Chapter 5

The Preparation of Chicken Tracheal Organ Cultures for Virus Isolation, Propagation, and Titration

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Abstract

Chicken tracheal organ cultures (TOCs), comprising transverse sections of chick embryo trachea with beating cilia, have proved useful in the isolation of several respiratory viruses and as a viral assay system, using ciliostasis as the criterion for infection. A simple technique for the preparation of chicken tracheal organ cultures in glass test tubes, in which virus growth and ciliostasis can be readily observed, is described.

Key words Tracheal organ culture, Ciliostasis, Respiratory virus, Viral assay

1 Introduction

Tracheal organ cultures (TOCs) have been used for the study of a number of respiratory tract pathogens [1]. The first human coronavirus (HCoV) was isolated using human ciliated embryonal trachea [2], and studies on persistent infection with Newcastle disease virus [3], isolation of the Hong Kong variant of influenza A2 virus [4], and studies on the pathogenicity of mycoplasmas [5] using TOCs have all been reported. More recently, TOCs have been used in studies on the pathogenicity and induction of protective immunity by a recombinant strain of infectious bronchitis virus (IBV) [6].

Tracheal organ cultures derived from 20 day old chicken embryos are reported to be as sensitive as 9 day old embryonated eggs for the isolation and titration of IBV [7], and are more sensitive than TOCs from chickens up to 31 days of age with complete ciliostasis, the criterion for infection, being observed 3 days after infection.

With the ease of production and the proven usefulness of TOCs in virus isolation and in studies on pathogenicity and immunization strategies, their more widespread use for research into respiratory tract viruses should be considered. Whilst TOCs have been successfully prepared for assays using multiwell plates [8], the

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method described below is based on that previously reported [5] and utilizes chicken embryo TOCs on a rolling culture tube assembly, where TOCs are reported to be capable of maintaining ciliary activity for longer periods than in static cultures. Debris accumulating within the TOCs rings is reduced, making observation of ciliary activity easier.

2 Materials

2.1 Preparation of Tracheal Section

1. 19- to 20-day-old embryonated eggs from specific pathogen free (SPF) chicken flock.
2. Tissue chopper: the following method assumes the use of a McIlwain mechanical tissue chopper (Mickle Laboratory Engineering Co. Ltd.).
3. Sterile curved scissors (small).
4. Sterile scissors (large).
5. Sterile forceps.
6. Sterile Whatman filter paper discs 55 mm diameter (*see* **Note 1**).
7. 70 % industrial methylated spirits (IMS).
8. Double-edged razor blades.
9. Eagle's Minimum Essential Medium (MEM) with Earle's salts, 2 mM L-glutamine, and 2.2 g/L sodium bicarbonate.
10. Penicillin + streptomycin (100,000 U of each per ml).
11. 1 M HEPES buffer prepared from HEPES (free acid) and tissue culture grade water, sterilized in an autoclave at 115 °C for 20 min.
12. Culture medium: MEM, 40 mM HEPES buffer, 250 U/ml penicillin, and 250 U/ml streptomycin.
13. Sterile Bijou bottles or similar.
14. Sterile 100- and 150-mm-diameter petri dishes.

2.2 Culture of Tracheal Sections

1. Tissue culture roller drum capable of rolling at approximately 8 revolutions/hour at 37 °C.
2. Associated rack suitable for holding 16 mm tubes on roller drum.
3. Sterile, extra-strong rimless soda glass tubes 150 mm long × 16 mm outside diameter, suitable for bacteriological work (*see* **Note 2**).
4. Sterile silicone rubber bungs 16 mm diameter at wide end, 13 mm diameter at narrow end, and 24 mm in length (*see* **Note 3**).
5. Inverted microscope (60–100× magnification).

3 Method

To calculate the number of embryonated eggs required for an assay, assume that each trachea will yield 17–20 rings. Expect a loss of up to 20 % of the cultures during the preliminary incubation step, owing to damage to the rings during preparation or spontaneous cessation of ciliary activity.

3.1 Preparation of Tracheal Sections

1. On a clean workbench spray the top of the eggs with 70 % IMS (*see* **Note 4**).
2. Using curved scissors remove the top of the shell, lift the embryo out by the wing and cut off the yolk sac. Place the embryo in a 150 mm petri dish and discard the egg and yolk sac.
3. With a sharp pair of scissors decapitate the bird, severing the spinal cord just below the back of the head and angling the cut to just below the beak (*see* **Note 5**).
4. Position the embryo on its back and, using small forceps and scissors, cut the skin along the length of the body from the neck to the abdomen. Care must be taken not to damage the underlying structures.
5. Locate the trachea and using small scissors and forceps, dissect it away from the surrounding tissues (*see* **Note 6**).
6. Cut the trachea at the levels of the carina and larynx (the larynx may have been removed on decapitation) and remove it from the embryo, placing the tissue in a Bijou bottle containing culture medium (*see* **Note 7**).
7. Repeat **steps 2–6** for all available embryos.
8. Place one trachea at a time on a disc of filter paper in a petri dish and, using two pairs of fine forceps, gently remove as much fat as possible (*see* **Note 8**).
9. Place the cleaned tracheas in a 100 mm petri dish containing culture medium.
10. Swab the tissue chopper with 70 % IMS.
11. Place two filter paper discs on top of the plastic cutting table disc and slide the assembled discs under the cutting table clips on the tissue chopper.
12. Raise the chopping arm of the of the tissue chopper and attach the razor blade.
13. Position the arm over the center of the cutting table (*see* **Note 9**).
14. Place the tracheas on to the filter paper under, and perpendicular to, the raised blade and moisten with a small amount of culture medium (*see* **Note 10**).
15. Adjust the machine to cut sections 0.5–1.0 mm thick and activate the chopping arm.

16. Once the arm has stopped moving, discard the first few rings from each end of the cut tracheas; then with a scalpel, scrape the remaining rings into a 150-mm petri dish containing culture medium.
17. With a large bore Pasteur pipette or similar gently aspirate the medium to disperse the cut tissue into individual rings.
18. Repeat **steps 11–17** until all the tracheas have been sectioned (*see* **Note 11**).

3.2 Culture of Tracheal Sections

1. With a large bore Pasteur pipette or similar dispense one TOC ring together with approximately 0.5 ml of culture medium into a glass tube (*see* **Note 12**).
2. Seal the tube with silicone bung and check that each tube contains one ring (*see* **Note 13**).
3. Put the tubes in the roller tube rack, place on the roller apparatus and set to roll at approximately 8 revolutions/hour, at approximately 37 °C. Leave the tubes rolling for 1–2 days (*see* **Note 14**).
4. Check each tube culture for complete rings and the presence of ciliary activity, using a low power inverted microscope
5. Discard any tubes in which less than 60 % of the luminal surface has clearly visible ciliary activity.
6. The remaining tubes may be used for viral assays (*see* **Note 15**).

4 Notes

1. Batches of sterile Whatman filter papers can be prepared by interleaving individual discs with slips of grease-proof paper and placing them in a glass petri dish. Wrap the dish in aluminum foil and sterilize in a hot air oven (160 °C for 1 h).
2. Batches of sterile tubes can be prepared by placing them, open end down, in suitable sized lidded tins lined with aluminum foil. Sterilize in a hot air oven as above.
3. Batches of sterile silicone rubber bungs can be prepared by placing them, narrow end down, in shallow, lidded tins. Sterilize by autoclaving at 120 °C for 20 min.
4. Preparation of TOCs can be performed on the open laboratory bench after cleaning the surfaces with 70 % IMS or any other suitable disinfectant.
5. Care must be taken at this stage not to damage the trachea.
6. The trachea can be identified by the presence of transverse ridges seen down its length owing to the underlying rings of cartilage.

7. The carina and larynx can be identified by the increased diameter at either end of the trachea.
8. To avoid damage to the trachea hold it as close to one end as possible with the first pair of forceps and use the second pair to strip away the fatty tissue.
9. At this stage gently lower the arm on to the cutting area disc, loosen the screw holding the blade slightly, check that the blade is aligned correctly (the full length of the blade must be in contact with the cutting area), tighten the screw again, and raise the arm.
10. A maximum of five tracheas can be laid side by side on the cutting bed at any one time. Gently stretch each trachea as it is placed on the cutting area, and when all five are in the correct position, wet them with a few drops of culture medium.
11. It is important to use a fresh blade and paper discs for each set of five tracheas to be sectioned and ensure used blades are disposed of in an appropriate sharps bin.
12. Check for damaged glass tubes at this stage, particularly around the rims. Discard any with cracks as these can fail when bungs are inserted, leading to injured fingers.
13. Make sure the tracheal rings are fully submerged in culture medium and not stuck on the wall of the tube. Discard any that are ragged or incomplete.
14. Make sure that the drum is aligned correctly on the apparatus and that the roller is actually moving before leaving the cultures to incubate; the speed of the roller apparatus is slow.
15. A simple quantal assay for infectivity of IBV has been described by Cook et al. [7] and is used extensively in our Institute. Five tubes of TOCs per tenfold serial dilution of virus give sufficiently accurate results for most purposes. A simplification of the method of Cook et al. [7], used for many years by Cavanagh and colleagues, is to add 0.5 ml of diluted virus per TOC tube without prior removal of the medium already in the tube. TOCs are scored as positive for virus when ciliary activity is completely abrogated. If a virus is poorly ciliostatic, its presence can be demonstrated using indirect immunofluorescence, with TOCs conveniently not fixed [9].

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