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A MICRO CELL CULTURE METHOD UTILIZING MODIFIED MICROSCOPE SLIDES FOR USE IN FLUORESCENT ANTIBODY AND ENZYME IMMUNOASSAYS

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SUMMARY: A technique for culturing small quantities of mammalian cells on modified microscope slides is described. The modified microscope slides were Bellco Glass, Inc., toxoplasmosis slides and the cell cultures used were early passage bovine embryonic lung cells and continuous cell lines of porcine and canine origins. The slide cell cultures were either uninfected or infected with selected viruses or the obligate intracellular protozoan *Encephalitozoon caniculi* for utilization in direct and indirect fluorescent antibody testing or in peroxidase antiperoxidase immunosorbant assays.

Key words: immunofluorescence; enzyme immunosorbant assays; micro cell culture methods; microscope slide cell culture methods.

I. INTRODUCTION

Mammalian cell cultures infected with viruses, or other microorganisms, are utilized routinely as substrates for direct or indirect fluorescent antibody studies (1,2) or for enzyme immunoassay (3,4). Cell cultures for such purposes have been prepared in Leighton tubes, chamber slides, or microtiter plates; however, these systems require moderate to large quantities (0.2–2 ml) of reagents and are cumbersome to manipulate. Described herein is an inexpensive and simple micro cell culture technique that utilizes commercially available toxoplasmosis microscope slides; these slides have eight circumscribed areas (each six mm in diameter) which hold 0.025 ml of fluid. Additionally, these slides can be prepared *en masse* and easily stored for later use.

II. MATERIALS

A. Equipment

Controlled-atmosphere incubator, 37° C, equipped for 5% CO_2 environment
Table-top centrifuge, with swinging bucket rotor
Epi-illuminated fluorescent microscope
Inverted microscope
Jerne tray incubator, No. 11-716, Fisher[1]

B. Medium and chemicals

Tissue culture media appropriate to systems being utilized.
Fluorescein-conjugated antisera appropriate to viruses utilized (4).
Ethyl alcohol, reagent, No. A-962[1]

0271-8057/82/0013-0017 $1.50/0

Methyl alcohol, Certified, No. A-936[1]
Acetone, Certified, No. A-20[1]
Hydrogen peroxide, technical, No. H-327[1]
Permount, No. So-P-15[1]
Ethylene diamine tetraacetic acid (Versene) Certified ACSm Bi, No. E-478[1]
Phosphate buffered saline (PBS), pH 7.2
HEPES buffer (25 m*M*), No. H3373, Sigma[2]
Trizma® base, Reagent grade, No. T-1503[2]
Trizma® HCl, reagent grade, No. T-3253[2]
Diaminobenzidine, No. D-5637[2]
Hematoxylin solution, Delfield formula, No. SC 12564, Sargent-Welch[3]
Trypsin, 1:250, Difco, No. DF0152-13[4]
FA Mounting Fluid, No. DF2329-57[4]

C. Glassware and other

Jerne plaque tray, Norbo[5]
Toxoplasmosis slides, No. 5638-01940, Bellco[6]
Petri dish, 150 × 15 mm, No. 08-747E[1]
Coplin staining dish, No. 08-816[1]
Cover glasses No. 1, 22 × 60 mm, No. 12-5446[1]
Wooden applicators, No. 01-340[1]
Filter paper, Whatman 40, 12½ cm d, No. 09-845E[1]
Tissue culture flasks, Corning, No. 10-126-30[1]
Rabbit peroxidase antiperoxidase, No. 0100-1222[7]

III. PROCEDURE

A. Cell culture preparation

1. Culture cells, using standard techniques, in 25 cm² plastic culture vessels.
2. Infect the cultures with the appropriate concentration of virus to give maximum fluorescence, or antigen production, with minimum cytopathic effect within 36–48 hours post-infection.

NOTE: Remove cells by scraping with a Pasteur pipette every 4 h and place one drop of suspension in an area of a toxoplasmosis slide and air dry. When all areas are filled, fix in acetone at room temperature for 10 min. Examine by direct fluorescent antibody staining to determine appropriate harvest time for infected slide cultures (1).

3. Harvest cells, at appropriate time post-infection, with 0.05% trypsin and 0.025% Versene (EDTA) solution. Also harvest uninfected cells for controls.
4. Centrifuge cells at 150 ×*g* for 10 min in a non-refrigerated table-top centrifuge.
5. Resuspend the cells harvested from one 25 cm² culture vessel in 3–5 ml of growth medium for seeding of toxoplasmosis slides (See B.3).

B. Slide culture preparation

1. Place toxoplasmosis slides into a 150 × 15 mm petri dish containing two applicator sticks resting on a filter pad (Fig. 1).
2. Sterilize by steam autoclaving.
3. Place 0.025 (1 drop) of cell suspension on each circumscribed area of the slide. Seed two areas with uninfected cells.
4. Moisten filter pad with sterile PBS.

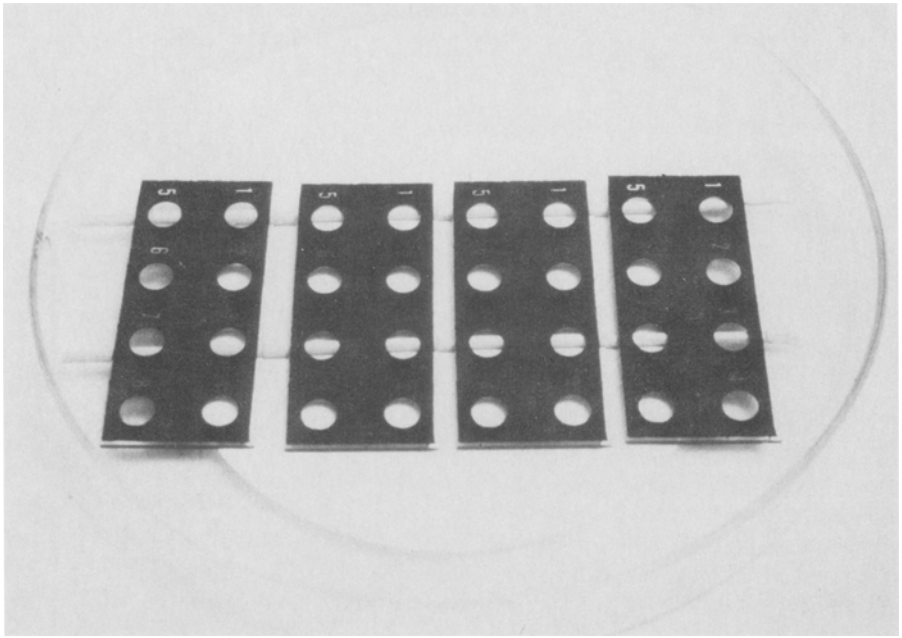

FIG. 1. Toxoplasmosis slides in a petri dish for use in slide culture preparation.

5. Incubate at 37° C for 2-4 h or until monolayer is confluent; use a 5% CO_2 in air system if HEPES buffering is not utilized.
6. Remove slides and rinse thoroughly with PBS.
7. Fix slides for 10 min at 20-22° C in acetone for fluorescent antibody studies or in methanol for peroxidase studies.
8. Store fixed slides at 4° C until needed.

C. Fluorescent antibody staining

1. Rehydrate acetone fixed slide cultures for 10 min at room temperature in PBS.
2. Utilize standard direct or indirect fluorescent antibody techniques (5).
3. Mount slides with FA mounting medium and examine with an epi-illuminated FA microscope.
4. Stained slides may be kept indefinitely by sealing the cover slip to the slide with fingernail polish and storing the slides in the dark at 4° C.

D. Enzyme immunoassay

1. Rehydrate methanol fixed slide cultures for 10 min at room temperature in 0.1 *M* Tris buffer.
2. Place 0.025 ml of serial two-fold dilutions (beginning with 1:50) of rabbit anti-virus serum on the six infected areas. One uninfected area receives 0.025 ml of undiluted specific antiserum while the remaining uninfected area receives 0.025 ml of undiluted normal rabbit serum.
3. Incubate slides at 37° C in a humid chamber for one hour then rinse thoroughly in 0.1 *M* Tris buffer.
4. Place the slides in a Jerne tray and flood with 1:100 dilution of goat anti-rabbit IgG and incubate for one hour at 37° C in a Jerne incubator.
5. Rinse the slides with 0.1 *M* Tris buffer, flood with 1:300 dilution of peroxidase antiperoxidase complex and incubate for 1 h at 37° C in a Jerne incubator.
6. Rinse slide for 20-30 min in a Coplin jar with 100 ml of 0.1 *M* Tris buffer containing 15 mg diaminobenzidene and 0.003% H_2O_2.
7. Counterstain the slides in 2-4% hematoxylin for 0.5-2 min.
8. Rinse the slides with running tap water.
9. Dehydrate as follows: 3 min each in 50%, 70%, 90% and 95% ethyl alcohol, three 5-min rinses in 100% ethyl alcohol, and two 10-min rinses with zylene.
10. Mount the slides with Permount and examine.

IV. DISCUSSION

The systems described herein were utilized in several routine diagnostic procedures involving

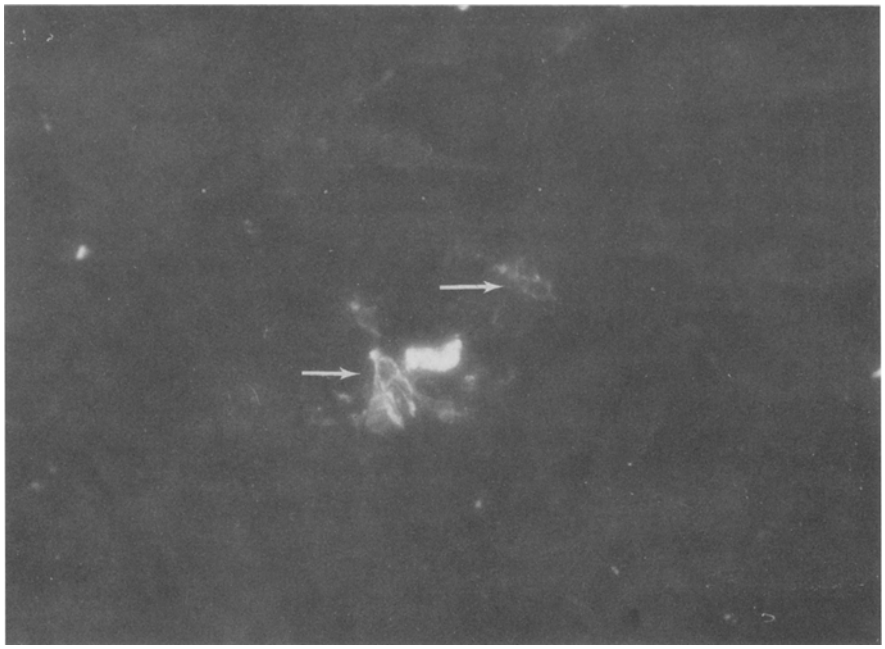

FIG. 2. Direct FA staining of BVD infected BEL cells: Note cytoplasmic fluorescence in infected cells (*arrows*). Negative cells can be distinguished in the background. ×400.

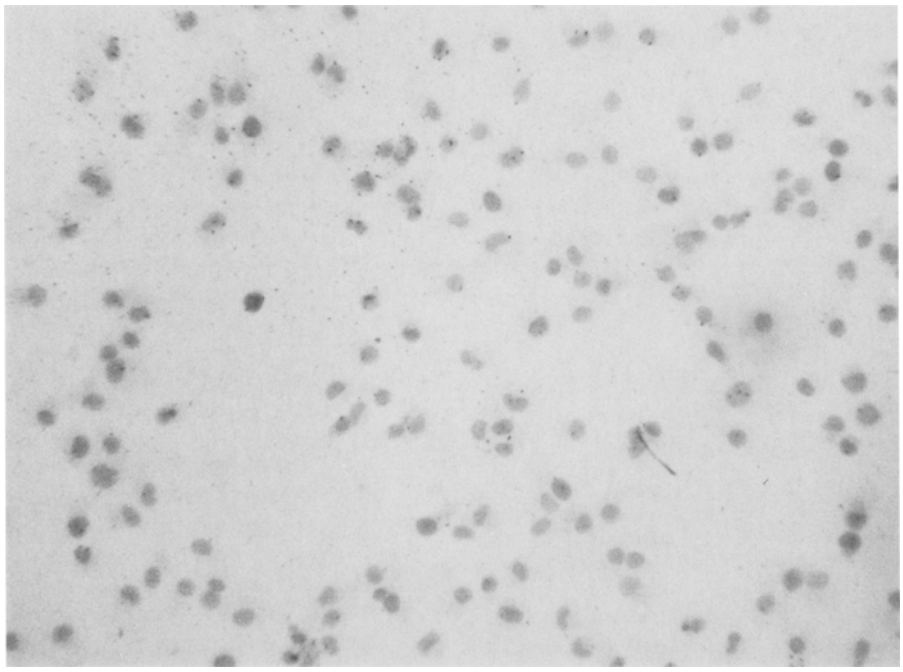

FIG. 3. Negative EIA reaction in uninfected BEL cells. Note prominence of counterstained nuclei and lack of detail of the cytoplasm. ×400.

direct (6) and indirect (7) fluorescent antibody techniques (4). The enzyme immunosorbant assay (EIA) technique used was the standard Sternberger unlabeled antibody technique (8). The viral systems used consisted of the herpesvirus of infectious bovine rhinotrachtheitis, the togavirus of bovine virus diarrhea, and the coronavirus of transmissible gastroenteritis of swine; these systems were analyzed by both fluorescent antibody (Fig. 2) and EIA (Fig. 4) techniques. Cell culture systems utilized in the toxoplasmosis slide culture system consisted of

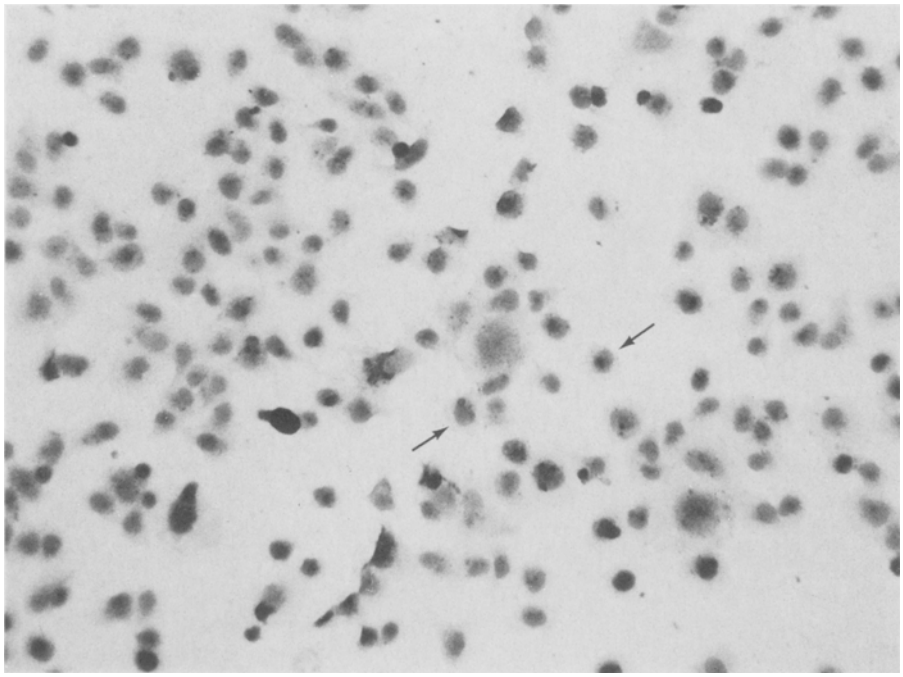

FIG. 4. Positive EIA reaction in IBR infected BEL cells. Note prominence of perinuclear area (*arrows*) due to uptake of peroxidase. ×400.

low (5-15) passage bovine embryonic (BEL) lung and continuous cell lines of porcine kidney (PK-15) and of canine osteosarcoma (COS) (kindly supplied by Dr. J. Shadduck, Dept. of Pathology, South West Medical School, Dallas, TX).

The cell culture systems utilized in this study all grew satisfactorily on the toxoplasmosis slides. Continuous cell lines grew more efficiently than low passage cells. Users of this technique must utilize a humid chamber and either a CO_2 incubator or HEPES buffer in the culture fluid to prohibit evaporation or pH change since each circumscribed area holds only 0.025 ml of fluid. The configuration of the toxoplasmosis slides permits the use of at least six wells for test purposes and one area each for positive and negative controls.

Use of these systems for EIA tests has shown considerable promise since serial dilutions may be used and since controls for each slide are also present. Of special interest was the ease with which these slides may be developed *en masse* using Jerne racks, thus facilitating the use of minimal amounts of reagents with uniformity of reaction time for the peroxidase techniques. Counterstaining with hematoxylin greatly facilitates reading EIA slides by providing a contrast between the nucleus and cytoplasm of the cells (Figs. 3, 4).

Use of these slide cultures for fluorescent antibody testing permitted the preparation of stock cultures for use as needed. These slides also may be rinsed using conventional hematology wash racks, and they may be easily cover slipped using FA mounting medium for FA examination. In addition, the slides used herein were of sufficient quality to permit direct examination with fluorescent microscope systems.

V. REFERENCES

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[1] Fisher Scientific Co., Englewood, CO.
[2] Sigma Chemical Co., St. Louis, MO.
[3] Sargent-Welch Scientific Co., Denver, CO.
[4] VWR Scientific, Inc., Denver, CO.
[5] Norbo Machine Co., 378 Simmon S.E., Huron, SD.
[6] Bellco Glass, Inc., Vineland, NJ..
[7] Cappel Laboratories, Cochranville, PA.

Approved by Author

James J. England 9/1/81
Signature Date