Short Communication

Comparison of Lowenstein Jensen Media and Ogawa Media Usage for Viability Test of BCG Vaccine Pasteur P11732 and Russian (Moscow) – 384 sub-strains

Erdiansyah, M[1]*

1. *National Quality Control Laboratory of Drug and Food (NQCLDF), Indonesian FDA, Central Jakarta, Indonesia.*

How to cite this article: Erdiansyah M. Comparison of Lowenstein Jensen Media and Ogawa Media Usage for Viability Test of BCG Vaccine Pasteur P11732 and Russian (Moscow) – 384 sub-strains. Archives of Razi Institute. 2024;79(1):240-245. DOI: 10.32592/ARI.2024.79.1.240

Article Info:
Received: 28 April 2023
Accepted: 25 July 2023
Published: 29 February 2024

Corresponding Author's E-Mail:
erdiansyah@pom.go.id

ABSTRACT

The BCG vaccines on the market have employed a *Mycobacterium bovis* (M. bovis) sub-strains derived from the initial strain. To date, there has been no recommendation regarding the sub-strains with the highest effectiveness when administered to humans. Because it remains the standard for Tuberculosis treatment, the quality of the BCG vaccine must be verified. The viability test is one of the parameters for BCG vaccine quality control. The culture method has become the gold standard for viability testing with various testing media. The present study aimed to evaluate the performance of Lowenstein Jensen (LJ) and Ogawa media for the viability test of Pasteur 1173P2 and Russian (Moscow) – 384 sub-strains of *M. bovis* in the BCG vaccine. The number of culturable particles of each sub-strain in the BCG vaccine was estimated and statistically evaluated using the t-test. The colonies of the Pasteur 1173P2 have characteristics; tended to clump on both mediums with tiny, rough, and pale yellow/cream colors. Although the colony character of the Russian (Moscow) – 384 generally has similar feature, it did not cluster and had a smooth texture. In terms of growth rate, LJ and Ogawa media performed similarly for Pasteur 1173P2 and Russian (Moscow) – 384 sub-strains. Maximum growth is reached by the fifth week. The culturable particles of Pasteur P1173P2 sub-strains did not differ between mediums. Whereas the growth of the Russian (Moscow) - 384 sub-strains was statistically better on Ogawa media. The results of this study reveal that the performance of the media used for determining the number of culturable particles is based on the sub-strains of *M. bovis* present in the BCG vaccine.

Keywords: BCG Vaccine, Culture Media, Microbial Viability, Vaccine potency, *Mycobacterium bovis*

1. Introduction

The BCG is the only vaccine now available and approved by the World Health Organization (WHO) to prevent Tuberculosis in humans using a preventive immunization approach. Considering that BCG vaccinations in humans are routinely administered using many organisms provided by various manufacturers worldwide. That fact has sparked concerns regarding the potential contribution of known variations among different BCG sub-strains to variable efficacy; however, there is currently insufficient information to determine whether Different BCG sub-strains has genuine clinical significance (1). Recent research has shown that licensed BCG vaccines differ drastically in terms of bacterial viability, RNA content, and innate immune activation. However, these data are inadequate to suggest one sub-strain over another. One of the parameters for the BCG vaccine quality control test is the determination of the culturable particles or the test for viability. Conventional culture methods are still the gold standard for BCG vaccine viability testing, although it takes 3 to 6 weeks to complete. Lowenstein Jensen (LJ), Ogawa, Middlebrook 7H11, Middlebrook 7H10, Stonebrink's, and Blood agar are among the available medium for viability testing using culture methods. Solid mediums containing eggs, such as LJ and Ogawa, are the least expensive within these mediums yet can provide reliable results. It has been demonstrated that production through fermentation and specific chemical medium affects viability (2). Consequently, data regarding the influence of culture medium on the growth of *Mycobacterium bovis* (M. bovis) is still required for optimum quality control of BCG vaccines. The available research on the impact of culture medium on growth was carried out on mycobacterium isolated from clinical samples (3–6). Meanwhile, no particular research in quality control test for the BCG vaccine with multiple sub-strains has ever been performed. By determining the culturable particle of the BCG vaccine, the present study intended to evaluate the performance of LJ and Ogawa medium for the growth of *M. bovis* sub-strains; Pasteur 1173P2 and Russian (Moscow) – 384.

2. Materials and Methods

2.1. Medium Preparation

Sauton media, Ogawa media, and LJ media were the mediums used in the current research. The Sauton media preparation protocols are based on Jensen (1954)(7). The procedure for preparing Ogawa media is based on a publication by Obayashi (1955)(8). The Lowenstein Jensen media were prepared following the manufacturer's guidelines (Difco™ & BBL™ Manual, 2nd Edition).

2.2. Sample Preparation and Inoculation

This study utilized two freeze-dried BCG vaccine samples containing either the Russia (Moscow)-384 or Pasteur 1173P2 sub-strains. The vaccine was reconstituted in a Sauton medium until it reached the required concentration for human administration. Two times of three dilution levels with 2×10^{-4} as the highest dilution rates for the Russian (Moscow) - 384 and 10^{-4} for the Pasteur 1173P2 sub-strains was used. The suspensions were inoculated into both Ogawa and LJ medium, incubated at 37°C, and monitored weekly. The calculation of culturable particle follows the WHO Technical Guide (9)

2.3. Statistical Analysis

The t-test was performed to evaluate the differences between dilutions, observation weeks, and culturable particle count. Moreover, a *P*-value of less than 0.05 was considered statistically significant. The calculations were performed using the data analysis tool provided with the Microsoft Excel 365 software.

3. Results

The following experiment evaluated *M. bovis* growth in Pasteur P1173P2 and Russian (Moscow) – 384 sub-strains at the fourth and fifth weeks on LJ and Ogawa media. Table 1 displays the number of cultivated colonies that grew on LJ and Ogawa media for each sub-strain. The number of colonies that grew during the fifth week of observation for BCG Russian and Pasteur strains on LJ and Ogawa media was always higher than during the fourth week. On the Ogawa medium, the BCG Russian strain grew more abundant. In the meantime, the BCG Pasteur strain's colony growth was similar on both LJ and Ogawa medium. The deviation value of BCG Pasteur sub-strains growth in LJ media (16.79-54.59%) and Ogawa media (25.79-47.88%) compared to BCG Russian strain growth in LJ media (6.53-15.62%) and Ogawa media (8.08-18.37%). The higher dilution concentration ($>1.00\ 10^{-4}$) tends to result in a higher deviation value for the number of cultures in the Ogawa medium than in the LJ medium at the time of observation during weeks four and five, consistent for both BCG strains. In particular, the culturable particle of the Russian BCG strain in the two mediums (LJ and Ogawa) during the fifth week of observation was significantly greater than that in the fourth week. These observations were similar to the culturable particle of Pasteur's BCG sub-strains; the culturable particle in the fifth week of observation was

significantly greater than that in the fourth week in the two mediums used. The t-test regarding the type of cultivation media revealed no potential for differences between the BCG Russian and Pasteur sub-strains at the time of observation in the fourth week. However, there was a significant difference between LJ and Ogawa media for the BCG Russian strain in the fifth week of observation; the Ogawa media viability test yielded a higher culturable particle than the LJ media test. In comparison, the growth of Pasteur's BCG strain was not substantially different between LJ and Ogawa media during the fifth week of observation.

Table 1. The number of M. Bovis Russian (Moscow)-384 and Pasteur 1173P2 colonies on Lowenstein Jensen and Ogawa media at 4 and 5 weeks

Sub-strains	Dilution concentration	Culturable particle (CFU)			
		Lowenstein Jensen medium		Ogawa medium	
		4^{th} week	5^{th} week	4^{th} week	5^{th} week
Russian (Moscow) - 384	2.00×10^{-4}	51.42 ± 3.36	59.63 ± 6.01	60.50 ± 9.31	65.50 ± 10.84
	1.00×10^{-4}	25.17 ± 2.89	30.75 ± 3.93	28.75 ± 3.42	43.22 ± 7.94
	0.50×10^{-4}	13.50 ± 2.11	15.73 ± 2.07	12.74 ± 1.12	16.21 ± 1.31
Pasteur 1173P2	1.00×10^{-4}	37.04 ± 6.22	39.81 ± 7.38	31.54 ± 9.81	38.32 ± 10.09
	0.50×10^{-4}	17.29 ± 3.99	18.50 ± 4.99	17.13 ± 4.89	18.96 ± 4.89
	0.25×10^{-4}	6.96 ± 3.80	7.79 ± 4.03	5.27 ± 2.37	6.39 ± 3.06

4. Discussion

Neither the Pasteur nor the Russian sub-strains had grown by the end of the third week. In the fourth to the fifth week, colonies of Pasteur began to form, gradually grew, and tended to clump on both mediums with tiny, rough, and pale yellow/cream colors. In general, the colony character of the Russian sub-strains was similar to that of the Pasteur sub-strains. Although the colonies grew between the fourth and fifth weeks, they did not cluster and had a smooth texture. The Pasteur and Russian sub-strain colonies began to dry and shrink after six weeks. On both LJ and Ogawa media, the Pasteur P1173P2 BCG vaccine's culturable particle deviated more than the Russian (Moscow) - 384 BCG vaccine (Figure 1). Clumping-related bias during observations is one of the causes contributing to the high deviation. In contrast to the Pasteur P1173P2 strain, the Russian (Moscow) - 384 strain colonies did not exhibit clumping, resulting in a relatively smaller standard deviation. The determination of vaccine viability based on the number of colonies growing at the fourth and fifth weeks was statistically significant ($P<0.05$). This relevance applies to both the medium used and the two *M. bovis* sub-strains cultivated. The research by Franco-Sotomayor et al. (2020) demonstrated the same outcome using clinical samples as at the fourth week, mycobacterium growth was only 80.7% (LJ) and 94.8% (Ogawa). This discovery is also positively correlated with the studies of Naveen and Peerapur (2012)(4), who determined that the average growth period in LJ medium is five weeks. In this work, observations were continued until the sixth week; however, no growth was found in the number of colonies. It suggests that determining the culturable particle of a vaccine requires at least five weeks. If it is done less than that, there is a risk of underestimating the vaccine's culturable particle. A comparison of medium performance is conducted to evaluate the feasibility of the following vaccine using data on the growth of colonies at the fifth week. The culturable particle of Pasteur sub-strain cultivated on LJ or Ogawa medium was not significantly different. In comparison, Ogawa media performed better than LJ media for the Russian sub-strains. Although the composition of LJ and Ogawa media was the same, except for the source of nitrate and the concentration of malachite green. Colonies grew much more on Ogawa media and the difference was statistically significant. Tanoue et al. (2002) also observed that variations in malachite green concentration, egg homogenate volume, pH, and nitrate supply did not affect Mycobacterium's growth ability. The data implies that various sub-strains have distinct growth properties, prompting the optimization of the medium used to conduct the viability test. Based on the findings of this study, the growth rate of Ogawa media was equivalent to that of the LJ media. Both sub-strains need five weeks to reach peak growth. The culturable particle of the BCG vaccine Pasteur sub-strain can be assessed using Ogawa and LJ medium, with the growth characteristics to develop clumping. Meanwhile, the culturable particle of the BCG vaccine Russian sub-strain was more optimally grown utilizing Ogawa medium, with growth characteristics that did not create clumping. The sub-strain of *M. bovis* in the vaccine influences the viability of the BCG vaccine as tested by the culture method. Therefore, information on growth properties between various sub-strains is essential for testing the viability of BCG vaccine quality control.

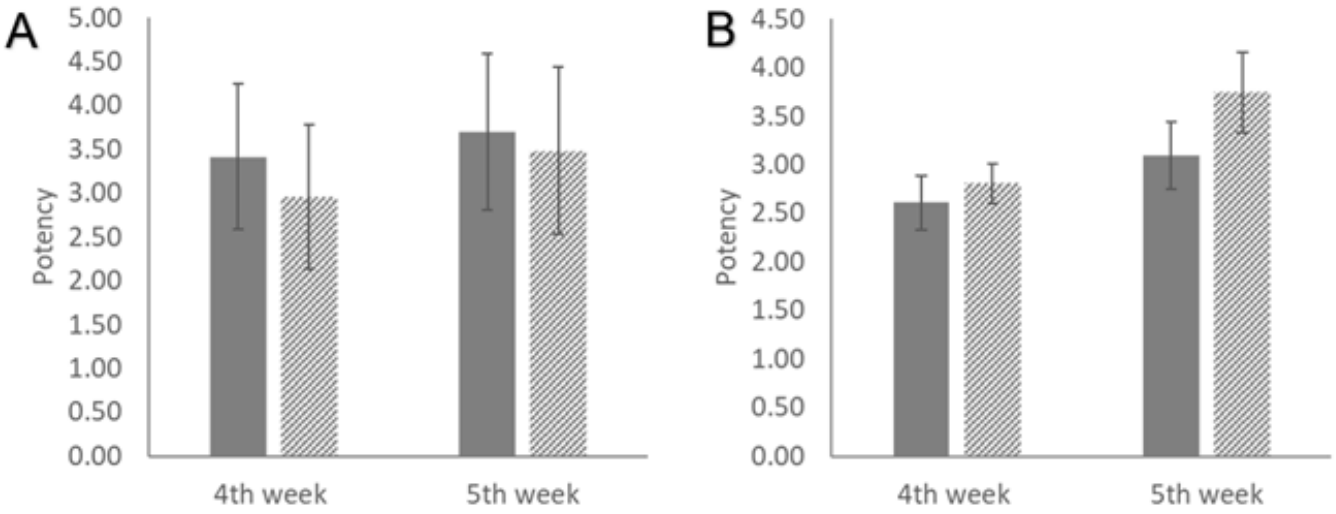

Figure 1. Culturable particle measured on LJ and Ogawa medium. (A) Vaccine containing Pasteur strain 1173P2; (B) The vaccine contains the Russian (Moscow) – 384 strain

Acknowledgment

This research was fully supported by National Quality Control Laboratory of Drug and Food (NQCLDF) of Indonesia

Authors' Contribution

M.E was solely responsible for all aspects of the study, including conceiving and designing the study, collecting and analyzing the data, interpreting the results, drafting and revising the manuscript and performing statistical analysis.

Ethics

It is declared that all ethical considerations were considered in the preparation of the submitted manuscripts.

Conflict of Interest

The author has no confilicts of interest to disclose.

References

1. Ritz N, Dutta B, Donath S, Casalaz D, Connell TG, Tebruegge M, Robins-Browne R, Hanekom WA, Britton WJ, Curtis N. The influence of bacille Calmette-Guérin vaccine strain on the immune response against tuberculosis: A randomized trial. Am J Respir Crit Care Med. 2012; 185:213–222. doi: 10.1164/rccm.201104-0714OC. PMID: 22071384.

2. Pascoe J, Hendon-Dunn CL, Birch CPD, Williams GA, Chambers MA, Bacon J. Optimisation of mycobacterium bovis BCG fermentation and storage survival. Pharmaceutics. 2020; 12:1–14. doi: 10.3390/pharmaceutics12090900.

3. Palange P, Narang R, Kandi V. Evaluation of Culture Media for Isolation of Mycobacterium Species from Human Clinical Specimens. Cureus. 2016; 8:e757. doi: 10.7759/cureus.757.

4. Naveen G, Peerapur B v. Comparison of the Lowenstein-Jensen medium, the middlebrook 7H10 medium and MB/BACT for the isolation of Mycobacterium tuberculosis (MTB) from clinical specimens. Journal of Clinical and Diagnostic Research. 2012; 6:1704–1709. doi: 10.7860/JCDR/2012/4603.2635. PMID: 23373033.

5. Costa RR da, Silva SF da, Fochat RC, Macedo RL, Pereira TV, Silva MR, Pinto CPG, Leite ICG. Comparison between Ogawa-Kudoh and modified Petroff techniques for mycobacteria cultivation in the diagnosis of pulmonary tuberculosis. Einstein (Sao Paulo). 2018;

16:eAO4214. doi: 10.1590/S1679-45082018AO4214. PMID: 29898027.

6. Franco-Sotomayor G, Rivera-Olivero IA, Leon-Benitez M, Uruchima-Campoverde SE, Cardenas-Franco G, Perdomo-Castro ME, Cardenas-Franco CS, Ortega-Vivanco J, Abad-Ruiz AS, de Waard JH, et al. Fast, Simple, and Cheap: the Kudoh-Ogawa Swab Method as an Alternative to the Petroff-Lowenstein-Jensen Method for Culturing of Mycobacterium tuberculosis. J Clin Microbiol. 2020; 58:e01424-19. doi: 10.1128/JCM.01424-19.

7. Jensen KA. Bulletin of the International Union against Tuberculosis. 1954; 24:78–112. PMID: 1756295.

8. Obayashi Y. Dried BCG Vaccine [Internet]. Geneva: World Health Organization; 1955. Available from: https://apps.who.int/iris/handle/10665/41663.

9. World Health Organization. In Vitro Assays of BCG Products [Internet]. 1977. Available from: https://apps.who.int/iris/handle/10665/99372.

10. Tanoue S, Mitarai S, Shishido H. Comparative study on the use of solid media: Löwenstein-Jensen and Ogawa in the determination of anti-tuberculosis drug susceptibility. Tuberculosis. 2002; 82:63–67. doi: 10.1054/tube.2002.0323.