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Comparison of Viable Units Counts of BCG Vaccines on Four Different Culture Media

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=국문초록=

BCG 생균수 검사방법의 개선에 관한 연구

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BCG vaccine의 생산과, 접종을 목적으로 현지에서 보관중인 vaccine의 품질관리에 있어서는 vaccine의 생균수 측정이 vaccine평가의 중요 수단의 하나로 이용되고 있다. 세계보건기구의 추천에 의해 현재까지는 Löwenstein-Jensen(L-J)배지를 BCG 생균수 검사에 사용해왔으나 L-J 배지는 배지상의 생균수와 배지간의 변이가 심하여 다양한 결과를 얻는 경우가 많았다. 따라서, L-J 외의 기존 결핵균용 배지와 균집락형성 양상을 비교 관찰하여 생균수 검사에 더 적합한 배지를 선정코자 본 실험을 실시하였다.

시험결과에 의하면, 현재 널리 사용되고 있는 L-J 배지가 비교 시험된 KIT, 1% Ogawa, Middlebrook 7 H 11 배지에 비해, 백신 균주나 제품에 관계없이 발육한 균집락수도 떨어질 뿐 아니라 발육 속도도 느렸다. 그러므로 앞으로의 BCG 생균수 검사에서는 KIT 배지나 1% Ogawa 배지를 사용함이 더욱 경제적이고도 적합할 것으로 생각되었다.

INTRODUCTION

Despite the long controversy over its effectiveness, BCG vaccination remains an important component of national tuberculosis control programme in many developing countries and in some developed countries. BCG vaccination has been practiced since early 1950s in Korea so that well over 80% of people aged less than 30 years have been vaccinated as it was revealed in 1985 survey.

Determination of viable units in BCG vaccines is an utmost important control measure not only for production but also for storage status in the fields prior to use. The Löwenstein-Jensen medium has been most widely used to determine viable

particles in BCG vaccines in many countries including Korea. However we often observed a significant variation in viable units counts between replicate slants and sometimes it took more than 4 weeks incubation to obtain maximum counts of viable units even if WHO technical report series recommended 4 weeks incubation at 37°C.

In this study, four different culture media have been compared to count viable units in BCG vaccines and the results are presented in this paper.

MATERIALS AND METHODS

Vaccines: Three different vaccine products have been used in this study. Domestic BCG vaccines

Table 1. Formula of Three Egg-based Media Used in This Study

Name of medium Ingredients		L-J	1% Ogawa	KIT
Basal medium	KH ₂ PO ₄	0.4%	1.0%	2.7%
	Na ₂ HPO ₄			1.0%
	Sodium glutamate		1.0%	0.9%
	MgSO ₄ ·7H ₂ O	0.04%		
	Magnesium citrate	0.1%		
	Asparagine	0.6%		
	Pyruvic acid			0.1%
	Distilled water	1,000 ml	1,000 ml	1,000 ml
Whole egg homogenate		1,667 ml	2,000 ml	2,000 ml
2% malachite green		33.3 ml	60 ml	60 ml
Glycerine		20 ml	60 ml	40 ml

(Lot No. 85-1) were produced with French strain 1173-p2 and contained 1 mg of bacilli per ml. One of the imported vaccines was also produced with French strain 1173-p2, but the strain of the other one was Tokyo 172. Bacterial contents of both imported vaccines were 0.5 mg per ml and their lot numbers were F 5004 M and 220 L. Seven ampoules of each freeze-dried vaccines kept at 4°C were used to heat stability tests. Fourteen ampoules (9 ampoules of domestic vaccine and 5 ampoules of imported vaccine) were collected from the peripheral health centers where the health workers kept for vaccination, and included in this study.

Media: Four different culture media were used in this study. Three egg-based media are Löwenstein-Jensen medium without potato starch, 1% KH₂PO₄ Ogawa medium, and KIT (Korean Institute of Tuberculosis) medium, which formulas are shown in Table 1. Agar based 7H11 medium was also used and the medium was dispensed into petri-dishes instead of test tubes.

Determination of viable units in BCG vaccines: Vaccine ampoule was reconstituted with Sauton's broth medium (1/4 strength) and diluted to 10,000, 20,000, and 40,000 folds prior to inoculate onto media. Parts of vaccines tested were kept at 4°C and 37°C and their viable units counted after 1 and 6 weeks treatment. Viable units in vaccine

ampoules from the health centers were determined without heat (37°C) treatment. 0.1 ml of each dilution was inoculated onto 10 or 20 slopes or plates of each medium according to dilutions, then seeded egg-based media were incubated at 37°C in an ordinary hot room, while seeded agar-based media (7H11 agar) were incubated in a CO₂ (10% pressure)-incubator. The growth appearance has been observed every week after two weeks incubation until maximum counts attained. Viable numbers of each BCG strain were calculated by WHO recommendation.

RESULTS

Of the four different culture media compared, colony appearance of BCG has been delayed much longer on the L-J medium than on the other media. As seen in Table 2, the maximum number of viable particles were obtained with 4 out of 21 vaccine ampoules after 5 weeks incubation on the L-J medium and the remaining 17 ampoules after 6 weeks incubation, while 10 to 11 vaccine ampoules yielded maximum counts after 3 weeks incubation on the other medium.

The growth of BCG was much faster on 1% Ogawa, KIT, and 7H11 media, thus beyond 4 weeks some colonies coalesced thereby resulting

Table 2. Comparison of Incubation Times to Attain Maximum Counts of Viable Units in BCG Vaccines on Different Culture Media

Culture media	Total number of vaccine ampoules tested	Incubation times(weeks)			
		3	4	5	6
L-J medium	21	0	0	4	17
1% Ogawa medium	21	10	11	—	—
KIT medium	21	11	10	—	—
7 H11 medium	21	10	11	—	—

Table 3. Viable Units Counts in Three Products of Vaccines on the Four Different Culture Media

Vaccine strain**	French 1173-p2		Tokyo-172 220L
	85-1	F 5004M	
Bacterial content	1 mg/ml	0.5 mg/ml	0.5 mg/ml
Culture media*			
L-J medium	5.72±0.16	5.96±0.10	6.84±0.09
1% Ogawa medium	6.37±0.13	6.22±0.04	6.96±0.07
KIT medium	6.36±0.08	6.24±0.14	7.02±0.05
7H11 medium	6.30±0.12	6.27±0.09	7.10±0.09

Four ampoules of each vaccine have been tested. Numbers of viable particles are expressed in mean (log number) and standard deviation.

{Between BCG products **F=56.60 F.99(3.6)=9.78
{Within media used *F=6.659 F.95(2.6)=5.14

in reduction of colony counts.

The viable units counts were also very significantly less on the L-J medium than on the other media as seen in Table 3 ($P<0.01$). Mean log number of viable units in two French strain 1173-p2 were 5.72 and 5.96 respectively on the L-J medium, but more than 6.30 and 6.22 on the other media. Viability of bacilli in Lot No. F5004 M and 220L vaccine seemed to be superior to Lot No. 85-1 vaccine because viable units counts of the former were almost same with those of the latter even if bacterial contents of the former were merely half of the latter. Tokyo strain vaccine also grew better on 1% Ogawa, KIT, and 7H11 media, however difference in viable counts between L-J and the other media was less remarkable than French strain vaccine.

Heat (37°C) treatment lowered viable units counts in all tested vaccines, however decrease of viable counts was much more drastic in the 85-1

Fig. 1. Heat (37°C) Stability of Lot No. 85-1 French Strain 1173-p 2 Vaccine.

than in two others as shown in Figures 1,2, and 3. Merely 0.5~5.0% or 2.0~4.0% of viable counts have been reduced in French strain 1173-p2 and

Table 4. Viable Units Counts in Vaccines Collected from Health Centers

Name of health centers from which vaccines were collected	Lot No.	Viable units counts expressed in Log.	
		L-J medium	KIT medium
Gunsan, Chun-buk	83-4	0	6.68
Buahn, Chun-buk	83-10	6.47	6.63
Sungbuk, Seoul	83-7	6.76	6.85
Choong-gu, Seoul	83-10	6.04	6.46
Yongsan, Seoul	83-10	6.61	6.64
Chongro, Seoul	83-13	6.30	6.66
Kangso, Seoul	84-1	6.76	7.00
Dongjak, Seoul	84-1	6.57	6.90
Youngdeungpo, Seoul	84-1	6.42	6.94
Mapo, Seoul	F2003M	6.68	6.86
Pusan	F2003M	6.25	6.72
Eunpyung, Seoul	F2004M	6.68	6.85
Seoul	F2004M	6.47	6.67
Seoul	F2004M	6.30	6.75

Fig. 2. Heat (37°C) Stability of Lot No. F5004M French Strain 1173-p2 Vaccine.

Tokyo-172 strain vaccines by 37°C incubation for 6 weeks, while 10.0~15.0% reduction have been noticed in Lot No. 85-1 French strain 1173-p2 vaccine.

BCG vaccines collected from health centers also showed less viable units counts on the L-J medium than on the KIT medium. Most viable

Fig. 3. Heat (37°C) Stability of Lot No. 22OL Tokyo-172 Strain Vaccine.

counts of tested vaccine ampoules obtained on the KIT medium were more than double of those on the L-J medium.

DISCUSSION

Viable units counts of BCG vaccines have long been carried out as a control measure for both production and storage status of vaccines in the fields. Therefore the counts must be scored as many particles are culturable as possible. In this connection this study revealed that the L-J medium is not suitable medium to determine viable units in BCG vaccines regardless of vaccine strain and quality. Some investigator also noticed unacceptable disadvantages of the L-J medium in viable units counts of BCG vaccines, so that they recommended silica-gel medium, which may not be applicable to all levels of tuberculosis laboratories. We, however, found that 1% KH_2PO_4 Ogawa or KIT medium is economic and suitable, thus they can be replaced with the L-J medium.

It has been also found that, although viable units counts of domestic vaccine based on bacterial content could be graded a good quality, the viability and heat stability of them seemed to be

somewhat inferior to the imported vaccine of same strain, suggesting that freeze-drying condition may affect viability and heat stability of bacilli to a significant extent.

SUMMARY

Viable units counts in BCG vaccine on the L-J medium, which has been most widely used, have been compared with those on the other media, 1% KH_2PO_4 Ogawa medium, KIT medium, and 7H11 medium. The viable particles were counted least on the L-J medium and this medium also required much longer incubation to obtain maximum counts than the other media.

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