



## Original Study



### *Effect of fetal bovine serum concentration towards vero cells growth on culture in DMEM medium*



Bella Niken Ikasari<sup>1,2</sup> , Salman Alfarizi<sup>1</sup> , Shifa Fauziyah<sup>3</sup> , Puspa Wardhani<sup>3,4</sup>   
 Aryati<sup>3,4</sup> , Soegeng Soegijanto<sup>3</sup> , Teguh Hari Sucipto<sup>3,\*</sup> 

- <sup>1</sup> Internship student, Research Center on Global Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Universitas Airlangga, Indonesia
- <sup>2</sup> Chemistry Department, Faculty of Science and Technology, Universitas Airlangga, Indonesia
- <sup>3</sup> Dengue Study Group, Institute of Tropical Disease, Universitas Airlangga, Indonesia
- <sup>4</sup> Clinical Pathology Department, Faculty of Medicine, Universitas Airlangga, Indonesia

**Abstract:** Cell culture requires a suitable medium and environment for in vivo conditions. Conditions are created by regulating temperature, pH, oxygen, CO<sub>2</sub>, osmotic pressure, surfaces to adhere to cells, nutrients, and vitamins, protection against toxic substances, hormones, and growth factors that regulate cell growth and differentiation. In general, good cell growth occurs at a pH of 7.0 - 7.6. Fetal Bovine Serum (FBS) is one of the supplement media to support cell growth. The purpose of this study was to determine the effect of using FBS supplementation with various concentrations on Vero cell culture media on DMEM (Dulbecco's modified eagle medium) media. Vero cell culture on DMEM media has been successfully carried out using FBS by observing its growth for 14 days. Vero cell culture process begins with the manufacture of media consisting of DMEM and FBS. The cell density of each well at 5% and 10% concentration media was 1017 cells/well. By varying the concentration of FBS in the culture medium, the difference in the number of cells produced can be observed under the microscope. The results showed that the number of Vero cells produced from DMEM media with 10% FBS concentration was higher than 5% FBS concentration. Thus, this study proves that the higher the concentration of FBS in the medium, the more it supports the cell growth process in the Vero cell culture process.

**Keyword:** *Vero cells; DMEM; FBS*

## INTRODUCTION

Vero cells are cells that were first taken from the kidney of an adult African green monkey *Cercopithecus aethiops*, Vero cells are one of the most common mammalian continuous cell lines used in research<sup>1,2,3</sup>. It is used as a positive control that represents normal cells in the human body and is often used in toxicity test studies because of its easy handling, unlimited replication capability, and easy replacement of frozen stock in case of contamination<sup>4</sup>. Cell culture relates to a mode of reproduction of dispersed cells. Cell culture requires a suitable medium and environment for in vivo conditions. Conditions are created by regulating temperature, pH, oxygen, CO<sub>2</sub>, osmotic pressure, the surface to adhere to cells,

Corresponding author.

E-mail address: [teguhharisucipto@staf.unair.ac.id](mailto:teguhharisucipto@staf.unair.ac.id) (Teguh Hari Sucipto)

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nutrients, and vitamins, protection against toxic substances, hormones, and growth factors that regulate cell growth and differentiation.

The serum is a biological fluid that is proven to support cell growth outside the body<sup>5</sup>. Fetal Bovine Serum (FBS) is one of the IVM medium supplements derived from fetal bovine blood which was frozen and collected aseptically. It is used to stimulate the growth of large amounts of tissue culture cells. Serum concentrations are needed to support cell growth. For best cell growth a concentration of 5% is usually used. The function of FBS as a growth factor, penicillin as an antibiotic, and fungizon as an antifungal. Generally, good cell growth occurs at a pH of 7-7.6. The growing medium also requires a buffer because of two conditions: the use of an open flask causing the entry of O<sub>2</sub> which can increase the pH, and a high concentration of cells causing the production of CO<sub>2</sub> and lactic acid, which causes a decrease in the pH. Both of these conditions are faced by providing a buffer into the medium and the incubator CO<sub>2</sub> flows from outside. The condition culture consisting of a medium, Growth Hormone concentration and use of CO<sub>2</sub> incubator (old culture time) supports growth from follicular culture<sup>6</sup>.

## MATERIAL AND METHOD

### Research materials and tools

Vero cell purchased from The European Collection of Authenticated Cell Cultures (ECACC). Dulbecco's modified eagle medium (DMEM) purchased from Gibco that contains 4.5 g/L D-glucose, L-glutamine, and sodium pyruvate. FBS purchased from Gibco, and *trypsin-ethylenediaminetetraacetic acid* (Trypsin-EDTA).

### Medium Preparation and Cell Culture

The Vero cell culture process begins with the making of a medium consisting of DMEM and FBS. In this study, variations in the concentration of FBS were 5% and 10% in 100 mL medium volume. Vero cells in the initial medium were observed to ensure their presence. Normal vero cells are like small leaves attached to the bottom of the flask, while dead cells are round and do not stick<sup>7</sup>.



Figure 1. Vero Cell (Misra et al., 2010)

After the presence of Vero cells was observed, the initial medium was removed and trypsin-EDTA was added to exfoliate the cells from the vial it is called the passage process<sup>8</sup>. The medium was added again to neutralize trypsin-EDTA then centrifuged to separate the Vero cell with other substances. The cells are taken a slight to be counted using a counting chamber and microscope which aims to determine the amount of cell dilution in a new medium. Finally, a new medium was added with concentrations of FBS is 5% and 10%<sup>9</sup>. *Cell culture is the most popular method of virus propagation because of its high sensitivity. However, the need for high cost liquid nitrogen for cell line storage is one of the main limiting factors for its widespread use in developing countries. Freezer at -85°C can be used as an alternative to liquid N<sub>2</sub> for cell line preservation*<sup>10</sup>. Respectively, then it was saved in a 6-well culture cell and incubated in a cell incubator. The cell density of each well in 5% and 10% concentration media were 1017 cells/well. Cells in the medium were incubated to observe cell growth for 14 days. To reduce the risk of contamination, almost all activities above are carried out a sterile, laminar flow

hood, make sure all equipment and solutions that come into contact with the cells are sterile, and use proper sterile technique when working in the hood.

## RESULTS AND DISCUSSION

Vero cell growth in this study was observed on days 5, 7, 12, and 14 by comparing the approach to the number of Vero cells produced between the medium with 10% and 5% FBS. Cells can be in 3 states, namely being dividing (proliferative cycle), in a resting state (not dividing), and not dividing permanently. Vero cells that are dividing are found in several phases, namely mitosis (M), postmitotic (G1), DNA synthesis phase (S), and premitotic phase (G2) (Campbell and Farrell, 2003; Johnson dan Walker, 1999). At the end of the G1 phase there is an increase in RNA followed by the S phase which is the time of DNA replication<sup>11,12</sup>. After the S ends, the cell enters the premitotic phase (G2) with the following characteristics: the cell is tetraploid, contains twice as much DNA as other cells, and continues to synthesize RNA and proteins. During mitosis (phase M), protein and RNA synthesis decreases abruptly, and division into 2 cells occurs. After that, cells can enter interphase to re-enter the G1 phase, when cells proliferate or enter a resting phase (G0)<sup>13,14</sup>. Cells in the G0 phase that still have the potential to proliferate are called peak cells. So that the increase in the number of cells is cells that are in the proliferation cycle and G0 phase. The addition of serum will induce cells to reenter the cell cycle to the point of restriction for the next process<sup>15</sup>. The results of cell observation using a microscope are shown in figure 2.

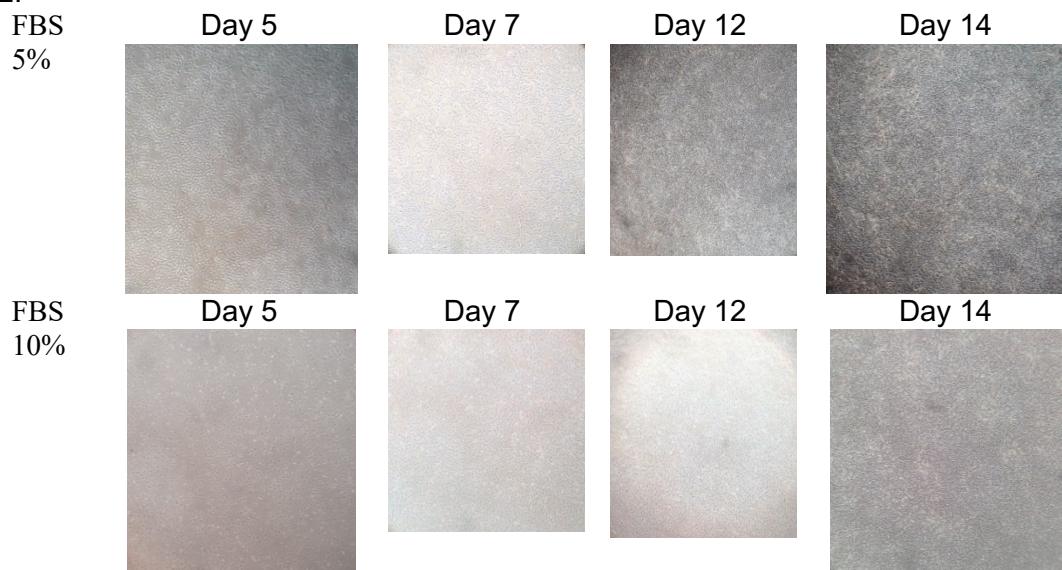


Figure 2. Vero cell development at day 5, 7, 12 and 14.

The figure shows that the more days, the number of cells contained in the medium are getting much and dense. Furthermore, there were differences in the approach number of cells produced from cultures with 10% and 5% FBS medium in DMEM. Vero cells produced by medium containing 10% FBS more than 5% FBS medium. This proves that the higher the concentration of FBS in the medium will support the growth of Vero cells in the culture process.

## CONCLUSION

Based on the research, it can be concluded that the use of FBS in DMEM medium with concentrations of 5% and 10% effect the number of Vero cells produced in the culture process. Observations on cell growth on days 5, 7, 12, and 14 using a microscope, proved that the number of cells increased day by day. Thus, from this study, it was reported that FBS 10% produced the most cells.

## AUTHOR'S CONTRIBUTION STATEMENT

all authors have equal responsibility in the research and preparation of the manuscript.

## FUNDING INFORMATION

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## DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

## DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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