robust pair of scissors just below where a wing attaches to the body and sever across the body, through the spinal cord to where the second wing attaches, taking care to avoid piercing the gut.
6. From the ends of this first cut, once again taking care to avoid piercing the gut and your own hand, cut along each side of the body towards the legs and through the top of each leg.

7. Carefully fold back the cut section of the bird to reveal the internal organs. Move the intestines, which should have remained attached to this retracted section, to reveal the kidneys.
8. Remove the kidneys using a small pair of scissors and forceps and place in a 250 ml beaker of PBSa.
9. Repeat **steps 4–8** for every bird.
10. When all the kidneys required have been removed from the birds, agitate them in the beaker and discard the PBSa. Repeat this process until the wash PBSa looks clear (*see* **Note 6**).
11. Tip the drained kidneys into a large glass petri dish and using two scalpels shred and mince the kidneys into very small pieces removing as much clotted blood, connective tissue, and kidney core as possible.
12. Transfer the minced tissue into a tightly capped bottle (for example a 150 ml medical flat) and wash with approximately 80 ml of PBSa until the supernatant runs clear, allowing the tissue fragments to settle for 1 min in between the washes and discarding the PBSa washes (*see* **Note 6**).
13. Add 50–80 ml Trypsin/EDTA to drained tissue and shake moderately hard for 2 min. Allow the tissue to settle and discard the supernatant (*see* **Note 6**).
14. Add another volume of Trypsin/EDTA and shake for 4 min. Allow the tissue to settle and this time pour the supernatant into the conical flask containing NBBS. Gently swirl the flask to distribute the isolated cells in the NBBS.
15. Repeat **step 14** until no more tissue remains (*see* **Note 7**).
16. Filter the cell suspension/NBBS mix collected in **Step 14** through the metal gauze filter supported in a funnel placed in a fresh conical flask (*see* **Note 8**). Decant filtered cells into centrifuge tube(s) and centrifuge at approximately $300 \times g$ for 10 min to pellet the cells.

3.2 Culture of Kidney Cells

1. Warm growth medium to 37 °C.
2. Working in a Microbiological Safety Cabinet (Class 2), carefully discard the supernatant from the centrifuge tubes and resuspend the pelleted cells in growth medium, triturating at least five times.
3. Using a 50 ml syringe pass the cell suspension through Swinnex filter 1 then through Swinnex filter 2 collecting the filtrates from filter 1 and 2 in a fresh flask each time (*see* **Note 8**).
4. Measure and record the volume. Take 0.1 ml of cell suspension add to 0.9 ml of trypan blue and count the viable cells (*see* **Note 9**).
5. Dilute cell suspension in growth medium to the cell concentration required, seed culture flasks and place in incubator until intact monolayer forms (*see* **Note 10**).

4 Notes

1. This should be done immediately prior to the removal of the kidneys to minimize the buildup of blood clots.
2. Wire mesh is obtainable from Locker Wire Weavers, www.wiremesh.co.uk.
3. As one batch of serum may not support the growth of CK cells as well as another, we recommend batch testing of serum prior to purchase and use.
4. Swinnex filter holders, support screens and silicone gasket are obtainable from Millipore. To assemble the Swinnex filters, cut a 25 mm diameter disc from 50 mesh × 0.200 mm dia wire for Filter 1 and 100 mesh × 0.100 mm dia wire metal gauze for Filter 2. Place mesh disc and O-ring in the Swinnex 25 holder, pack in autoclavable bag, label Filter I or Filter 2 as appropriate and sterilize at 121 °C for 20 min. Filter holders and mesh discs can be reused after disassembling and washing.
5. The volume of NBBS, which is used to inactivate the Trypsin/EDTA, will depend on the number of birds. Use at least 50 ml for up to 6 birds and increase the volume by 10 ml for each extra bird. The flask volume should be at least 500 ml.
6. Whilst some kidney cells may be lost in this process, it is an effective way of removing many of the red blood cells that are still present at this stage of the preparation.
7. This may require 6–8 repeats.
8. This process helps to remove some of the larger aggregates of cells that remain in the preparation at this stage. Removing the aggregates makes the cell counting process more accurate and the resultant monolayers more evenly dispersed.
9. There will still be a number of red blood cells at this stage. These should be excluded from the cell count. The red blood cells can be distinguished from other kidney cells by their size and shape.
10. For plaque assays and viral growth seed flasks at approximately $0.3 \times 10^6/cm^2$ and incubate for 72 h prior to virus introduction.

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