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RESEARCH ARTICLE

QUALITY EVALUATION OF BCG VACCINE BY CONVENTIONAL AND MOLECULAR METHODS

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ABSTRACT

BCG vaccine is an attenuated derivative of Mycobacterium bovis which is widely used as a vaccine against tuberculosis and conventional as well as molecular methods have been described as identification test for BCG vaccine. In present study, we compared the results obtained with Ziehl Neelsen staining and multiplex PCR (mPCR) methods for identification of different batches of BCG vaccine. We found that all the BCG vaccine batches tested showed specific amplified DNA band indicating that mPCR method may be used as a rapid and specific alternative method for the identification of BCG vaccine.

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INTRODUCTION

BCG vaccine contains live, attenuated strain of *Mycobacterium bovis* that was originally isolated from cattles with tuberculosis and cultured for period of 13 years and a total of 231 passages (Calmette and Plotz, 1929).BCG is currently the only vaccine available for tuberculosis and also the most widely used vaccine in human history. It is estimated that over 4 billion people worldwide have been given BCG vaccines, with more than 100 million doses of BCG administered each year. Quality control testing of BCG vaccine is performed as per the pharmacopoeia requirements to ensure the critical quality attributes of the vaccine (Indian Pharmacopoeia, 2014; European Pharmacopoeia, 2011). Various quality parameters are used to assure the quality of BCG vaccine among them one of the most important parameter is the identification which is usually carried out by microscopic examination of the bacilli in stained smears demonstrating their acid fast properties. However, the pharmacopoeia monographs on BCG vaccine mention that molecular biology techniques may also be used as an alternative for identification (Indian Pharmacopoeia, 2014; European Pharmacopoeia, 2011). In the literature, a multiplex PCR (mPCR) method has been described which differentiates *M. bovis* BCG strain from *M. bovis* and *M. tuberculosis* (Talbot *et al*., 1997). In view of above, we undertook present study with the objective to compare the conventional Ziehl Neelsen (ZN) staining and mPCR methods for the identification of BCG vaccine.

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MATERIALS AND METHODS

BCG Vaccine Batches

Three BCG vaccine batches each were obtained from two different manufacturers in India. International reference standard of BCG vaccine was sourced from National Institute for Biological Standards and Control (NIBSC).

ZN Staining

BCG vaccine batches were tested by ZN staining method using ZN acid fast stains kit (HiMedia, India). Reference standard from NIBSC was used as control during the staining.

mPCR

DNA was extracted from the BCG vaccine and NIBSC standard using Nucleo-Pore DNA Sure® Tissue Mini Kit (Genetix, India) followed by PCR amplification. The following PCR primers were used:

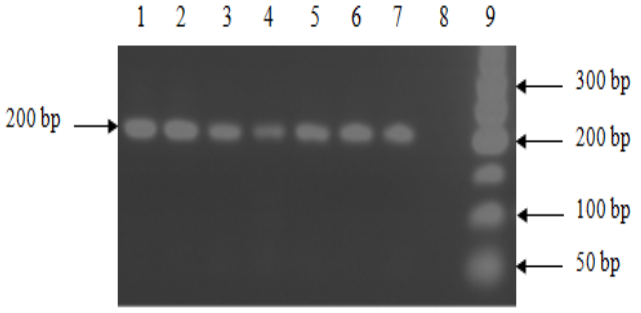

Figure 1. Ethidium bromide stained gel of mPCR products. Lane 1-3, BCG vaccine batches from manufacturer 1; lane 4-6, BCG vaccine batches from manufacturer 2; lane 7, NIBSC reference standard; lane 8, negative control; lane 9, 50 bpmolecular marker

ET1, 5′-AAGCGGTTGCCGCCGACCGACC-3′;ET2, 5′-CTGGCTATATTCCTGGGCCCGG-3′; and ET3,5′-GAGGCGATCTGGCGGTTTGGGG-3′. PCR was performed using GeneTaq PCR kit (Genetix, India) with 5µl of DNA sample in a total volume of 25 µl of PCR mix. The thermal cycling was performed in Gene Amp PCR system 9700 (Applied Biosystems, USA) with Initial Denaturation at 95°C for 3 min followed by 40 cycles consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min and final extension at 72°C for 10 min. PCR products were separated by electrophoresis on a 1.5 % agarose gel. Size of amplified PCR product was determined by UV transilluminator of ethidium bromide stained gel using a molecular weight marker.

RESULTS AND DISCUSSION

In ZN staining, the observations were based on the microscopic examination of acid-fast bacilli in the stained smears prepared from BCG vaccine. The bacilli exhibit the characteristics of an authentic strain of BCG by demonstrating their acid fast properties. All the six BCG batches showed positive smears in ZN staining confirming the identity of BCG vaccine. In the mPCR method, BCG vaccine batches were tested for detecting specific amplification product. Results of mPCR method are shown in Figure 1. The gel image shows a single band of 200 bp in all the tested batches indicating a positive identification. Microscopic examination of smears of acid fast bacteria by ZN staining is the quickest method of detecting mycobacteria but it is insensitive and nonspecific. Techniques based on molecular biology have facilitated the development of rapid means to identify the BCG vaccine (WHO, 2004; WHO, 2013).

PCR is the technique which is based on amplification of particular segment of mycobacterial DNA which helps to rapidly identify the BCG vaccine strains. According to recent studies, a genomic region designated RD1 was found to be present in all virulent *M. bovis* and *M. tuberculosis* strains tested but found deleted from all BCG vaccine strains (Talbot *et al*., 1997).Based on this genomic region mPCR method has been developed which shows amplified DNA band of 200 bp in BCG strains. However, *M. bovis* and *M. tuberculosis* give amplified band of 150 bp thus enabling differentiation of BCG stains from other related mycobacterial species (Talbot *et al*., 1997).

In our study also RD1 region has been found deleted from all BCG vaccine batches tested. Results of our study show that mPCR method is 100% sensitive and specific for the identification of BCG vaccine. Conventional ZN staining method has its limitations in identification of BCG vaccine whereas PCR method can be used for genetic level identification of BCG vaccine. Thus mPCR method has significant advantages over existing conventional method and may be used as alternative by the manufacturers and regulatory laboratories for quality evaluation of BCG vaccine.

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