Osong Public Health Res Perspect 2016 7(2), 77–82
http://dx.doi.org/10.1016/j.phrp.2015.11.011
pISSN 2210-9099 eISSN 2233-6052

KOREA CENTERS FOR DISEASE CONTROL & PREVENTION

REVIEW ARTICLE

Traditional and Modern Cell Culture in Virus Diagnosis

Ali Hematian [a], Nourkhoda Sadeghifard [a], Reza Mohebi [a], Morovat Taherikalani [b], Abbas Nasrolahi [a], Mansour Amraei [a], Sobhan Ghafourian [a,*]

[a]*Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.*
[b]*Razi Herbal Medicines Research Center and Department of Microbiology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.*

Received: November 17, 2015
Revised: November 29, 2015
Accepted: November 30, 2015

KEYWORDS:
cell culture,
gold standard,
traditional culture,
viruses

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

*Corresponding author.
E-mail: Sobhan.ghafurian@gmail.com (S. Ghafourian).

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.