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Short communication

Quality control of injectables based on ATP Bioluminescence

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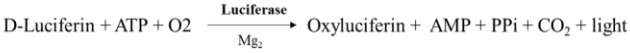
Introduction

Adenosine triphosphate (ATP) is a universal intermediate of energy in all living cells. The hydrolysis of ATP provides enough force for energy-dependent processes that occur in the cell**.** The presence of ATP in cells makes its detection a direct measure of life. Promicol method is based on the measurement of ATP using the firefly enzyme which converts the substrate luciferin into oxyluciferin and light by dephosphorylating the ATP present in the cells (McElroy, W.D and Green A., 1956). The light produced is measured and expressed in relative light units (RLU).



ATP bioluminescence is a long-stablished method for sterility control in food, beverages, and cosmetics (Griffins M.W., 1993; Monica S. et al, 2021; Jimenez L. et al, 2001). However, the outbreak of SARS-COV 2 prompted us to develop a rapid microbial sterility test for the pharmaceutical industry. Where modern vaccines and other pharmaceutical therapeutics could be tested in a simpler, faster and truthful manner as an alternative to the turbidity assessment with 14 days incubation protocol, described in the compendial method.

Materials and methods

The American (USP) and the European pharmacopeia (Ph. Eur.) state the use of harmonized protocols for sterility testing of all pharmaceutical products. The compendial method is limited by long incubation times and it was not intended for cell-based therapies. Promicol protocol provides a rapid microbial method (RMM) that tests material in a reduced incubation time where samples are preincubated in different medias to promote microbial growth. Specifically, we have chosen three types of media which are specific for aerobic (TSB) and anaerobic (TSB anaerobic) organisms, and yeast/mold (SDB) (Promicol, Geleen, The Netherlands).

Bioluminescence assay was carried out with the Novilite® Instrument with the PRENOVA® COV-1 platform and measurements were executed using PROMICOL PRENOVA® software (Promicol, Geleen, The Netherlands). The protocol was performed as follows: One milliliter (1mL) of mRNA vaccines (S1, S2 and S3) were added to 20 mL of each Promicol culture media (TSB, TSB anaerobic and SDB). In the case of the antibiotic, 100 mL of prediluted sample was filtered with 0.45 µm cellulose acetate membrane filter (Cytiva, USA) and later incubated with the media in sterile plastic jars. Samples were then spiked with 5-45 CFU/mL of Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus brasiliensis, Candida albicans and Clostridium sporogenes (ATCC 6538, 6633, 9027, 16404, 10231 and 11437 respectively. ATCC, Manassas, Virginia, USA), as stated in the USP < 71 > and Ph. Eur. 2.6.1. All samples were kept at 30 °C no shaking for 48 to 96 hours (depending on the sample type) post-inoculation.

After incubation, 100 µL of sample was added in quadruplicates to a 96-well microplate (Promicol, Geleen, The Netherlands) and analyzed in the fully automated device. Following 15 minutes of equilibration time, firefly enzyme and microbial extract were added to the sample. Results were expressed in RLU. For a correct interpretation of the results, blank values (control) were set by incubating and analyzing the different products in culture medias without inoculums. Sterile samples (negative) were confirmed when RLU sample values were equal or less than 2 times than the mean RLU of the blank value; positive results were confirmed when the mean RLU were 3 times equal or over the blank; re-test values were found when the RLU sample was found to be higher than 2 and

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less than 3 times the blank value. In case of re-test, samples were incubated for further six hours.

Results and discussion

The capability to detect sterility by PRENOVA® COV-1 platform was investigated in this preliminary study. Our ATP bioluminescence assay can detect bacteria, yeast and mold growth in three different mRNA vaccines in all promoting cultures (Figure 1). Although the RLU values for *S. aureus*, in samples 2 and 3 were lower, they were above the threshold set for positive samples. Figure 2 confirms the possibility of testing antibiotic-containing samples that were previously filtered. The highly specific media designed by Promicol to support the selective growth of the aerobic and anaerobic bacteria and yeast and mold, has proven the fast and effective detection of sterility by ATP bioluminescence.

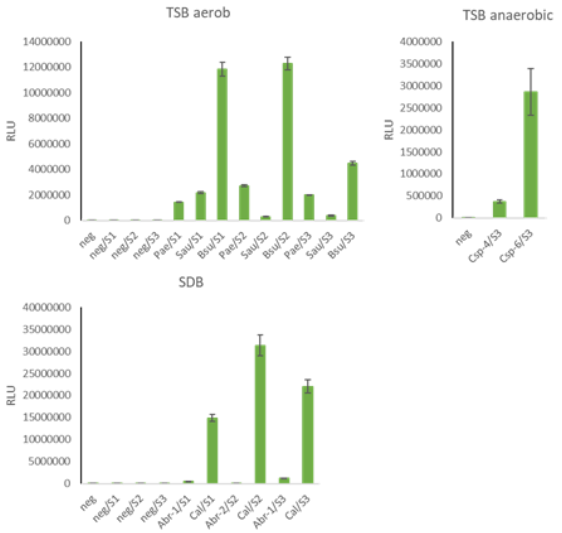

Figure 1. mRNA vaccines measured after 48h of pre-enrichment. Direct inoculation of sample into TSB, TSB anaerobic and SDB media.

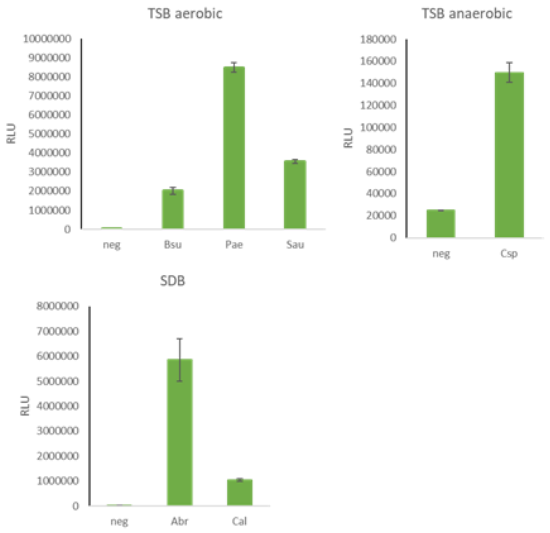

Figure 2. Antibiotic-containing ointment measured after 96h of pre-enrichment. Filtration set-up of sample into TSB, TSB anaerobic and SDB media.

Conclusion

Here we show how the principle of detection of ATP bioluminescence for sterility testing is successfully applied to cell-free products either by direct inoculation or membrane filtration with results available after 48 to 96 hours. It could be applied to cell-based therapies shortening the product release period which could benefit terminally ill patients.

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