動物用藥品細菌內毒素重組因子 C 試劑檢驗技術

動物用藥品檢定分所

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摘要

細菌內毒素主成分為脂多醣(lipopolysaccharide, LPS)，為革蘭氏陰性菌細胞壁成分佔外膜 70%左右。在細菌成長階段，內毒素會以微量釋放，當細菌分解或死亡時會大量釋放，其中脂質 A(lipid A)引發動物體免疫反應，產生細胞素、活化凝集反應及補體，造成發熱、低血糖、低血壓或者瀰漫性血管內凝血等症狀。目前「動物用藥品檢驗標準」將細菌內毒素檢驗法(bacterial endotoxins test, BET)列入檢測項目，以確保動物用藥注射劑安全性；動物用藥製造廠除依法檢測細菌內毒素外，廠內應建立風險評估機制，管控原料、生產設備、環境、水質及包材等各項細菌內毒素來源因素，以降低藥品中細菌內毒素限量值(endotoxin limit)。目前各國藥典對於細菌內毒素檢驗法有凝膠法(gel-clot technique)、濁度法(turbidimetric technique)及呈色法(chromogenic technique)等三種，係利用鱟阿米巴變形細胞水性抽提液(amoebocyte lysate)製成之 BET 試劑，與細菌內毒素結合形成凝膠特性，檢測或定量藥品中細菌內毒素含量。由於近年全球鱟數量逐年銳減，國際推動實驗動物 3R 原則，以人工合成重組 C 因子(recombinant factor C, rFC)或重組蛋白試劑(recombinant cascade reagent, rCR)替代

鱟源 BET 試劑，而 2022 年歐洲藥典 10.3 更新版已將細菌內毒素 rFC 試劑檢驗法列入正式方法。本研究為建立動物用藥品細菌內毒素 rFC 試劑檢驗技術，依據歐洲藥典規範執行分析方法驗證，進行 13 件動物用藥品注射劑產品進行細菌內毒素檢測。在預試驗結果方面，0.005-5.0 EU/mL 試驗範圍內之標準曲線相關係數(r)皆大於 0.980，而干擾因子試驗回收率皆介於 50-200%間，皆符合藥典規範。故細菌內毒素 rFC 試劑檢驗可應用於動物用藥品檢測，未來可推廣國內動物用製藥廠使用，期能減少國內對鱟源試劑依賴。

The Recombinant Factor C Assay Technique for Bacterial Endotoxins in Animal Drugs

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Abstract

The main component of bacterial endotoxins is lipopolysaccharide (LPS), which makes up about 70% of the outer membrane of Gram-negative bacterial cell walls. During the bacterial growth stage, endotoxins are released in minute quantities, but when bacteria decompose or die, they are released in large amounts. Lipid A triggers immune responses in animals, producing cytokines, activating clotting reactions and complements, which cause symptoms such as fever, hypoglycemia, hypotension, or disseminated intravascular coagulation. The bacterial endotoxins test (BET) is currently included in the inspection standards for animal drugs to ensure the safety of injections. In addition to legally testing for bacterial endotoxins, domestic animal drug manufacturers should establish a risk assessment mechanism to consider factors such as raw materials, production equipment, environment, water quality, and packaging materials as potential sources of bacterial endotoxins, reduce the endotoxin limit in products. Currently, pharmacopoeias have three methods for bacterial endotoxin testing: gel-clot technique, turbidimetric technique, and chromogenic technique. These methods utilize the BET reagent made from the aqueous extract of horseshoe crab amoebocytes to form a gel characteristic when combined with bacterial endotoxins, allowing for the detection or quantification of bacterial endotoxins. However, due to the global decline in the number of horseshoe crabs and the international promotion of the 3R principle for animal testing, synthetic recombinant Factor C (rFC) or recombinant cascade reagent (rCR) are being used as alternatives to horseshoe crab source BET reagents. The 10.3 version of the European Pharmacopoeia updated in 2022 has officially listed rFC reagent as an official method. This study aims to establish the rFC assay technique for bacterial endotoxins in animal drugs. We followed the European Pharmacopoeia specifications to perform analytical method validation and tested 13 animal injectable products for bacterial endotoxins. In the preliminary test, the correlation coefficient (r) of the standard curve within the 0.005-5.0 EU/mL test range was all greater than 0.980, and the recovery rate of the interference factor test was between 50-200%, all in compliance with pharmacopoeia specifications. Therefore, the rFC assay can be used for testing in animal drugs. It is hoped that it can be promoted for use in domestic animal pharmaceutical factories in the future, reducing our reliance on horseshoe crab sourced reagents.