撰文：岑祥技術部

PyroSmart NextGen™ 重組蛋白試劑的確效方法

Associates of Cape Cod (ACC) 為了保育鱟魚而研發的重組蛋白試劑 PyroSmart NextGen™ Recombinant Cascade Reagent (PSNG rCR) 較生物性原料具有批次間一致性佳、來源穩定、能避免葡聚醣 (1,3-β-D-Glucans) 交叉污染等等優勢，目前為檢測內毒素的替代方法學，因此終端使用者必須確認此替代方法效能 (Performance) 等同或優於傳統鱟魚血試劑 Limulus Amoebocyte Lysate (LAL)。本研究根據 USP <85>、USP <1085>、USP <1225>以及 ICH Q2 指南設計了 PSNG rCR 的確效方法，是第一篇直接比較 PSNG rCR 和 LAL 試劑在檢測檸檬酸鈉注射藥物 (Sodium Citrate for Injection) 內毒素時的效能並且確認 PSNG rCR 能有效檢測注射藥物內毒素的研究。

驗證方法為三位分析人員分別在數天內使用 ACC 試劑：PSNG rCR、濁度法試劑 Pyrotell®-T LAL、呈色法試劑 Pyrochrome® LAL 各三個批號，檢測三個批號的檸檬酸鈉注射藥物，每次試驗皆為三重複，再依據各方法學標準線所計算出的內毒素濃度，比較分析三種試劑的確效特性 (Validation Characteristics)：線性 (Linearity)、準確度 (Accuracy)、精密度 (Precision)、範圍 (Range)、最低定量濃度 (Quantitation Limit)、專一性 (Specificity) 和耐變性 (Robustness)，其實驗結果為 PSNG rCR、Pyrotell®-T LAL、Pyrochrome® LAL 皆符合允收標準，也代表 PSNG rCR 和 LAL 具有相同的分析性能 (Analytical performance) (表一、表二)。

Table 3. Assessment of PyroSmart NextGen® Method Suitability Compared to Pyrochrome® According to USP <1085>, USP <85>, USP <1225> and the ICH Q2 Guideline (quantitative test for impurities)

Method Suitability Characteristics		PyroSmart NextGen® Results	Pyrochrome® Results	Acceptance Criteria
1. Linearity (absolute value, correlation coefficient)	**0.05-5.0 EU/mL**	0.990	0.994	|r| ≥0.980
2. Accuracy (PPC recovery)	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	50-200%
	0.05 EU/mL	91-138	90-122	
	0.5 EU/mL	122-177	127-186	
	5.0 EU/mL	88-134	115-152	
3. Precision	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	CV ≤30%
3-1 Repeatability (CV)	0.05 EU/mL	1-12	1-9	
	0.5 EU/mL	7-17	7-18	
	5.0 EU/mL	11-19	10-23	
4. Range		**0.05-5.0 EU/mL**	**0.05-5.0 EU/mL**	Precision, accuracy, and linearity at suitable level
5. Quantitation Limit		**At 0.05 EU/mL** Accuracy: 91-138% Repeatability: 1-12%	**At 0.05 EU/mL** Accuracy: 90-122% Repeatability: 1-9%	The lowest concentration of endotoxin that can be quantitatively determined with suitable precision and accuracy
6. Specificity		**Lot 1-3 Samples** Sample Concentration: <1.11 EU/mL Repeatability: 1-19% PPC Recovery: 88-177%	**Lot 1-3 Samples** Sample Concentration: <1.11 EU/mL Repeatability: 1-23% PPC Recovery: 90-186%	For a sample matrix that does not contain endotoxin, the endotoxin concentration is determined as undetected with suitable precision and accuracy (PPC recovery)
7. Robustness				
7-1 Intermediate Precision (95% CI for CV)	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	CV ≤30%
	0.05 EU/mL	9-13	8-11	
	0.5 EU/mL	13-18	13-18	
	5.0 EU/mL	15-20	15-20	

Note: The pH of the mixture of the reagent and sample solution was determined to be between 6.0 and 8.0.

表一 PSNG rCR 和 Pyrochrome® LAL 具有相同的分析性能

Table 4. Assessment of PyroSmart NextGen® Method Suitability Compared to Pyrotell®-T According to USP <1085>, USP <85>, USP <1225> and the ICH Q2 Guideline (quantitative test for impurities))

Method Suitability Characteristics		PyroSmart NextGen® Results	Pyrotell®-T Results	Acceptance Criteria
1. Linearity (absolute value, correlation coefficient)	**0.003-0.3 EU/mL**	0.994	0.996	|r| ≥0.980
2. Accuracy (PPC recovery)	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	50-200%
	0.003 EU/mL	82-128	97-168	
	0.03 EU/mL	107-171	158-199	
	0.3 EU/mL	94-129	110-133	
3. Precision				
3-1 Repeatability (CV)	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	CV ≤30%
	0.003 EU/mL	3-21	1-27	
	0.03 EU/mL	2-17	1-17	
	0.3 EU/mL	1-11	3-14	
4. Range		**0.003-0.3 EU/mL**	**0.003-0.3 EU/mL**	Precision, accuracy, and linearity at suitable level
5. Quantitation Limit		**At 0.003 EU/mL** Accuracy: 82-128% Repeatability: 3-21%	**At 0.003 EU/mL** Accuracy: 97-168% Repeatability: 1-27%	The lowest concentration of endotoxin that can be quantitatively determined with suitable precision and accuracy
6. Specificity		**Lot 1-3 Samples** Sample Concentration: <1.11 EU/mL Repeatability: 1-21% PPC Recovery: 82-171%	**Lot 1-3 Samples** Sample Concentration: <1.11 EU/mL Repeatability: 1-27% PPC Recovery: 97-199%	For a sample matrix that does not contain endotoxin, the endotoxin concentration is determined as undetected with suitable precision and accuracy (PPC recovery)
7. Robustness				
7-1 Intermediate Precision (95% CI for CV)	**Spiked Sample**	**Min-Max (%)**	**Min-Max (%)**	CV ≤30%
	0.003 EU/mL	13-18	13-18	
	0.03 EU/mL	14-19	7-10	
	0.3 EU/mL	8-11	7-10	

Note: The pH of the mixture of the reagent and sample solution was determined to be between 6.0 and 8.0.

表二 PSNG rCR 和 Pyrotell®-T LAL 具有相同的分析性能

當原本使用 LAL 的用戶想要導入 PSNG rCR，本研究的比較分析確效方法可以做為應用於自身產品的範例。

原廠完整文章：

Madeline Kelley, etc., A Demonstration of the Validation Process for Alternative Endotoxin Testing Methods Using PyroSmart NextGen® Recombinant Cascade Reagent, BPB Reports, Vol. 6 No. 2 p.68-75, 2023

PSNG介紹：

PyroSmart NextGen™-你還在用鱟魚試劑偵測內毒素嗎