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REVIEW ARTICLE

Traditional and Modern Cell Culture in Virus Diagnosis

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Abstract
Cell cultures are developed from tissue samples and then disaggregated by mechanical, chemical, and enzymatic methods to extract cells suitable for isolation of viruses. With the recent advances in technology, cell culture is considered a gold standard for virus isolation. This paper reviews the evolution of cell culture methods and demonstrates why cell culture is a preferred method for identification of viruses. In addition, the advantages and disadvantages of both traditional and modern cell culture methods for diagnosis of each type of virus are discussed. Detection of viruses by the novel cell culture methods is considered more accurate and sensitive. However, there is a need to include some more accurate methods such as molecular methods in cell culture for precise identification of viruses.

1. Introduction

In the 1900s, embryonated eggs and laboratory animals were used for isolation of viruses. Typically, cell cultures are developed from tissue samples and then disaggregated by mechanical, chemical, and enzymatic methods to extract cells suitable for isolation of viruses. With the utilization of cell culture technique, use of laboratory animals in experiments has decreased significantly [1]. In addition, by selection of suitable cell lines, the number of viruses indexed has increased dramatically. Isolation of viral pathogens in cell cultures commenced in the 1960s; however, at this point, some limitations existed, including very limited services available for diagnosis of viral infections. In 1970, commercial development of purified reagents and cell lines opened a new window for diagnosis of viral infections [2]. With the discovery of cell culture, many human viruses were grown *in vitro*. In comparison with eggs and animals, cell culture is more convenient and cost effective. This method is considered gold standard for virus isolation and identification [2].

The aims of the current review are to explain the current role of cell culture in viral diagnosis and the

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advantages (e.g., cost, culture time) of the new methods of culture over traditional cell culture methods.

2. Traditional cell culture for virus diagnosis

In 1913, for the first time ever, a virus (vaccinia) was grown in cell culture, and then in the 1930s, yellow fever and small pox viruses were grown in cell culture that aimed for vaccine production [3–5]. However, it was only in 1950 that the first virus (poliovirus) was isolated [6]. Cell culture was developed by adjustment of antibiotic for prevention of contamination with bacteria and use of some chemical to media, which provided the cell culture media [7]. Although culture media and cell lines can be purchased commercially, some laboratories still prepare culture media in-house. Cell culture can be accomplished in any container, however, the standard container is a screw-cap tube glass (16 mm × 125 mm; Figure 1) in which monolayer cells can grow on one side of the glass. For accurate identification of viruses, different types of cell lines should be prepared to inoculate the suspected sample. The most important cell lines widely used for viral diagnosis are primary rhesus monkey kidney cells (RhMK), primary rabbit kidney cells, MRC-5, human foreskin fibroblasts, HEp-2, and A549.

The type of specimens to be used are determined based on the number and cell types needed for virus diagnosis. The cost of cell culture ranges from US $1.5/tube to US $6.50/tube. The success of virus isolation depends on the best selection, collection, and transportation of clinical samples.

Figure 1. Standard screw-cap tubes (16 mm × 125 mm) used for cell culture.

Table 1. CPE formation and confirmation test in different viruses.

Viruses	CPE in			Final identification of isolates
	Fibroblasts	A549 cells	RhMK cells	
Adenovirus	Some produce clusters	Grape-like clusters or "lacy" pattern; 5–8 d	Some produce clusters	IF for group and neutralization for type
Cytomegalovirus	Foci of contiguous rounded cells; 10–30 d	—	—	CPE
Herpes simplex virus	Rounded large cells; 2–6 d	Rounded large cells; 1–4 d	Some produce CPE	IF for group and neutralization for type
Influenza virus	—	—	Undifferentiated CPE, cellular granulation; 4–8 d	IF for group and neutralization for type
Rhinovirus	Degeneration, rounding; 7–10 d	—	—	CPE

CPE = cytopathic effect; IF = immunofluorescence; RhMK = rhesus monkey kidney cells.

2.1. Sample collection

The specimen processing protocol varies between laboratories, but the main steps followed are as follows:

First, the medium containing the sample is vortexed and the swap is discarded. The liquid medium is then centrifuged. The supernatant obtained is used in cell culture. In this method, fungi, cells, bacteria, and blood remain at the bottom of tube (pellet form), whereas viruses remain dispersed in the liquid.

Then, 0.2—0.3 mL of the liquid is added to the cell culture medium for absorption of the virus (inoculation). The cell culture tube containing the virus for absorption is then incubated at 35°C and 5% CO_2 for 90 minutes, following which the inoculum is discarded and substituted with fresh medium. The cell culture tube is incubated until the virus begins to grow. This process may take 1 day to several weeks depending on the type of virus. The cell culture tube is examined everyday using an inverted microscope [8,9].

The standard protocol applied for estimating the proliferation of the virus on monolayer cells involves examination of unstained cells on monolayer cells. Changes in monolayer cells (e.g., swelling, shrinking, syncytium formation) indicate the presence of viruses. These changes in cell culture are defined as the cytopathic effect (CPE), which is due to the presence of the virus [10].

In most cases, the CPE appears after 5—10 days of incubation; however, an exception is herpes simplex virus (HSV) in which the CPE is observed after just 24 hours. In some viruses, including cytomegalovirus (CMV), 10—30 days are needed after first incubation for CPE observation. According to the type of cell line used for cell culture, type of specimen, the incubation period, and form of the CPE, the type of virus can be predicted; however, confirmatory testing such as immunofluorescence (IF) assay is needed for better diagnosis. This assay is based on the reaction between the antibody and viral antigen. Table 1 shows the CPE of some viruses in different cell lines. Figure 2 shows the CPE formation by different types of viruses.

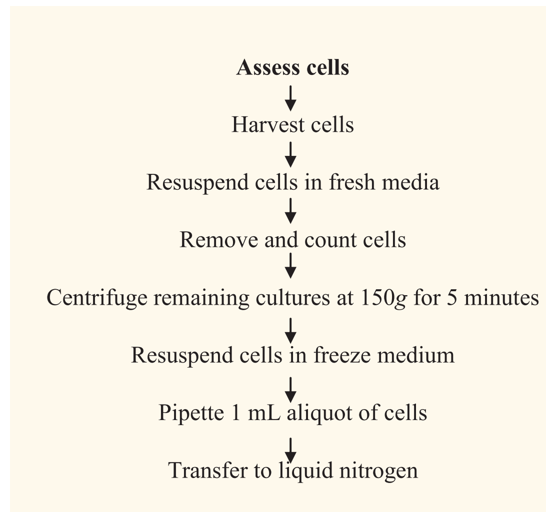
However, it is not possible to detect all viruses by IF staining. Numerous serotypes are observed in the enterovirus family, and all these are not identified by IF staining. Furthermore, monoclonal antibody used for identification of enterovirus lacks sensitivity and there are reports indicating crossreaction between monoclonal antibody and enteroviral serotypes [11—13].

Using the traditional cell culture methods, a variety of viruses can be detected in different cell lines. However, the long time needed for incubation and observation of the CPE are significant disadvantages; additionally, the high cost associated with the purchase and maintenance of different monolayer cells is another limitation.

3. The new format of cell culture

The traditional screw-cap cell culture tube (16 mm × 125 mm) is now replaced by a 1-dram vial or a shell vial, which is smaller (Figure 3). Using this vial, it is possible to grow monolayer cells at the bottom of the vial. In addition, this method also allows for easy centrifugation.

Another type of new container used in recent times is the microwell plate, which is also called a "cluster plate." This is available as a 24- or 96-well plate, however, 24 wells are more popular.



3.1. Cryopreserved cell culture

Some laboratories prepare their cell lines in-house, whereas some buy the commercial cell lines. With the introduction of cryopreservation, the maintenance of prepared cells became easier. Using this technique, the monolayer cells are grown in shell vials and then stored at −196°C. Prior to use, the shell vial is removed from liquid nitrogen and samples are incubated in a 37°C water bath. Then, the cell culture is prepared according to the standardized protocol, and then clinical samples are applied on the cell culture. It was reported that cryopreserved monolayer cells are sensitive to chlamydiae, CMV, HSV, and other pathogens in respiratory tract [14]. The various steps in cryopreserved cell culture are as follows:

3.2. Virus isolation in cocultured cells

Using this method, different types of cells are grown as a monolayer in a vial and various monoclonal antibodies are applied on these cells for diagnosis of different viruses. Using this technique, different viruses can be detected in the same vial.

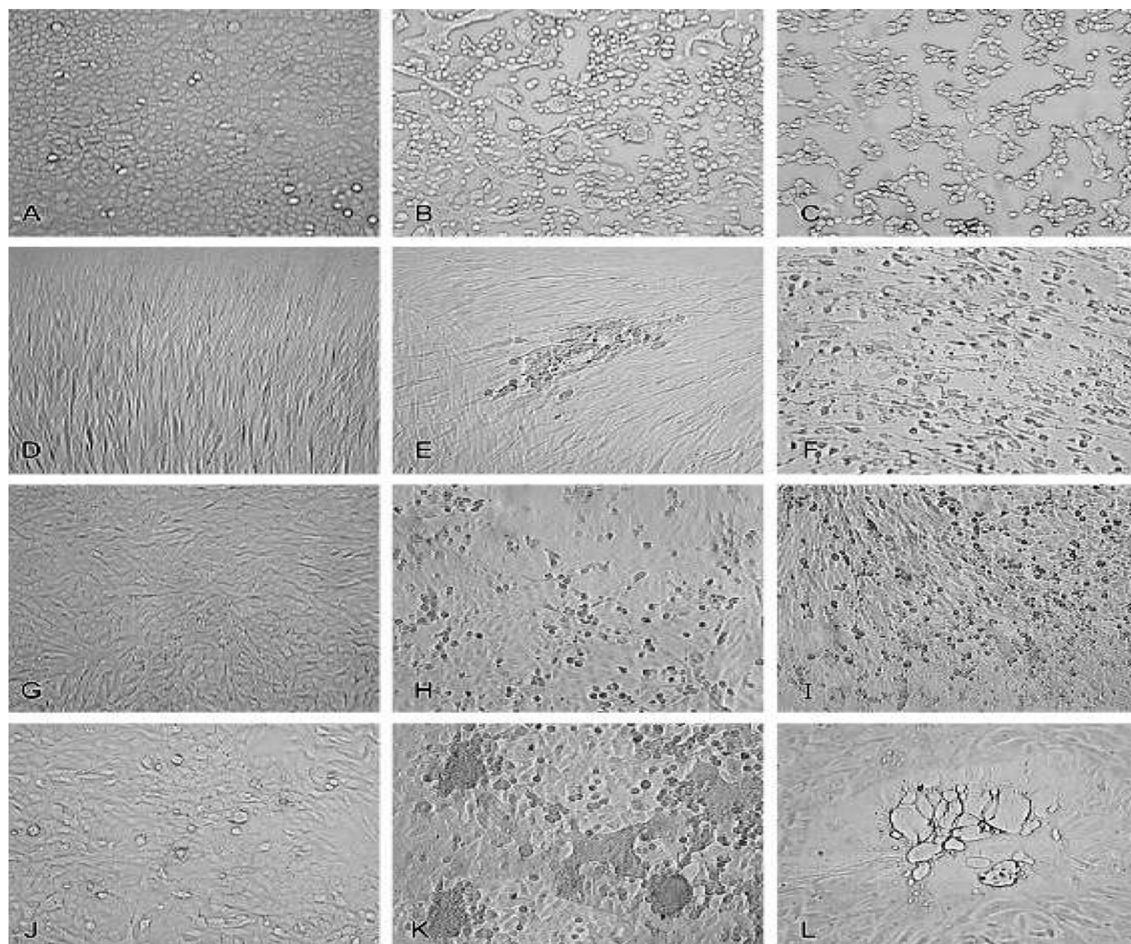


Figure 2. Cell and virus culture.(A) Untreated A549 cells, (B) HSV2 inoculated with A549, (C) adenovirus inoculated with A549, (D) untreated MRC-5 fibroblasts, (E) cytomegalovirus-inoculated MRC-5 fibroblasts, (F) rhinovirus inoculated with MRC-5 fibroblasts, (G) untreated RhMK, (H) enterovirus inoculated with RhMk, (I) influenza A inoculated with RhMk, (J) untreated HEp-2, (K) respiratory syncytial virus inoculated with HEp-2, and (L) monkey virus inoculated with RhMk. HSV = herpes simplex virus; RhMK = rhesus monkey kidney cells.

MRC-5 and A549 cell lines were used as monolayer in a vial for diagnosis of CMV, HSV, and adenoviruses. Cocktail antibody was used for staining. A secondary antibody labeled with antispecies antibodies was then added. The labeled dyes are fluorescein isothiocyanate (FITC), Cy3, and 7-amino-4-methylcoumarin-4-acetate. The stained cells were examined with FITC and then with UV filters. The sensitivity of current experiment was shown to be 93.8% for adenovirus, 88.9% for CMV, and 100% for HSV [15].

Figure 3. Shell vial that can be directly centrifuged.

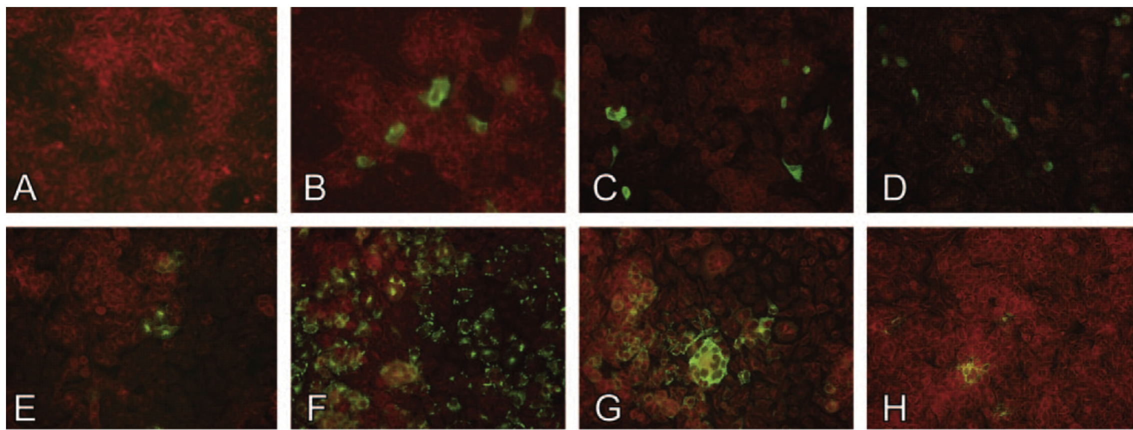


Figure 4. Immunofluorescence diagnosis of viral respiratory pathogens inoculated with R-Mix cells. (A) Untreated R-Mix, (B) adenovirus, (C) influenza type A, (D) influenza type B, (E) parainfluenza virus type 1, (F) parainfluenza virus 2, (G) parainfluenza virus 3, and (H) respiratory syncytial virus.

R-Mix cell is another cell line used for isolating a variety of viral respiratory pathogens. This cell line is a combination of A549 and mink lung cells in a shell vial. Three R-Mix cell lines are used for each sample. The vials were then centrifuged and incubated at 35°C/5% CO_2. After 24 hours, R-Mix was treated with different types of fluorescein-labeled monoclonal antibody against adenoviruses; parainfluenza virus Types 1, 2, and 3; influenza virus Type A; influenza virus Type B; and respiratory syncytial virus. Figure 4 shows IF diagnosis of viral respiratory pathogens inoculated with R-Mix cells.

3.3. Virus identification in transgenic cell lines

This is a rapid and accurate technique that uses transgenic cell lines. Some genetic elements are included in the cells, using which particular virus can be detected. These elements can be derived from any organism. In a previous study, for detection of human immunodeficiency virus (HIV), CD4-positive lymphoid cell line transformed into a retroviral vector containing long terminal repeat promoter in combination with chloramphenicol acetyltransferase gene and HeLa was used. Using this transgenic cell line, only HIV was detected; however, a limitation is that it cannot differentiate between HIV-1 and HIV-2 [16].

4. Conclusion

Since its discovery, many innovative methods for cell culture have been proposed (e.g., use of shell vial, cryopreservation). In addition, the time required for identification of viruses showed a significant decrease: from 5−10 days (traditional methods) to 24 hours (novel methods). Using different cells in one vial, the number of different cell lines used in laboratories for identification of viruses was decreased. Furthermore, detection of viruses by the novel cell culture methods is more accurate and sensitive. However, there is a need to include some more accurate experiments such as molecular methods in cell culture for precise identification of viruses.

Conflicts of interest

The author declares no conflicts of interest.

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