Comparative Study between Avian Cell and Mammalian Cell in Production of Influenza Vaccine Shariah Compliance

F N Aliya Mohamad Ros¹, Norliza Abd Rahman¹, Jarinah Mohd Ali¹, Nurina Anuar¹, Siti Rozaimah Bt. Sheikh Abdullah¹ & Amir Fazlin Bin Jusoh @ Yusoff²

¹Chemical Engineering Programme, 43600 UKM Bangi, Selangor, Malaysia
²Pusat Fiqh Kontemporari dan Pematuhan Syariah, Fakulti Pengajian Islam Faculty of Engineering & Built Environment, 43600 UKM Bangi, Selangor, Malaysia
*e-mail: fatinuraliyaros@gmail.com; norlizajkkp@ukm.edu.my

Abstract

Nowadays, the manufacturing industry of the influenza vaccine always make the improvement for their production. Most of them are looking for the new production cell line which are easily scalable, high yield production of viral and highly tolerant to multiple viruses. This situations happens as the high demand of the vaccine for every year from around the world. It can be seen that the capacity for the vaccines’ production are really critical in the pharmaceutical industry. However, certain Muslims’ community do not gain a trust from the vaccine’s ingredient itself. This is due to the production process involves blood consist of serum and cell components that may come from the prohibited animals such as pig and dog. Therefore, shariah compliance vaccine has been studied in this research. The avian cell lines are now competing with the mammalian cell currently at advance stage of commercial development for the manufacture of influenza vaccines. Avian cell line chosen are AGE1.CR.pIX cells and DuckCelt-T17 will be compared with the mammalian cells which is Vero and Madin Darby Canine Kidney (MDCK) cells in producing the shariah compliance vaccine. Different type of mediums also will be considered to produce shariah compliance vaccine. The artificial media will be compared with the natural media that contain the non-shariah components. Hence, the shariah status of the enzyme and media will be known in this research. In this study, the production of shariah compliance vaccine mainly focused on the bioreactor and simulated by using a software called SuperPro® Designer. Results gained from the simulation shows the highest yield at 72 hours which is 93.51 % conversion. These study will acknowledge the halal’s status of the vaccine and contribute the development of the halal vaccine processing industry.

Keywords: Influenza vaccine shariah compliance; Avian cell; Mammalian cells; Media; SuperPro® Designer

1 Introduction

Vaccine is a substance comes from protein, polysaccharide or known as a pathogenic nucleic acid used to stimulate the immune system in order to destroy and weaken the pathogens. In the other word, vaccine give a biological protection to help the immune against contagious diseases such as chicken pox influenza and others. Vaccine also can be derived from Latin which is *Variolae Vaccinae* that invented by Edward Jenner for a disease knowns chicken pox [1]. Edward Jenner successfully introducing a vaccine to cure chickenpox disease during 1796 which is this disease is very dangerous towards people [1]. The virus always undergo the genetic changes and form a new type of viruses that cannot be recognized by humans’ immune system. Hence, the production of influenza virus must be done annually and compatible to the new viruses [2]. Milián & Kamen (2015) discovered that there are many ways to produce a vaccine but the most common is by using the cell culture techniques. For example, Optaflu and Flucelvax are the vaccine that produced by cell culture by using Madin Darby Canine Kidney (MDCK) cell. In addition, Millian & Kamen (2015) also explained that by using a bioreactor to culture the cell in a large quantity is more convenient.

Furthermore, the host cell selection also play a main role in the production of the vaccines. Nowadays, avian cells are competing with the traditional mammalian cells as a host cell in the pharmaceutical industry. Both of them having a same compatibility needed in the process which is highly permissive to the multiple virus, ability to grow in suspension, in serum free conditions and at high cell densities. These characteristics are the most suitable to the host cell or known as a cell line. Avian cell is generated from the primary embryonic of the duck cell such as Duck EB66 cells and DuckCelt-T17. The main mammalian cell can be consisted from 2 types which is Vero cell and MDCK cell. Each host cells have their own production capacity for influenza virus as shown in the Table 1.
2.2 Media

yield of the hemagglutination assay for the DuckCelt species duck embryonic investigated to produce a yield of the influenza vaccine. MDCK cell also known as African green monkey [4]. It is the most effective cell line for the production of influenza vaccines. MDCK cell also known as Madin Darby Canine Kidney is another example for the mammalian cells which is used in the pharmaceutical industries too. MDCK cells are extracted from kidney tissues of a cocker spaniel species of a dog. The uses of this cell are really widely as the high yield of production of vaccine can be achieved [5].

Besides that, avian cell is now competing with the current cell as both of the cells have same characteristic which is permissible to the multiple virus and easily scalable [6]. AGE1.CR.pIX_ cells and DuckCelt-T17 will be investigated to produce a yield of the influenza vaccine shariah compliance. Both of them comes from the different species duck embryonic which is from Muscovy sp. and regular duck, respectively. Based on Table 1, it shows a yield of the hemagglutination assay for the DuckCelt-T17 cell is 1.5–1.8 log HAU/100ml proves that the influenza vaccine shariah compliance can be produced.

2.2 Media

Medium culture is used to help the growth of the microorganism or cell in the host cell in the production of the vaccine. Media provide a sufficient nutrients, vitamin, mineral and glucose to make sure the replication of the virus

| Cell Line       | Type       | Infectious titers (logTCID50/ml HA titer) | Hemagglutination Assay (HA titer) (log HAU/100ml) | References |
|-----------------|------------|-----------------------------------------|--------------------------------------------------|------------|
| MCDK adherent   | Mammalia   | 10⁻⁹⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-~|
cell growth rapidly according to the time given. Media also can maintain the pH and the osmotic pressure throughout the cultivation process. **Figure 1** shows a type of culture media.

![Diagram of culture media]

**Figure 1:** Types of culture media (Meenakshi Arora, 2018).

The suitable media for the production of the influenza vaccine compliance shariah will be selected based on their performance and compatibility in the process. L-Glutamine, Dulbecco’s Modified Eagle’s Medium (DMEM), Minimum Essential Medium (MEM) are the example of the media that will be tested in this research.

### 2.3 Development of Bioreactor Modelling

The main operation involves in this operation is at the bioreactor. Normally, mammalian cells will be used and cultured at 37 °C. Later, it will be treated with 5% carbon dioxide [1]. Cell cannot survive in the high temperature which is more than 42 °C [8]. The optimum temperature for the cell growth is between 15 °C to 30 °C. In this study, temperature of the cell culture is fixed at 25 °C. The pressure in the bioreactor can be assumed 1.01325 bar or 101 kPa as bioreactor has air holes to release the gas produced. Optimal cell growth need neutral pH of 5.5 to 8.5 (Oyeleye et al, 2016). Without enough oxygen, the cell will produce acetic acid known as cultured anaerobically. By providing adequate ventilation, pH will remain at close to neutral. pH will be controlled in range of pH 6 to pH 8 in this study. SuperPro® Designer v9.5 software was used to develop a simulation model of bioreactor for production of shariah compliance influenza vaccine. The time will be varies into 4 which is 24, 36, 48 and 72 hours.

### 3 Result and discussion

Bioreactor modelling has be done by simulation software which is SuperPro® Designer v9.5. **Figure 2** shows a conventional process of the influenza vaccine in the industry

![Diagram of conventional process]

**Figure 2:** Conventional process of the influenza vaccine by simulation

This research focuses only on the seed reactor which is located at the upstream process. This reactor plays a main role in the production of the influenza vaccine and several concerns are needed. Firstly, the cell line must be cultured
in the shake flask with the optimum condition before to the bioreactor or seed reactor. The equation for the reaction in the seed reactor shown in equation (1):

\[ \text{Glucose + Media + O}_2 \rightarrow \text{NH}_3 + \text{Biomass} + \text{CO}_2 + \text{Hemagglutinin} + \text{Lactate} + \text{H}_2\text{O} \]  

(1)

The downstream process involve 6 type of operations which are heat exchanger, centrifugation, storage tank, freeze dry and ion exchange. These process is purposely to separate and purifies the vaccine from the supernatant from the seed reactor. Rutty CJ (2012) stated that most of the virus is inactivated by chemically using ‘B-propiolactone’ (B-PL) and Cetyltrimethyl Ammonium Bromide’ (CTAB).

3.1 Reaction Time

The simulation is done by the software called SuperPro® Designer. The data for the production of the influenza vaccine is used for this simulation. Hence, the reaction time are 24, 36, 48 and 72 hours. This range is based on their ability to growth the cell in the bioreactor [9]. In the bioreactor, the component will be preserved by the nutrients and vitamin that known as media. It will help to maintain the pH, osmotic pressure and provide sufficient oxygen for the growing process happens. Figure 3 shows a number of cell can be achieved varies with time using Vero cell.

![Figure 3: Number of cell achieved varies with time using Vero cell](image)

From Figure 3, it shows the highest number of cell is $7.73 \times 10^6$ cells/mL at 72 hours compare to the lowest yield which is 0.03261 at 24 hours. Meghrous et al (2009) discovered that the growth of the cell is proportional to the time based on the equation (2)

\[ N = N_0 \times e^{\lambda t} \]  

(2)

This happens as the cell must achieve the optimum condition to replicate the product in the bioreactor. Meghrous et al. (2009) also recommended to set at time for the process to be 72 hours. But it is different with the DuckCelt-T17 cells which is the concentration reaching the highest at time between 20 to 40 hours. The concentration of the hemagglutinin can up to $6.5 \times 10^6$ cells/mL compare to the very cell which is $7.73 \times 10^6$ cells/mL. This research prove that the application of the Vero cell should be better compare to the avian cell which is DuckCelt-T17 by their yield production. However, the optimum time for the avian cell is less than the mammalian cell. The result of the simulation will be not significant if the time exceeds to 84 hours as the growth of the cell is already complete (Meghrous et al, 2009).

3.2 Media

There are 2 types of media which is artificial and natural media. Natural media can be in form biological liquid, the extraction from the tissue and clog. Most of them are made of the plasma and serum from human’s body such as
amniotic water, liver, backbone and the coagulant [7]. In addition, artificial media can be classified into 4 types which are serum containing media, serum free media, protein free media and chemically defined media. Mary Johnson (2012) stated that this media made from the coagulation of the cow or horses’ embryo blood. The media chosen in this research is L-Glutamine. Based on the simulation, conversion of the yield by using L-Glutamine from 37.77% until 93.51% based on the time. Figure 4 shows a yield conversion from simulation varies with time.

The highest conversion of the yield is at 72 hours using a Vero cell. It also proved by the Meghrous (2009) said that the optimum condition for the vaccine production is 72 hours. The longest the time, the production will increase as the cell requires certain time to growth. However, it will be not significant if the percentage approaching 95% of conversion [12]. It is because the production of the virus cannot be growth anymore when exceeding the percentage. L-Glutamine act as reaction limiting component. It provides nitrogen for NAD, NADPH as secondary energy source for the metabolism process. L-Glutamine is the nonessential amino acid that produced from the fermentation process. These fermentation process require a few of material which are glucose, ammonia, mineral and growth factors under certain optimum condition [12]. Meenakshi Arora (2018) discovered that L-glutamine have a high compatibility to the host cell for the cultivation process occur.

Conclusion
The production of the influenza vaccine shariah is more effective and achieve the highest yield at 72 hours of time reaction which is 93.51% conversion. However, it will be not significant when the percentage reach 95%. Hence, the application for the influenza vaccine shariah compliance at 72 hours using a Vero cell and L-Glutamine as a media are accepted significantly.

Acknowledgement
The authors would like to extend thanks to Universiti Kebangsaan Malaysia (UKM) for supporting this research through grant GUP-2018-009.
References

[1] Brown, I. R. 1990. Induction of heat shock (stress) genes in the mammalian brain by hyperthermia and other traumatic events: A current perspective. *Journal of Neuroscience Research* 27(3).

[2] Wong SS, Webby RJ. 2013. Traditional and new influenza vaccines. *Clin Microbiol Rev.*

[3] Milián and Amine A. Kamen. 2015. Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines. *BioMed Research International. Volume 2015*

[4] Newport Biotech, 2013. Breaking Bad Biotech

[5] Huang, D et al. 11 March 2015. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients

[6] Emma Petiot, Anaïs Proust et. Al 2017. Influenza viruses production: Evaluation of a novel avian cell line DuckCelt_ -T17. *Journal of Vaccine*

[7] Meenakshi Arora, 2018. All about cell culture media and its types

[8] Oyeleye, O. O., Ogundoji, S. T., Ola, S. I. & Omitogun, O. G. 2016. Basics of animal cell culture: Foundation for modern science. *Biotechnology and Molecular Biology Reviews* 11(2): 6–16.

[9] Rutty CJ, Barreto L, Van Exan R, et al. Conquering the cripper: Canada and the eradication of polio. *Can J Public Health* 2005; 96:I1–I24

[10] Meghrous, J., Mahmoud, W., Jacob, D., Chubet, R., Cox, M. & Kamen, A. A. 2009. Development of a Simple and high-yielding fed-batch process for the production of influenza vaccines. *Vaccine* 28(2): 309–316.

[11] Mary Johnson 2012. Fetal Bovine Serum. *Synatom Research*, Princeton, New Jersey, United States

[12] Isao Kusumoto, 2011. Industrial Production of L-Glutamine. *Journal of Nutrition* 131(9 Suppl):2552S

[13] Lohr, V. et al. 2010. Elements in the Development of a Production Process for Modified Vaccinia Virus Ankara. *Journal of Microorganisms*. 2013 Dec; 1(1): 100–121.

[14] Genzel, Y et al. (2010). Metabolic effects of influenza virus infection in cultured animal cells: Intra- and extracellular metabolite profiling. *BMC Syst Biol*. 2010 May 13;4:61.