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WHITE PAPER

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-resistant.[3] 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 tubercules in the lungs.[2]

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 prevalent.[4]

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 infection.[4] 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 efficacy.[8] 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 development.[9] 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 efficacy.[10-13]

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 nonprofit 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.

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TB-112021-v03