DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di progetto per una borsa di Dottorato (Finanziata da Merck non da Sapienza)

Titolo della ricerca:
Validation of sterility ATP-based methods for biotech drug product analysis

Docente guida *(includere E-mail; se esterni al Dipartimento anche affiliazione. Il docente non dovrà avere altri dottorandi in corso con borsa[1])*:

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Altri docenti guida:

Summary (max 300 parole)

The sterility test is a compendial method performed according to the pharmacopoeia chapter EP 2.6.1 Sterility and USP <71>. The compendial sterility test is a qualitative test that requires 14-day incubation to obtain a valid analytical result, in which the turbidity developed in the culture media is indicative of microbial contamination and is verified by a qualified operator's visual inspection. The ATP-based rapid Sterility method is a growth-based sterility test, which detects microbial contamination based on the presence of Adenosine triphosphate (ATP) in the sample. The bioluminescence occurs when luciferase and luciferin come into contact with the molecule adenosine triphosphate (ATP), which is present in all living organism. The use of alternative methods allows the reduction of the incubation period to 7 days.
This project is aimed to validate the alternative Sterility ATP-based method to use it for biotech drug product analysis. The validation will fulfil the entire process from the verification of the method suitability, to the regulatory discussion, until the financial analysis. Since the ATP is present in all the living organisms and because of the most biotech production process involves in the use of living organisms (Prokaryotic or eukaryotic) to stimulate the production of useful substances, one goal of this project will be to evaluate if the biotech products matrices have some interference with the ATP based detection system. To reach this goal the products will be clustered based on their composition and tested for possible interferences.
In addition, the method verification must be performed also using microorganisms under stress conditions and slow-growing microorganisms to simulate the real contamination. For this purpose, a robust stress protocol will be designed and used during the method validation.
Finally, the financial advantages derived from the implementation of the rapid Microbiological method will be evaluated through the construction of a business case.

- [1] *Sono esclusi dottorandi stranieri con Borsa Sapienza*

Referenze

United States Pharmacopeia -USP 40/NF 35. <1223> Validation of Alternative Microbiological Methods. Rockville, MD: The United States Pharmacopeial Convention, 2017.

Parenteral Drug Association (PDA). Technical Report No.33 (Revised 2013): Evaluation, validation and implementation of alternative and rapid microbiological testing methods. Bethesda, MD: Parenteral Drug Association, Inc.; 2013.

Gray JC, Stark A, Berchtold M, Mercier M, Neuhaus G, Wirth A. Introduction of a rapid microbiological method as an alternative to the pharmacopoeial method for the sterility test. Am Pharm Rev. 2010;13(6):88–94.

Parveen S, Kaur S, David SA, Kenney JL, McCormick WM, Gupta RK. Evaluation of growth based rapid microbiological methods for sterility testing of vaccines and other biological products. Vaccine. 2011;29(45):8012–23.

Bugno, A., Almodovar, A.A.B., Saes, D.P.S. et al. Evaluation of an Amplified ATP Bioluminescence Method for Rapid Sterility Testing of Large Volume Parenteral. J Pharm Innov 14, 152–158 (2019).

Gordon O, Gray JC, Anders HJ, Staerk A, Schlaefli O. Overview of rapid microbiological methods evaluated, validated and implemented for microbiological quality control. Eur Pharm Rev. 2011;16(2)

Chen Y, Zou B, Zhu S, Ma Y, Zhou G. Detection of low-level microorganism by concomitant use of ATP amplification and bioluminescence assay. Acta Microbiol Sin. 2009;49:826–30

DESCRIZIONE DELLA RICERCA *(max 2 pagine, Arial 12, interlinea singola, esclusa bibliografia)*

Obiettivi della ricerca (generale e specifici)

The production process of drug substance or drug product can be more or less long and complex requiring several phases, in which can occur a random contamination that, if not controlled, could endanger the patient's life.
Indeed, generally all the activities of pharmaceutical companies are under the control of regulators to guarantee the quality and the safety of the products, like Food and Drug Administration (FDA), European Medicines Agency (EMA), Japanese Pharmaceuticals and Medical Devices Agency (PMDA), etc.
A typical total quality control starts from the procurement of raw materials to the finished product, until it gets consumed by patient. The main microbiological tests performed are the bioburden test, the sterility test and the endotoxin test, carried out following the traditional, or compendial, methods as described in the pharmacopoeia chapters. The present project will be focused principally on the implementation of rapid sterility test.
The sterility test is applied to substances, preparations or articles which, according to the pharmacopoeia, are required to be sterile and it is performed according to the pharmacopoeia chapter EP 2.6.1 Sterility and USP <71>. The compendial sterility test is a qualitative test that requires 14-day incubation to obtain a valid analytical result, in which the turbidity developed in the culture media is indicative of microbial contamination and is verified by a visual inspection carried out by a qualified operators. The traditional sterility test has some limits: it last several days, a 14 days incubation period is required to obtain results; the cultural methods can be not optimal for the recovery of slow grown microorganism and for stressed microorganism; and finally the results of conventional sterility testing methods are based on subjective evaluations of microbial growth carried out by a qualified operator.
In this context, Rapid Microbiological Methods (RMM) or alternative microbiological methods, that allow to obtain results faster than the compendial method and also guarantee objective results in compliance with the data integrity requirements are emerged. RMMs have several advantages as compared to traditional methods such as faster product release, faster reaction time to non-compliance or deviations, and increased automation, resulting in a better control of the manufacturing process.
Health authorities encourage pharmaceutical companies to use rapid microbiological methods.
Any alternative method to be implemented in the pharmaceutical field must be "validated". The validation process involves in a robust demonstration of the non-inferiority of the alternative method as compared to the traditional method currently in use.
The present PhD project has the final objective to validate the alternative Rapid Sterility method based on the ATP detection to use it for biotech drug product analysis.
The rapid microbial screening system uses ATP bioluminescence and sensitive light instruments (luminometers) to reveal the presence of contamination in the samples. The bioluminescence occurs when luciferase and luciferin come in contact with the molecule adenosine triphosphate (ATP), which is present in all living organism. The result is the emission of light that is directly proportional to the amount of ATP eventually present in the sample (Bussey and Tsuji, 1986).
The ATP-based rapid Sterility method is a growth-based sterility test, which detects microbial contamination reducing the incubation period to 7 days starting from 14 day of the compendial method.

The validation will fulfil the entire process from the verification of the method suitability, to the regulatory discussion, until the financial analysis.
The most biotech production process involves in the use of living organisms (Prokaryotic or eukaryotic) that are genetically manipulated to stimulate the production of useful substances, for industrial or medical application. Since the ATP is present in all the living organisms one goal of this project will be to evaluate if the biotech products matrices have some interference (enhancement) with the ATP based detection system that could led to false positive results. To reach this goal the drug products will be clustered based on their composition and tested for possible interferences.
Moreover, the method verification must be performed also using microorganisms under stress conditions and slow-growing microorganisms to simulate the real contamination. Indeed, the microorganisms from the manufacturing environment could be starved or stressed and the cultural conditions can be not optimal for their growth. More than one stress factor could arise injuring the microbial cells for example heat stress or oxidative stress etc. Based on the importance of challenge the alternative method by using the stressed microorganisms during the method verification, one part of this project will be focused on the review and identification of the most common stress occurring in the manufacturing environment/ during the manufacturing process with the final goal to design a robust and reproducible stress protocol.
Finally, the company must fully understand the economic benefits of carrying out the implementation of the rapid Microbiological method. To support this project a comprehensive economic analysis will performed to justify the costs associated with qualification and implementation of the rapid technology.

Stato delle conoscenze

In the Microbiological quality control laboratory with the aim to implement a rapid sterility test we have performed a technology assessment in order to evaluate the technologies available on the market. The technology assessment was aimed to analyze the aspects of science and technology of the alternative method in order to assessing and rating the new technology to identify the suitable technologies for our scope.
Following this preliminary evaluation, the ATP based detection system was selected as the suitable system. In addition, different pharmaceutical company has successfully validated the rapid Sterility test by using the ATP detection system Celsis and in some cases has yet received the regulatory approval.
To perform a robust validation of the new method a guidance on test procedures and acceptance criteria has primarily come from three documents:

- PDA Technical Report No. 33, Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods
- United States Pharmacopoeia Informational Chapter <1223>, Validation of Alternative Microbiological Methods
- European Pharmacopoeia Chapter 5.1.6, Alternative Methods for Control of Microbiological Quality

Metodologie:
The general aim of the validation was to compare two methodologies (Rapid and compendial sterility method) in order to demonstrate the equivalence based on defined validation parameters.

A guidance on the parameters, the procedures and acceptance criteria has primarily come from three documents:

- PDA Technical Report No. 33, Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods
- United States Pharmacopoeia Informational Chapter <1223>, Validation of Alternative Microbiological Methods
- European Pharmacopoeia Chapter 5.1.6, Alternative Methods for Control of Microbiological Quality

For the implementation of the Rapid alternative method the principal microbiological assay will be used, in particular: Sterility test, microbial growth assay and microbial identification.
In addition, a statical analysis approach will be used to evaluate the obtained results by using statistical tools.

Bibliografia

United States Pharmacopeia -USP 40/NF 35. <1223> Validation of Alternative Microbiological Methods. Rockville, MD: The United States Pharmacopeial Convention, 2017.

Parenteral Drug Association (PDA). Technical Report No.33 (Revised 2013): Evaluation, validation and implementation of alternative and rapid microbiological testing methods. Bethesda, MD: Parenteral Drug Association, Inc.; 2013.

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Gray, JC, Staerk, A, Berchtold, M, Hecker, W, Neuhaus, G, Wirth, A, 2010, 'Growth promoting properties of different solid nutrient media evaluated with stressed and unstressed micro-organisms: Prestudy for the validation of a rapid sterility test', PDA Journal of Pharmaceutical Science and Technology, vol. 64, pp. 249–263

Gordon, O, Gray, JC, Anders, HJ, Staerk, A, Neuhaus, G, Schlaefli, O, 2011, 'Overview of rapid microbiological methods evaluated, validated and implemented for microbiological quality control', European Pharmaceutical Review, vol. 16(2), pp. 9–13.

Miller, MJ, 2012a, 'Case study of a new growth-based rapid microbiological method (RMM) that detects the presence of specific organisms and provides an estimation of viable cell count', American Pharmaceutical Review, vol. 15(2), pp. 18–25.

Lavori pubblicati negli ultimi 5 anni dai docenti richiedenti la borsa:

Informazioni sulla sostenibilità finanziaria della proposta *(fondi per la ricerca, copertura economica del dottorando)*:

Collaborazioni del docente guida con laboratori nazionali ed internazionali rilevanti per questo progetto di ricerca