



The Need for Tuberculosis Reference Standards in Vaccine Development

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Tuberculosis (TB), a highly contagious respiratory disease caused by the bacterium *Mycobacterium tuberculosis*, annually results in over two million deaths worldwide^{1,2}. In the United States alone, the Centers for Disease Control and Prevention (CDC) reported a total of 9,588 new cases of TB in 2013; of which, 86 cases were multidrug-resistant³. This infection is commonly spread by the aerosolization of the bacteria via coughing, sneezing, speaking, or singing. Clinical symptoms of TB include chronic cough with blood-tinged sputum, fever, weight loss, and the formation of tubercles in the lungs².

To prevent the spread of TB in endemic countries, the Bacillus Calmette–Guérin (BCG) vaccine is used. This vaccine, which was first introduced in 1921, is derived from an attenuated live bovine tuberculosis bacillus, *Mycobacterium bovis*, which is non-virulent in humans. Following its introduction into the World Health Organization Expanded Programme on Immunization in 1974, use of the BCG vaccine has reached global coverage rates of >80% in countries where TB is prevalent⁴.

On average, the BCG vaccine has been found to reduce the risk of TB by 50%, with estimates of protection ranging from 0-80%^{5,6}. Moreover, it does not prevent primary infection or the reactivation of latent pulmonary infection⁴. These variations in vaccine efficacy have been attributed to a wide range of factors, including genetic or nutritional differences between populations, environmental influences, exposure to other microbial infections, or the methods used to prepare the vaccine^{6,7}. Overall, the impact of the current BCG vaccine on reducing the transmission of TB is limited.

In recent years, the genomic plasticity of BCG vaccine strains was offered as another possible explanation for variable efficacy⁸. In the early years of vaccine development, prior to the introduction of archival seed lots, vaccine strains were maintained by serial passaging. Following the implementation of proper cold-chain maintenance procedures, several different BCG seed strains were preserved for use in vaccine development⁹. However, by that point, years of subculturing ultimately resulted in significant differences in the genomes of each strain; where, comparative genomics have uncovered deletions, insertions, and single nucleotide polymorphisms that may have contributed to diminished vaccine efficacy¹⁰⁻¹³.


To help control for the intrinsic differences between BCG strains, the use of a single biological standard in vaccine development should be considered. Generally, a biological standard is defined as a well-characterized, authenticated, purified biological reference material – qualities of which are essential in minimizing variations between vaccine preparations. Through the use of a single, minimally-passaged *M. bovis* standard, one of the contributing factors affecting vaccine efficacy can be accounted for; thus, potentially improving the quality of the vaccine preparation and ensuring that all recipients are receiving the best possible protection against TB.

Currently, biological standards are developed and produced by a number of entities, including government agencies, commercial companies, and non-profit institutions. ATCC, for example, offers a number of *M. bovis* strains, including those known to demonstrate resistance to isoniazid. Each of these ATCC® Genuine Cultures is fully characterized using genotypic, phenotypic, and functional analyses to establish identity. Moreover, each strain is carefully preserved as low passaged stocks using a seed stock system to minimize subculturing and maintain the original culture characteristics.

Overall, there have been a number of factors attributed to the variations seen in BCG vaccine efficacy. Through the use of a single, consensus, biological standard that demonstrates high levels of protection when used in vaccine development, manufacturers can come one step closer to improving BCG vaccine performance.

References

1. World Health Organization (WHO). Global Tuberculosis Control: WHO report 2010. Geneva, Switzerland., <http://www.who.int/tb/publications/global_report/en/>, 2010.
2. Centers for Disease Control and Prevention (CDC). Tuberculosis (TB), <<http://www.cdc.gov/tb/>>, 2013.
3. Centers for Disease Control and Prevention (CDC). Trends in Tuberculosis — United States, 2013. Morbidity and Mortality Weekly Report (MMWR) 63: 229-233, 2014.
4. World Health Organization (WHO). BCG Vaccine, <<http://www.who.int/biologicals/areas/vaccines/bcg/en/>>, 2014.
5. Colditz GA, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA 271: 698-702, 1994.
6. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet 346: 1339-1345, 1995.
7. Venkataswamy MM, et al. *In vitro* culture medium influences the vaccine efficacy of *Mycobacterium bovis* BCG. Vaccine 30: 1038-1049, 2012.
8. Brosch R, et al. Genome plasticity of BCG and impact on vaccine efficacy. Proc Natl Acad Sci U S A 104: 5596-5601, 2007.
9. Fine PE, Carneiro IAM, Milstein JB, Clements J. Issues relating to the use of BCG in immunization programmes. <http://whqlibdoc.who.int/hq/1999/WHO_V&B_99.23.pdf>, 1999.
10. Behr MA, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 284: 1520-1523, 1999.
11. Belley A, et al. Impact of methoxymycolic acid production by *Mycobacterium bovis* BCG vaccines. Infect Immun 72: 2803-2809, 2004.
12. Mostowy S, Tsolaki AG, Small PM, Behr MA. The *in vitro* evolution of BCG vaccines. Vaccine 21: 4270-4274, 2003.
13. Gordon SV, et al. Identification of variable regions in the genomes of tubercle bacilli using bacterial artificial chromosome arrays. Mol Microbiol 32: 643-655, 1999.

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TB-0419-02

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