

TITLE: ALTERNATIVE METHODS APPLIED TO THE CONTROL OF THE VIABILITY OF BCG VACCINE EMPLOYED IN BRAZIL.

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ABSTRACT:

The BCG vaccine consists of a lyophilized suspension of alive bacilli Calmette Guérin, a strain originating from *Mycobacterium bovis*. It is indicated in prevention of the most severe forms of tuberculosis. The quality control of the BCG vaccine is conducted in National Institute of Quality Control in Health in Fiocruz with emphasis on biological safety tests, identity, thermostability and viability, being the last three accomplished in the Laboratory of BCG Vaccines. The viability conventional assay is performed by counting BCG colony forming units per milliliter of the vaccine (CFU / mL) after culture in solid medium of Lowenstein Jensen. Although this traditional method is considered reference to the BCG vaccine quality control and recommended by WHO, it presents limitations, such as the high variability inherent in the tendency to formation of cell aggregates and the delay to obtain results due to the prolonged time of growth characteristic of mycobacteria. Faced with these difficulties, the objective of this research is to analyse alternative methods for determining the viability of the vaccine, such as the dosage of ATP (adenosine triphosphate) and Flow Cytometer. To analyze by Flow Cytometer method, it was used a reconstituted national reference BCG vaccine ampoule in addition to 16 test samples, in order to verify the correlation between the cytometry test and the conventional method. The viability marker used was Fluorescein Diacetate (FDA). The samples were collected on the Cytoflex flow cytometer (Beckman Coulter) through the software CytExpert 1.1 (Beckman Coulter) in collaboration with the Flow Cytometry Platform of the Oswaldo Cruz Institute. From the same ampoules assayed by the cytometer, samples were taken, centrifuged, suspended with 7H9 medium and incubated at 37 °C for about 24 h. The overnight BCG suspensions were submitted to ATP extraction by heating with Tris-EDTA buffer at 96 – 98 °C for exactly 6 minutes. The ATP extracts were cooled at room temperature before ATP reaction (CellTiter-Glo®-Promega). ATP standard (Invitrogen™) curve was performed in parallel, and relative light units (RLU) converted into pmol/100mL by interpolation. Readings were performed in a CentroPRO LB 962 Microplate Luminometer (Berthold). In parallel with both methods the same samples, from national reference BCG vaccine and test samples were evaluated by the conventional method by colony counting. Pearson correlation indices show moderate to strong relation between methods. The strongest correlation of 0,806 was observed between results of conventional counting and ATP dosage. Moderated correlations were obtained respectively between counting colonies, and cytometer method (0,399), and ATP dosage (0,454). Results of assays still in progress will contribute to make clear differences or equivalence between results provided by the alternative methods. However, it appears from the correlations shown that these rapid methods are in fact good alternatives to the traditional culture, since well standardized and validated.

Keywords: vaccine, bcg, viability, method

