動物用藥品細菌內毒素重組替代試劑檢驗技術

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摘要

細菌內毒素試驗(bacterial endotoxins test, BET)是無菌製劑關鍵品質管制項目，長期以來多採用鱟試劑(limulus amebocyte lysate, LAL)，惟其涉及生態永續、供應穩定與批間差異之疑慮，促使非動物來源替代技術之建置。重組級聯試劑(recombinant cascade reagent, rCR)以重組 C 因子、重組 B 因子與重組凝固酶原重現 LAL 凝集級聯反應，得以進行 BET 定量。目前藥典採納 rCR 現況不一，美國藥典已於 2025 年生效第 86 章節，以作為第 85 章節的替代試驗，須依一般公告與方法學章節完成方法確效與可比性評估；歐洲藥典目前將 rCR 視為替代方法；日本藥典與中華藥典尚無 rCR 通用章節，採行時同樣要求方法確效與可比性。本研究旨在建立動物用藥品之 rCR 檢測技術，並依藥典原則執行分析方法驗證。細菌內毒素標準品於 0.005、0.05、0.5、5.0 EU/mL 範圍內，標準曲線相關係數(|r|) ≥ 0.980；各濃度重複性變異係數(coefficient of variation, CV)均 ≤10%(實驗室管控標準)。以細菌內毒素標準品 0.5 EU/mL 添加於無熱原水進行干擾性評估，結果以 LAL 濁度法、LAL 動力學呈色法與 rCR 檢測其回收率落在 71.02–84.54%，符合藥典對於回收率 50–200%標準。進一步測試

6 個動物用單株抗體(monoclonal antibody, mAb)品項，在最大有效稀釋度(maximum valid dilution, MVD)下，回收率為 55.97–84.48%，基質干擾性試驗 CV 均 ≤10%，符合藥典及實驗室管控標準。未來研究將擴及不同種類動物用藥無菌製劑產品進行基質干擾性評估研究及方法可比性，以確認 rCR 試驗方法適用於動物用藥品。

Bacterial Endotoxins Testing Using Recombinant Alternative Reagents for Veterinary Medicinal Products

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Abstract

The bacterial endotoxins test (BET) is a critical quality control step for sterile veterinary medicinal products and has long relied on limulus amebocyte lysate (LAL). Concerns regarding ecological sustainability, supply stability, and inter-lot variability, have driven adoption of non-animal alternatives. The recombinant cascade reagent (rCR), comprising recombinant Factor C, recombinant Factor B, and (pro) clotting enzyme to reconstitute the LAL coagulation cascade, enables quantitative BET. Pharmacopeial adoption of rCR in present is heterogeneous: the United States Pharmacopeia (USP) General Chapter <86> (effective 2025) recognizes BET using recombinant reagents as an alternative to Chapter <85>, with implementation requiring method validation and comparability assessment; the European Pharmacopoeia currently treats rCR as an alternative method; the Japanese and Chinese Pharmacopoeias have no rCR general chapter and likewise require validation and comparability when adopted. This study establishes rCR testing techniques for veterinary products and conducts method validation in line with pharmacopeial principles. Within 0.005–5.0 EU/mL of bacterial endotoxin standards, calibration correlations were ($|r|$) ≥ 0.980; repeatability coefficients of variation (CVs) at each level were ≤10% based on laboratory control standard. Interference was evaluated by spiking 0.5 EU/mL endotoxin into endotoxin-free water; positive product control recoveries measured by LAL turbidimetric, LAL kinetic chromogenic, and rCR methods ranged 71.02–84.54%, meet the 50–200% acceptance criterion of the pharmacopoeia. Six veterinary monoclonal antibody products were further tested at the maximum valid dilution (MVD), yielding recoveries of 55.97–84.48% with interference-test CVs ≤10%, were consistent with pharmacopeial expectations and laboratory quality controls. Future work will extend matrix-interference and method-comparability studies to a broader set of sterile veterinary dosage forms to confirm the suitability of rCR for veterinary medicinal products.