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Original Article

Seed History and In-process Control for Freeze-dried BCG Vaccine Produced in Taiwan

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Abstract

Tokyo 172 strain had been selected as seed for BCG vaccine production in Taiwan since 1979. Five lots of the seed were presented by Statens Serum Institut in Denmark to Taiwan during 1981 to 1991. These master seeds were originally prepared by Japan BCG laboratory in 1960. Currently, the working seed lot was established from lot B of master seed in 1993. This working seed has been employed in vaccine manufacture until now. The BCG vaccine is supplied for 10, 30, 50, 100 infants use in amber ampoule filled with BCG and sodium glutamate. Taiwan Centers for Disease Control provides 700 thousand doses of freeze-dried BCG vaccine for Expanded Programme on Immunization every year. The specification of BCG vaccine on potency test is above $15x10^6$ colony forming unit/mg/ml. For in-process control, 16 testing items were utilized to assure the consistency of manufacturing process for the quality of BCG vaccine. The results of in-process control were summarized from batch records as following. The average weight of harvested semi-dry BCG pellicle from three consecutive passages after one-week growth was estimated as 0.81g per 100 ml Sauton medium. The measured optical density at 470nm for intermediate product was 0.18 on average. Moreover, two other parameters were used to verify the reliability of manufacturing process. Ratio of semi-dry BCG weight to 1% sodium glutamate volume, 0.95% (weight/volume) was found for final bulk preparation. The BCG survival rate after lyophilization was above 30% by viability counting. The efficacy and safety of BCG vaccine produced in Taiwan were discussed in this report.

Key words: freeze-dried BCG vaccine, in-process control, Tokyo 172 strain

Introduction

Albert Calmette and Camille Guerin cultured *Mycobacterium bovis* with medium containing potato, bile and glycerin, and then subcultured every 3 weeks in the Institut

Pasteur, Lyon, France. The first attenuated mycobacterium strain, the Bacillus Calmette and Guerin (BCG), was developed in 1921 after 231 passages in 13 years effort [1]. Many laboratories requested this BCG strain for further subculture in similar culture media and environments. The different culture conditions created variants in colony morphology, growth feature, biochemical representation, and virulence to animals. Therefore, 49 BCG sub-strains were initially used for vaccine production [2]. Consecutive passage was the main cause of strain variation and thus, seed lot system was established since 1956 to stabilize BCG sub-strain characters. BCG strains can be classified into 2 groups, Group 1 and Group 2, based on secretion of methoxymycolates in culture medium and duplication number of BCG DNA insertion sequence 6110 (IS 6110) [3-5]. On the other hand, BCG strains may also be categorized as Weak strain and Strong strain by the reaction and immunity level in animal experiment [6-7]. However, these grouping results may not provide sufficient information in explaining the efficacy difference ranged from 0% to 80% in human clinical trials [8].

Virulent mycobacterium was attenuated within culture medium containing potato slice due to missing of Region of Difference 1 (RD1) and all BCG strains preserved this characteristic. Thus, RD1 is the key region for pathogenicity of mycobacterium. The phylogenetic and evolutionary relationship among BCG strains may be revealed by molecular biology techniques. The BCG strains distributed by Institut Pasteur before 1925 as early strains contained mpt 64 gene and 2 duplications of IS 6110 gene, such as Russian BCG-I, Tokyo 172, and Moreau RDJ stains. Later on, BCG strain lost one IS 6110 gene in 1925-1926, such as Sweden and Birkhaug strains. In 1926-1931, BCG strains lost gene of mpt 64. Thus, BCG strains categorized as “late strain” contained only one IS 6110 gene and no mpt 64 gene [9]. The virulent component, essential fatty acids as phthiocerol dimycocerosates and phenolic glycolipids, on cell wall of *Mycobacterium tuberculosis* and *Mycobacterium bovis* was analyzed in 2007. The result revealed that 3 different BCG strains (Tokyo 172, Moreau RDJ and Glaxo) contained less extent of fatty acid in cell wall. Of them, Tokyo 172 strain is considerable BCG on this study especially [10]. This demonstration was consistent with virulence experiment in mice for Tokyo 172 strain [7].

The expectation for BCG from World Health Organization

BCG plays a leading role in prevention of tuberculosis (TB) and is the only vaccine available for human use presently. World Health Organization (WHO) from 2003 to 2009 convened 5 formal and informal conferences under discussion about BCG strain features, quality control and international reference reagents. All BCG strains were originated from Institut Pasteur, France, in 1920. However, different culture conditions caused genetic variation among BCG strains, which indicated that BCG gene was not as fixed as it was expected. Multiplex Polymerase Chain Reaction (mPCR) was applied to examine BCG nucleic acid in the manufacturing process of BCG, from culture within Sauton potato

medium to final product. The results revealed that Russian BCG-I, Tokyo 172, Danish 1331, Glaxo 1077 and Connaught strains contained no evidence of molecular variation and these were gene-stable BCG [11]. In colony culture testing, Tokyo 172 strain showed mixed colony growth by type 1 (22 bp missing in zone RD) and type 2 (full gene sequence). However, Tokyo 172 strain had stable gene structure while no further gene variation was discovered after more 20 passages. In respiratory TB challenge model in guinea pig, these 2 types of Tokyo 172 strain presented identical protection potency. Furthermore, similar characteristic was also found in Pasteur 1173-P2 and Danish 1331 strains. In Pasteur 1173-P2 strain, the difference was noted in 2 and 3 sections of tandem duplication in zone DU2; while the difference of 2 types of Danish 1331 strain was revealed in 2 and 3 sections of 77 bp mycobacterial interspersed repetitive unit in zone SenX3-RegX3 [12].The mRNA translation revealed no gene difference in 2 types of Danish 1331 strain after 20 more passages which indicated that this strain was gene stable. Understanding on genomic differences between BCG strains was important to establish BCG identification examination, then to monitor BCG gene stability during manufacturing process.

Recently, both focal cutaneous reaction induced after BCG immunization and tuberculin skin test (TST), also known as purified protein derivative reaction (PPD reaction), are believed to be the major evidence of cell-mediated immune reaction caused by mycobacterium. In addition, TST is the tool for tuberculosis diagnosis, instead of significance of BCG vaccine on immune or protective efficacy [12]. Thus, the efficacy and safety evidence of BCG should be obtained from human clinical trials. Currently, the efficacy of all BCG vaccines in tuberculosis protection is not identical. About 50% of BCG-inoculated people acquire sufficient protection, while it is 75% for infants from getting tubercular meningitis and intestinal tuberculosis. However, BCG immunization may cause generalized tuberculosis in AIDS-infected infants. There is no sound evidence supporting which particular BCG vaccine provides better prevention efficacy by far [12]. No difference in TB prevention efficacy between early strains (Tokyo 172, Russian BCG-I, Moreau RDJ) and late strains (Danish 1131, Pasteur 1173 P2, Connaught, Tice) was studied in respiratory TB-challenged guinea pig model. Yet, of late strains, Glaxo presented low prevention efficacy in animal model. Analyzing BCG-induced cytokines (IL1β, IL6, IL8, IL12, TNFα) secreted by human myelomonocytic cell line revealed that early BCG strains may induce THP-1 generating higher level of cytokines. Late strains may not secret methoxymycolates, so lower level of cytokines are induced by these strains. Thus, it is suspected that late strains provide lower prevention efficacy against tuberculosis [12].

The first international reference preparation was created in 1965 by Statens Serum Institut, Denmark, the qualified international standard laboratory. However, this preparation was insufficient after 1975. Instead, Russian BCG-I, Tokyo 172, Danish 1331, Moreau RDJ and Pasteur 1173-P2 strains were used as seed strain for over 90% of BCG vaccine worldwide. Statens Serum Institut, Denmark, collected 4 candidate strains and

proceeded in vivo/in vitro experiments in 11 laboratories among 9 countries. The examinations included colony forming unit (CFU) per ampoule, ATP content (ng), and mPCR profiles. Three of 4 candidate strains (Tokyo 172-1, Danish 1331, Russian BCG-I) were proved constant characteristics and were qualified as second generation of reference reagent (RR) [13, 14]. Nowadays only 4 pre-qualified BCG vaccine plants were approved by WHO, and among them that Tokyo 172-1, Danish 1331 and Russian BCG-I strains were used for BCG vaccine manufacture.

Five RDs (RD 1, 2, 8, 14, 16) and SenX3-RegX3 were the examination targets for BCG mPCR as reliable identity test [15], which substituted traditional BCG microscopic examination by acid-fast stain and morphological differentiation.

Freeze-dried BCG vaccine was implemented for human inoculation since 1947 [16]. WHO amended requirements for dried BCG vaccine (see WHO Techn Rep Ser 329, 1966) and re-edited in 1985 and 1987 (see WHO Techn Rep Ser 745, 1987). In order to enhance CFU and stability for BCG vaccine, the requirements included: (1) use monosodium L-glutamate monohydrate as stabilizer for lyophilization; (2) maintain vacuity or fill the ampoule with nitrogen; (3) pack BCG vaccine in amber-colored ampoule. It also recommended to preserve reconstituted BCG vaccine under 4-8℃ environment and then to use within 4-6 hours [11].

The third edition of requirement was proposed by WHO in 2010 to assure the quality, safety and efficacy of BCG vaccines, which included: (1) BCG vaccine should be manufactured by dedicated facility and equipment; (2) less than 12 passages should be proceeded from master seed to final lot; (3) the safety and efficacy of BCG vaccine should be approved through human clinical trials; (4) the survival rate of guinea pig injected with 50 human doses of BCG should be increased from 60% to 90% to exclude possibility of pathogenic mycobacterium-contaminated vaccine; (5) nucleic acid typing technique (such as multiplex polymerase chain reaction) should be listed in identity test; (6) susceptibility concentration of antibiotic for BCG strain should be indicated; (7) CFU test may be substituted by fast test for potency; and (8) 3 international reference preparations are newly listed in the requirement. WHO considers 3 parts of requirements to be deleted in near future: (1) TST test for BCG-inoculated guinea pig to confirm Koch phenomenon reaction; (2) thermal stability examination under 37℃; and (3) oxygen uptake test [12].

The origin of mycobacterial strain of Taiwan BCG vaccine

In early days, BCG vaccine was liquefied formulation and the shelf life was short. Manufacture section had to provide 3 lots of BCG to replenish those expired vaccine every week. In order to extend the expiration date of BCG vaccine from 2 weeks (liquefied formulation) to 2 years (lyophilized formulation), production for freeze-dried BCG vaccine was started by Taiwan Serum and Vaccine Institute in 1970 and the technique was fully developed in 1975. Tokyo 172 strain was imported for comparing the relationship of BCG

titer and complication (lymphadenopathy) with Pasteur 1173 P2 that the BCG strain was utilized at that time [17]. After 2 times of evaluating experiment in children (7,564 cases) and 3 times of that in infants (5,840 cases), from 1977 to 1978, the incidence of lymphadenopathy complication was found lower in BCG vaccine produced by Tokyo 172 strain (0.37-1.05%) than by Pasteur 1173 P2 (8.49%). Thus, Pasteur 1173 P2 was substituted by Tokyo 172 as freeze-dried BCG vaccine strain since June 1, 1979 [18], and the dosage was determined as 0.05 mg/0.1ml.

Statens Serum Institut, Denmark provided 5 lots of Tokyo 172 strain containing 2.5 mg BCG and sodium glutamate as stabilizer, which produced by Japan BCG Laboratory in October 3,1960, since April 29, 1981 (Table 1). The lot B received in November 23, 1989, was selected as master seed for current BCG working seed lot in Vaccine Center at Taiwan CDC. The master seed was amplified through 4 passages of culture and then 1884 ampoules of BCG were produced in February 7, 1991. This lot of BCG vaccine contained 2.5mg of BCG per ampoule and was labeled as Tokyo 172A, lot A. This working seed lot was approved by quality control section in May 28, 1993, and then was officially utilized on BCG manufacturing since September 15 of the same year. Two ampoules, one with lot C (unknown year) label and one with lot D (1991) label, are reserved as master seed until now while other lots of BCG master seed had been manipulated for BCG vaccine before April 21, 1993.

Manufacture of intermediate product and end product of freeze-dried BCG vaccine

The facility and equipment for BCG manufacture in Taiwan are dedicated and not for other biological products, which is conformed to PIC/S: guide to good manufacturing practice for medicinal products, 2009, Annexes 2. Procedures for BCG manufacture includes: (1) culture working seed by Sauton potato medium for 3 weeks; (2) bacterial pellicle are transferred to Sauton medium and proceed 1 passage each week; (3) pellicles from bottles after 3 passages are collected and ground by steel balls, and then bulk bacterial suspension was adjusted to 50mg/ml. Intermediate product is collected from the 8th passage of master seed. In addition to optical observation of BCG growth on medium, pH value of Sauton medium after culture is also an index for evaluating BCG growth. The pH value of medium after 1 week of BCG growth should be higher than that of before culture (pH 7.0±0.2). In our process, the pH value of Sauton medium after Tokyo 172 strain growing was over 7.6.

Table 1. The date and number of ampoule of Tokyo 172 strain provided by Statens Serum Institut, Denmark.

Lot	Received Date				
	1981.04.29.	ND※	1985.12.16.	1989.11.23.	1991.05.18.
A	1	2	2	2	—
B	1	2	2	2	—
C	8	2	1	—	1
D	—	2	1	2	9
E	—	2	4	4	—

※none determine

BCG vaccine contains no preservative or other microbial organism. Thus, for sterility test, samples should be collected and subcultured by soybean-casein digest medium and fluid thioglycollate medium under 22.5±2.5 ^{0}C and 32.5±2.5 ^{0}C for 14 days, respectively, after each crucial procedure. No other microbial organism, except BCG, should be detected in this examination.

The bulk was modulated with 1% sodium glutamate by spectrophotometer to final bulk of $OD_{470nm} \leqq 0.2$. The filling amount for each ampoule from individual syringe should be weighed by electronic scale after filling out each box of BCG vaccine and recorded. The personnel to operate dispensing syringe should be also recorded. The filling amount of dispensing syringe should be adjusted while the dispensing dosage was strayed from scheduled amount. Then lyophilization and ampoule sealing procedures were performed. The temperature for ampoule to seal should be over 750 ^{0}C and the vacuum degree should be ≦10mBar. Vacuum condition was relieved when the temperature was cool to 580±20 ^{0}C, then sealed ampoule was collected. Monitoring of clean room environment should be conducted within all procedures, these data including record sheet for falling particles, record sheet for falling microorganism, record sheet for aero-floating microorganism and record sheet for microorganism on worktable surface. Microorganism examination for operating personnel should also be proceeded. Samples were inspected from all fingers by 2 dishes of soybean-casein digest agar medium (9 cm in diameter) and from both of elbows, forehead and chest by 4 contact plates of soybean-casein digest agar medium (5.5 cm in diameter). Collected samples were incubated under 22.5±2.5^{0}C for 5-7 days and then transfer to 32.5±2.5^{0}C for 2-3 days. Microscopic examination for non-mycobacterial contamination of final bulk and intermediate product through Ziehl-Neelsen stain was also necessary. Sixteen items on in-process control were conducted through whole BCG manufacture procedures (Table 2). The average weight of harvested semi-dried BCG was 0.81g / 100ml Sauton medium (Figure 1) and the average optical density was 0.18 for intermediate product (Figure 2), which was within the standard range (≦0.2). Sealed intermediate products were stored at 4^{0}C environment. Vacuum level of the intermediate product should be examined by electrostatic discharge (ESD) generator before ampoule labeling. Disqualified intermediate product possessed suspicions of titer recession and other microorganism contamination. Specimen from intermediate product and final product were regularly sampled for inspection by quality control section to ensure BCG quality (Table 3). Analysis and certification of BCG product by Food and Drug Administration, Department of Health, were essential before releasing into market in order to satisfy with the requirements of biological products in Chinese pharmacopoeia. In addition to these quality control items, 2 other indexes were measured to evaluate product consistency. First, the ratio of semi-dried BCG weight and 1% sodium glutamate volume should be maintained at 0.95% (Table 3). Second, the survival rate of BCG comparing before (final bulk) and after freeze-dried (intermediate product) was 30-40% (Table 4), which conformed to the expected BCG survival rate (20%) in WHO requirements for dried BCG vaccine Part A (5.5).

Table 2. Items of in-process control for BCG vaccine manufacture

Procedure	In-process control
Culture	1. microorganism test after passage 2. colony growth condition 3. pH value of culture medium 4. weight of harvested semi-dry BCG pellicle
Bulk deployment	1. optical density 2. microorganism test
Final bulk	1. optical density 2. filling volume 3. staining examination 4. microorganism test
Freeze-dry	1. time period for freeze-dry 2. relative humidity in dry cabinet
Vacuum sealing	1. vacuum level of intermediate product after ampoule sealing 2. optical density of intermediate product after ampoule sealing 3. microorganism test of intermediate product after ampoule sealing 4. staining examination

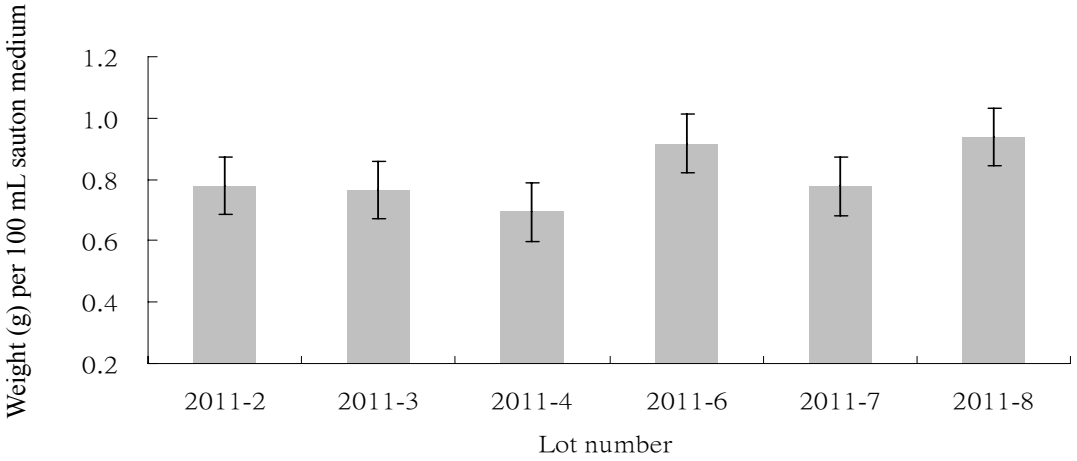

Figure 1. Weight of harvested BCG pellicle from sauton medium after one-week growth

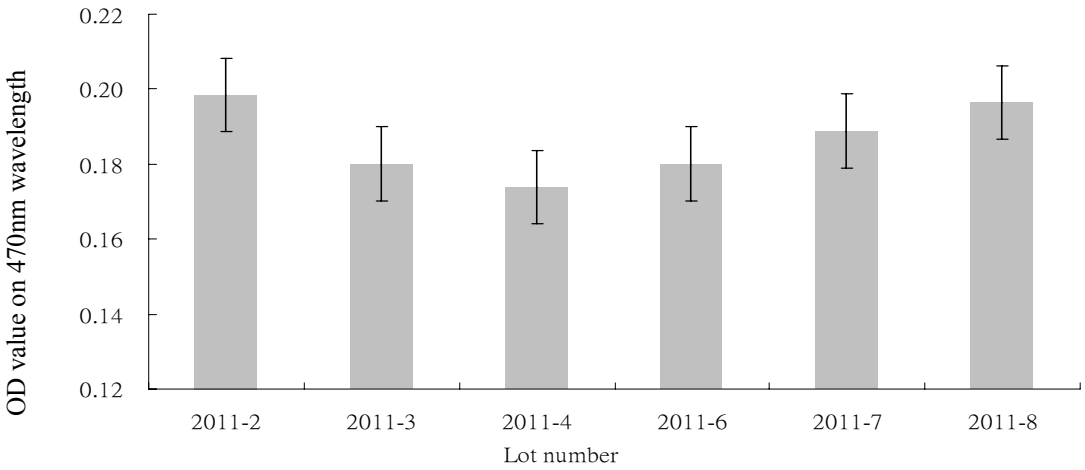

Figure 2. Opacity test for BCG intermediate products

Table 3. Quality control items of freeze-dried BCG vaccine

Product stage	Quality control items
Intermediate product	1. Potency test：total mycobacterial number>15x10^6（CFU/mg/ml）
	2. Safety test：no progressive mycobacterial infection in guinea pig
	3. Staining test：acid-fast stain
Final product	1. Identity test：staining test
	2. Safety：pH value, microorganism test, safety test
	3. Validity：Koch phenomenon, potency test

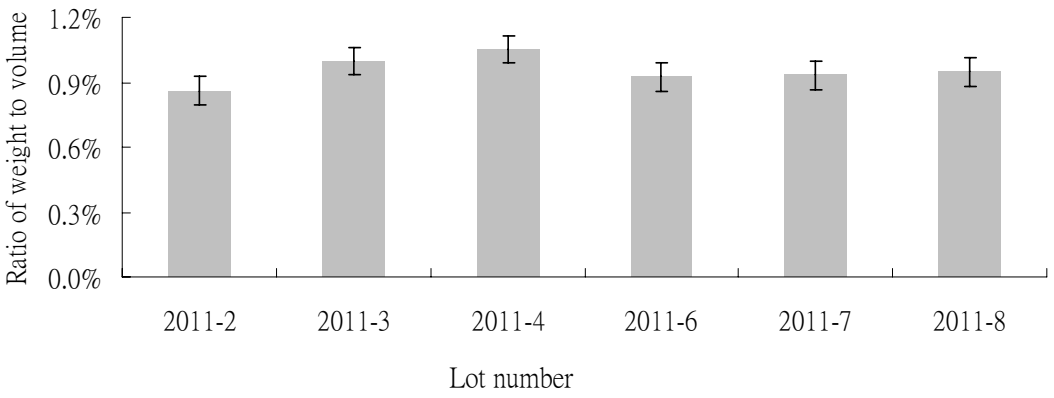

Figure3. Ratio of semi-dry BCG weight to 1% sodium glutamate volume for final bulk

Table 4. Survival rate of Tokyo 172 BCG before and after freeze-dried procedure

Lot No.	Intermediate product (after freeze-dried) (×10^6 CFU/mL)	Final bulk (before freeze-dried)	Survival rate (%)
2008-3	17.9	47.9	37.37
2008-5	18.8	50.2	37.45
2008-7	26.7	77.2	34.58
2008-9	38.3	98.6	38.84
2008-10	17.1	55.3	30.92
2008-11	22.6	56.5	40.00

The minimum requirement for survival colony number of Tokyo 172 strain is 15x10^6 CFU/ml, which is higher than that of Russian BCG-I strain (1.5x10^6 CFU/ml) [19]. Comparing among 3 WHO international reference preparations, the survival rate of Tokyo 172 strain is much higher than Russian BCG-I and Danish 1331 strain [14], and this characteristic may offset the disadvantage of weaker immune response induced by Tokyo 172 strain.

Discussion

The quality of vaccine can be evaluated by 2 approaches: (1) efficacy: decrease the incidence of disease; (2) safety: decrease complications caused by vaccine itself.

BCG coverage rate of infants in Taiwan in 2001 was about 97%, which was higher

than that of worldwide announced by WHO. Comprehensive BCG inoculation has decreased the incidence of infant extrapulmonary tuberculosis from 10/million infants (1965) to 0.1/million infants (1985) [20]. A research which analyzed TB cases from 1996 to 2003 revealed that BCG vaccine successfully decreased the incidence of TB as well as tubercular meningitis in infants under 5 years old. However, this protection was declined for children over 12 years old [21]. It is necessary to conduct generalized BCG inoculation in Taiwan due to uninterrupted record for tubercular meningitis cases.

The incidence of BCG complications were 100-1000 cases/million doses for suppurative lymphadenitis, 1-700 cases/million doses for BCG osteitis/osteomyelitis, and 2 cases/million doses for disseminated BCG- infection, announced by WHO in 2000 [22]. In countries using Tokyo 172 strain as BCG vaccine seed, BCG osteitis/osteomyelitis had been reported in Taiwan and Korea [23-24], but not in Japan and Thailand. At present, data from worldwide is still insufficient to evaluate complication of BCG vaccine produced by Tokyo 172 strain. According to previous studies, the incidence of BCG osteitis/osteomyelitis in Taiwan was increased from 3.68/million doses (2002-2006) [22] to 12.9/million doses (2005-2007) [23]. This result, although lower than WHO report, indicated that post-market surveillance of BCG was insufficient in Taiwan. Since 2007 Taiwan CDC requested medical organizations to send samples related to extrapulmonary tuberculosis for further examination. This procedure is conductive to evaluate the incidence of BCG complication and TB incidence, and to clarify BCG quality produced in Taiwan which may provide new information for BCG inoculation policy. BCG is attenuated, live vaccine and may induce disseminated BCG infection in infants with congenital immunodeficiency, especially in countries with high incidence of AIDS. It is important to diagnose this congenital disease before BCG inoculation to avoid severe BCG infection [12].

Quality for BCG vaccine in Taiwan is based on well-preserved BCG seed bank and consistent manufacturing procedures, and then is emphasis on each link during whole procedures, including operating personnel and manufacture facility. Due to low birthrate in Taiwan, the purpose of BCG manufacture should be emphasized on decreasing incidence of BCG complication instead of sufficient quantity to satisfy request.

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References

1. Smith KC, Starke JR. Bacille Calmette-Guerin Vaccine. In: Plotkin SA, Orenstein WA, ed., Vaccines. WB Saunders Co., 1999;111-39.

2. Corbel MJ, Fruth U, Griffiths E, et al. Report on a WHO consultation on the characterization of BCG strains, Imperial college, London 15-16 December 2003. Vaccine 2004;22:2675-80.
3. Abou-Zeid C, Smith J, Grange J, et al. Subdivision of daughter strains of Bacille Calmette-Gurerin（BCG）according to secreted protein patterns. J Gen Microbiol 1986;132:3047-3053.Behr MA, Small PM. A historical and molecular phylogeny of BCG strains. Vaccine 1999;17:915-922.
4. Fomukong NG, Dale JW, Osborn TW, et al. Use of gene probes based on the insertion sequence IS 986 to differentiate between BCG vaccine strains. J Appl Bacteriol 1992;72:126-33.
5. Minnikin DE, Minnikin SM, Dobson G, et al. Mycolic acid patterns of four vaccine strains of Mycobacterium bovis BCG. J Gen Microbiol 1983;129:889-91.
6. Brewer TF, Colditz GA. Relationship between Bacille Calmette-Guerin (BCG) strains and the efficacy of BCG vaccine in the prevention of tuberculosis. Clin Infect Dis 1995;20(1):126-35.
7. Ladefoged A, Bunch-Christensen K, Guld J. Tuberculin sensitivity in guinea-pigs after vaccination with varying doses of BCG of 12 different strains. Bull World Health Organ 1976;53:435-43.
8. Coldtiz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature, J Am Med Asssoc 1994;271(9):698-702.
9. Behr MA, Small PM. A historical and molecular phylogeny of BCG strains. Vaccine 1999;17:915-22.
10. Chen JM, Islam ST, Ren H, et al. Different productions of lipid virulence factors among BCG vaccine strains and implications on BCG safety. Vaccine 2007;25:8114-22.
11. WHO. Report on a WHO consultation on the characterization of BCG vaccines, Geneva, 8-9 December 2004.
12. WHO. Report on a WHO informal consultation on standardization and evaluation of BCG vaccines. Geneva, 22-23 September 2009a.
13. WHO. Report on a WHO discussion on the improvement of the quality control of BCG vaccines. Pasteur Institute, Paris, 7 June 2005.
14. WHO. Expert committee on biological standardization: International collaborative study to evaluate and establish WHO reference reagents for BCG vaccine of three different sub-strains. Geneva, 19-23 October 2009b.
15. Markey K, Ho MM, Choudhury B, et al. Report of an international collaborative study to evaluate the suitability of multiplex PCR as an identity assay for different sub-strains of BCG vaccine. Vaccine 2010;28:6964-9.
16. Yamamoto S, Yamamoto T. Historical review of BCG vaccine in Japan. Jpn J Infect Dis 2007;60:331-6.

17. Chang KJ, Wang YF, Chen CT, et al. Evaluation of efficacy of freeze-dried BCG in primary school children and infants. Collections of research reports of the National institute of preventive medicine, National health administration（1977-1979）:113-20.（In Chinese: English abstract ）
18. Chang KJ, Lee JD, Lee YC, et al. Production of lyophilized BCG vaccine and improvement of assaying technique. Collections of research reports of the National institute of preventive medicine, National health administration（1980-1981）:32-5.（In Chinese: English abstract）
19. WHO. Report on a WHO consultation on the characterization of BCG strains, Imperial college, London 15-16 December 2003.
20. Chan PC, Huang LM, Kuo HS. Is neonatal bacillus Calmette-Guérin vaccination protective in Taiwan? Journal of the Formosan Medical Association. 2008;107(3):195-7.
21. Chan PC, Huang LM, Wu YC, et al. Tuberculosis in children and adolescents, Taiwan, 1996-2003. Emerging Infectious Diseases. 2007;13(9):1361-3.
22. GC Sheu, SL Yang, CD Lee, et al. Adverse Events Induced by BCG Immunization in Taiwan. Taiwan Epidemiology Bulletin2008;24(5)：313-25.
23. Jou R, Huang WL, Su WJ. Tokyo-172 BCG vaccination complication, Taiwan. Emerging Infectious Diseases. 2009;15(9):1525-16.
24. Kim SH, Kim SY, Eun BW, et al. BCG osteomyelitis caused by the BCG Tokyo strain and confirmed by molecular method. Vaccine 2008;26: 4379-81.

Outbreak Investigation Express

The Report of Scrub Typhus Outbreak in Kinmen County, 2011

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Abstract

The outbreak of scrub typhus in Kinmen region in 2011 year is higher compared to the same period in previous years, and peaks early in the 26th week (June). According to Taiwan CDC's statistics, as of the 31st week this year, Kinmen has reported a total of 172 cases of scrub typhus with 59 confirmed, compared to 70 reported cases with 32 confirmed in the same period of 2010. The reason was presumably related to the climate, murine and chigger mites' population in the island, high risk groups and protective measures. Taiwan CDC worked with

local government to urgently mobilize all resources, and integrated control action, including trimming the focal environment of the island, the implementation of weeding and rodent control in high-risk areas, enhance public health education, strengthen the health care institutions in diagnosis and reporting, as well as cooperation with military. The outbreak is slowing down since the 32nd week (August).

Keywords: scrub typhus, Kinmen County

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