重组C因子的法规变化和全球验证策略简介

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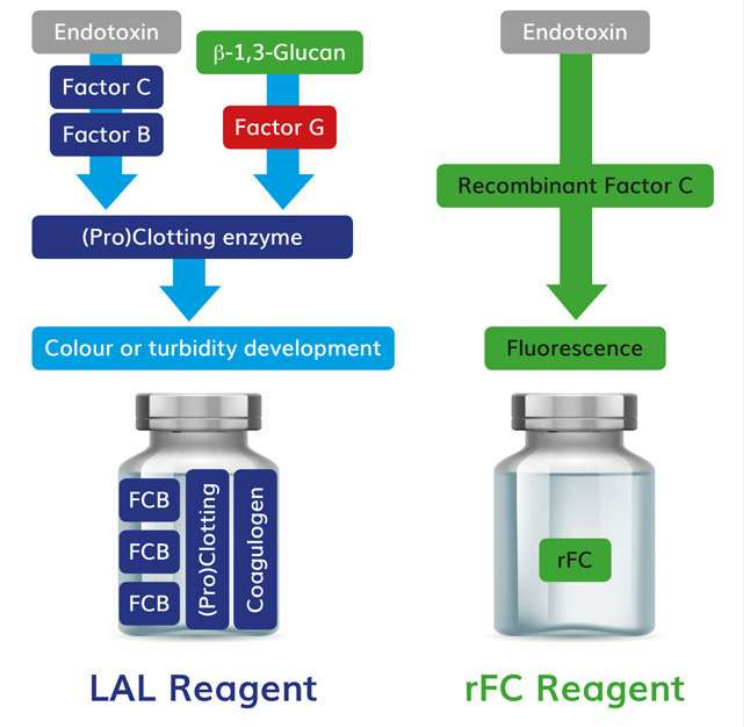
2025年04月16日

重组C因子的法规变化

	热原检查		内毒素检查		
	热原检查法	**单核细胞活化反应测定法（monocyte activation test, MAT)**	**内毒素检查法**	**rFC**	**指导原则**
《中国药典》	1142 热原检查法	*9301 注射剂安全性检查法应用指导原则* *附：单核细胞活化反应测定法（monocyte activation test, MAT)*	1143 细菌内毒素检查法	*9251 细菌内毒素检查法应用指导原则* *附：重组C 因子法*	9301 注射剂安全性检查法 应用指导原则 9251 细菌内毒素检查法应用指导原则
欧洲药典	2.6.8. Pyrogens *EDQM修订讨论中*	2.6.30. Monocyte-activation test	2.6.14. Bacterial endotoxins	2.6.32. Test for bacterial endotoxins using recombinant factor C	5.1.10. Guidelines for using the test for bacterial endotoxins 5.1.13. Pyrogenicity
美国药典	⟨151⟩ Pyrogen Test	/	⟨85⟩ Bacterial Endotoxins Test	⟨86⟩ Bacterial Endotoxins Test Using Recombinant Reagents *(Commenting Closed)*	⟨1085⟩ Guidelines On Endotoxins Test

重组C因子的法规变化



- 欧盟于2010年通过新的指令Directive 2010/63/EU，明示了保护科研目的用动物的立场
- EP 5.2.8章节《降低将动物海绵状脑病病原体通过人和兽药产品传播的风险》中也列明了“使用非动物来源的材料更加值得倡导”

重组C因子的法规变化

1971	1987	2010	2020
2.6.8. Pyrogens	**2.6.14. Bacterial endotoxins**	**2.6.30. Monocyte-activation test**	**2.6.32. Test for bacterial endotoxins using recombinant factor C**



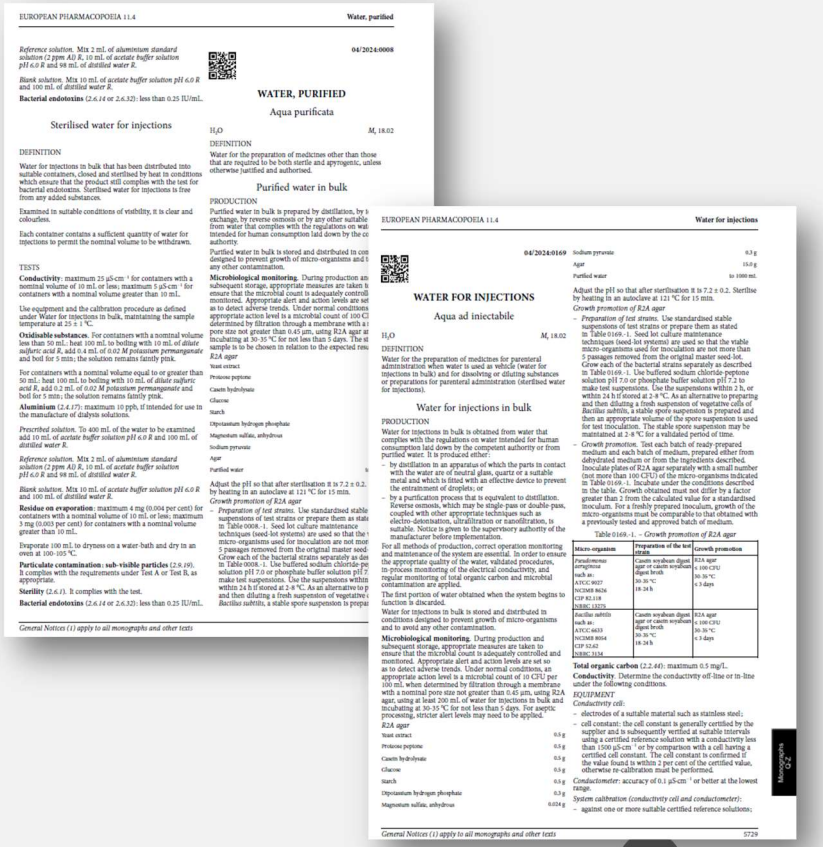
- The conference aimed to show how the European Pharmacopoeia intends to **remove the RPT from its texts by 2026**, facilitate the use of MAT, and identify gaps in the suppression of RPT. 3Rs concept of Reduction, Replacement and Refinement of laboratory animal use.
- If the presence of NEP can be excluded, BET (rFC or LAL) is sufficient. If the presence cannot be excluded, MAT is the appropriate method. This assessment can be achieved by using both MAT and BET methods for the products in scope.

The future of pyrogenicity testing: Phasing out the rabbit pyrogen test. A meeting report. 2023

EP: Supressed in accordance with the pyrogenicity strategy for removing the rabbit pyrogen test (RPT) from the Ph. Eur. Suppression of general chapter 2.6.8 Pyrogens from the Ph. Eur. on 1 January 2026 (Issue 12.1)".

重组C因子的法规变化

- 已采用rFC的章节：
  - Parenteral preparations
  - Gene therapy medicinal products for human use
  - 5.34. Additional information on gene therapy medicinal products for human use
  - 2024年已逐步开始出现各论中对rFC的援引
    - 4月1日11.4版本生效，制药用水已开始
      - 0008.纯化水
      - 0169.注射用水
- 2024年4月4日，发布Pharmeuropa 36.2
  - 5.32. Cell-based preparations for human use，细胞基制剂
  - 5.36. mRNA vaccines for human use，人用mRNA疫苗
  - 5.37. Recombinant viral vectored vaccines for human use，人用重组病毒载体疫苗
  - 5.39. mRNA substances for the production of mRNA vaccines for human use，生产用mRNA基质
  - 5.40. DNA template for the preparation of mRNA substances，mRNA基质的DNA模板



EUROPEAN PHARMACOPOEIA 11.4 — Water, purified

04/2024:0008

WATER, PURIFIED

Aqua purificata

Sterilised water for injections

Purified water in bulk

EUROPEAN PHARMACOPOEIA 11.4 — Water for injections

04/2024:0169

WATER FOR INJECTIONS

Aqua ad iniectabile

Water for injections in bulk

重组C因子的法规变化

FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers, 2012

5. May a firm use alternative assays to those in the USP for a compendial article?

- Yes, firms may use alternative methods and/or procedures if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, and in other special circumstances. Such alternative procedures and methods should be validated as described in the USP General Chapter <1225>, Validation of Compendial Procedures, and should be shown to achieve equivalent or better results. When a difference appears or in the event of a dispute, the final decision is made based upon the USP compendial gel clot method unless otherwise indicated in the monograph for the product being tested.
- Below are two examples of alternative assays.
- (1) **Recombinant Horseshoe Crab Factor C Assay**
- If a manufacturer chooses to use a recombinant factor C-based assay, then method validation should be in accordance with the requirements of USP Chapter <85>, Bacterial Endotoxins Test, as described in the section for Photometric Quantitative Techniques, and **USP Chapter <1225>, Validation of Compendial Procedures**.
- (2) Monocyte Activation Type Pyrogen Test
- Product-specific validation is necessary to establish whether a particular test substance or material is appropriate for evaluation of the monocyte activation method. The validation should include, but is not limited to, interference testing, accurate detection of pyrogen in individual test samples, and, for devices, ability of test system to provide direct contact to the monocytes.

重组C因子的法规变化

FDA POSITION

- The FDA supports the use of alternative tests for detection of pyrogens.
- Equivalence between the compendial and the alternative test should be demonstrated as per 21 CFR 610.9.
  - rFC should demonstrate equivalence to the LAL-BET for detection of endotoxins and suitability for the intended use as per USP <85>
  - MAT should demonstrate equivalence to the RPT for detection of endotoxin and non-endotoxin pyrogens.
- Validation may include a combination of non-product-specific validation and additional product-specific validation; the user may rely in non-specific validation conducted by the vendor or published in peer-reviewed literature.

FDA PERSPECTIVE ON RECOMBINANT ENDOTOXIN DETECTION SYSTEMS, 2021

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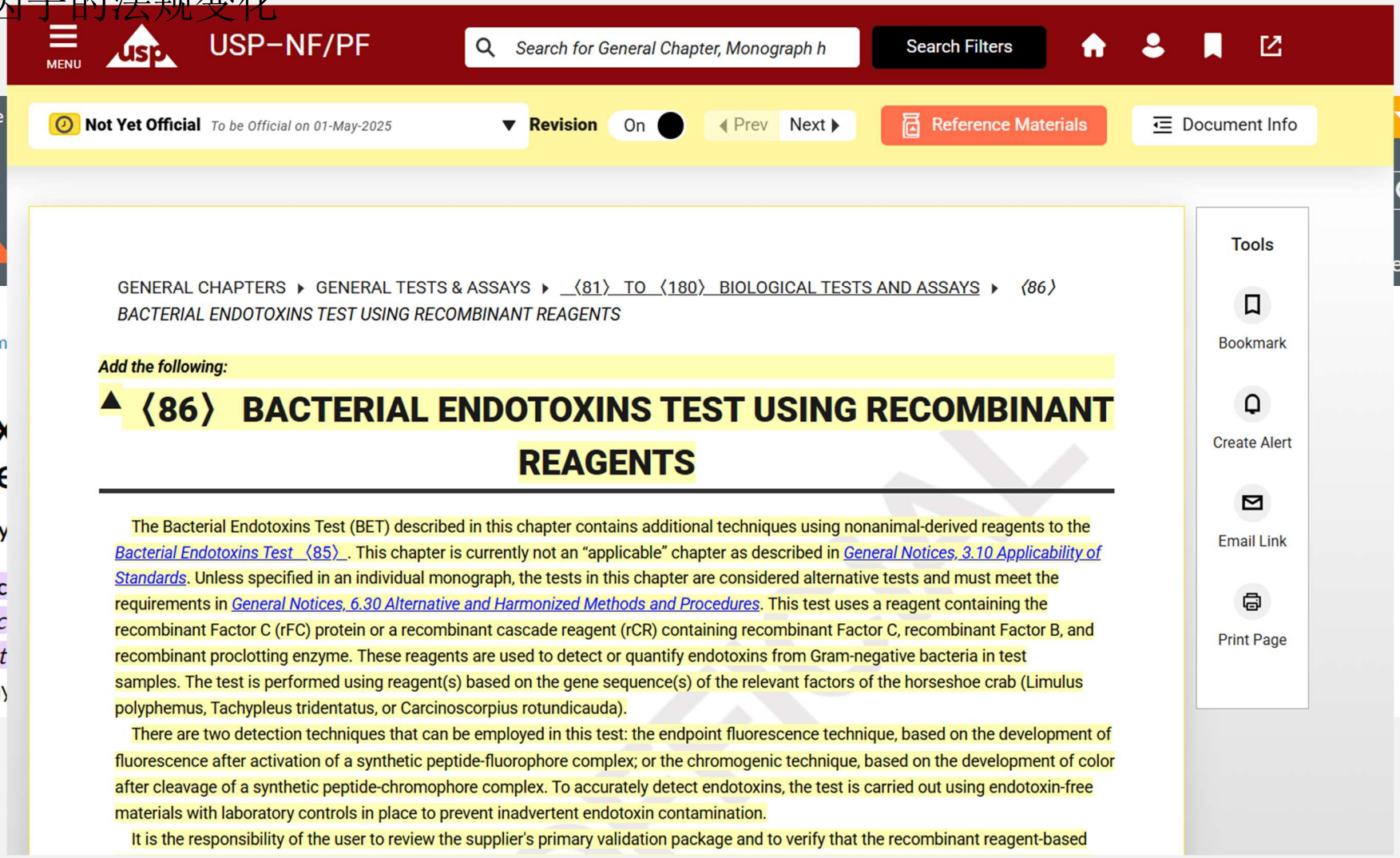
MENU
USP–NF/PF
Search for General Chapter, Monograph h
Search Filters
Not Yet Official To be Official on 01-May-2025
Revision On
Prev Next
Reference Materials
Document Info
Global Presence
GENERAL CHAPTERS ▸ GENERAL TESTS & ASSAYS ▸ ⟨81⟩ TO ⟨180⟩ BIOLOGICAL TESTS AND ASSAYS ▸ ⟨86⟩
BACTERIAL ENDOTOXINS TEST USING RECOMBINANT REAGENTS
Add the following:
⟨86⟩ BACTERIAL ENDOTOXINS TEST USING RECOMBINANT REAGENTS
The Bacterial Endotoxins Test (BET) described in this chapter contains additional techniques using nonanimal-derived reagents to the Bacterial Endotoxins Test ⟨85⟩. This chapter is currently not an "applicable" chapter as described in General Notices, 3.10 Applicability of Standards. Unless specified in an individual monograph, the tests in this chapter are considered alternative tests and must meet the requirements in General Notices, 6.30 Alternative and Harmonized Methods and Procedures. This test uses a reagent containing the recombinant Factor C (rFC) protein or a recombinant cascade reagent (rCR) containing recombinant Factor C, recombinant Factor B, and recombinant proclotting enzyme. These reagents are used to detect or quantify endotoxins from Gram-negative bacteria in test samples. The test is performed using reagent(s) based on the gene sequence(s) of the relevant factors of the horseshoe crab (Limulus polyphemus, Tachypleus tridentatus, or Carcinoscorpius rotundicauda).
There are two detection techniques that can be employed in this test: the endpoint fluorescence technique, based on the development of fluorescence after activation of a synthetic peptide-fluorophore complex; or the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromophore complex. To accurately detect endotoxins, the test is carried out using endotoxin-free materials with laboratory controls in place to prevent inadvertent endotoxin contamination.
It is the responsibility of the user to review the supplier's primary validation package and to verify that the recombinant reagent-based
Tools
Bookmark
Create Alert
Email Link
Print Page

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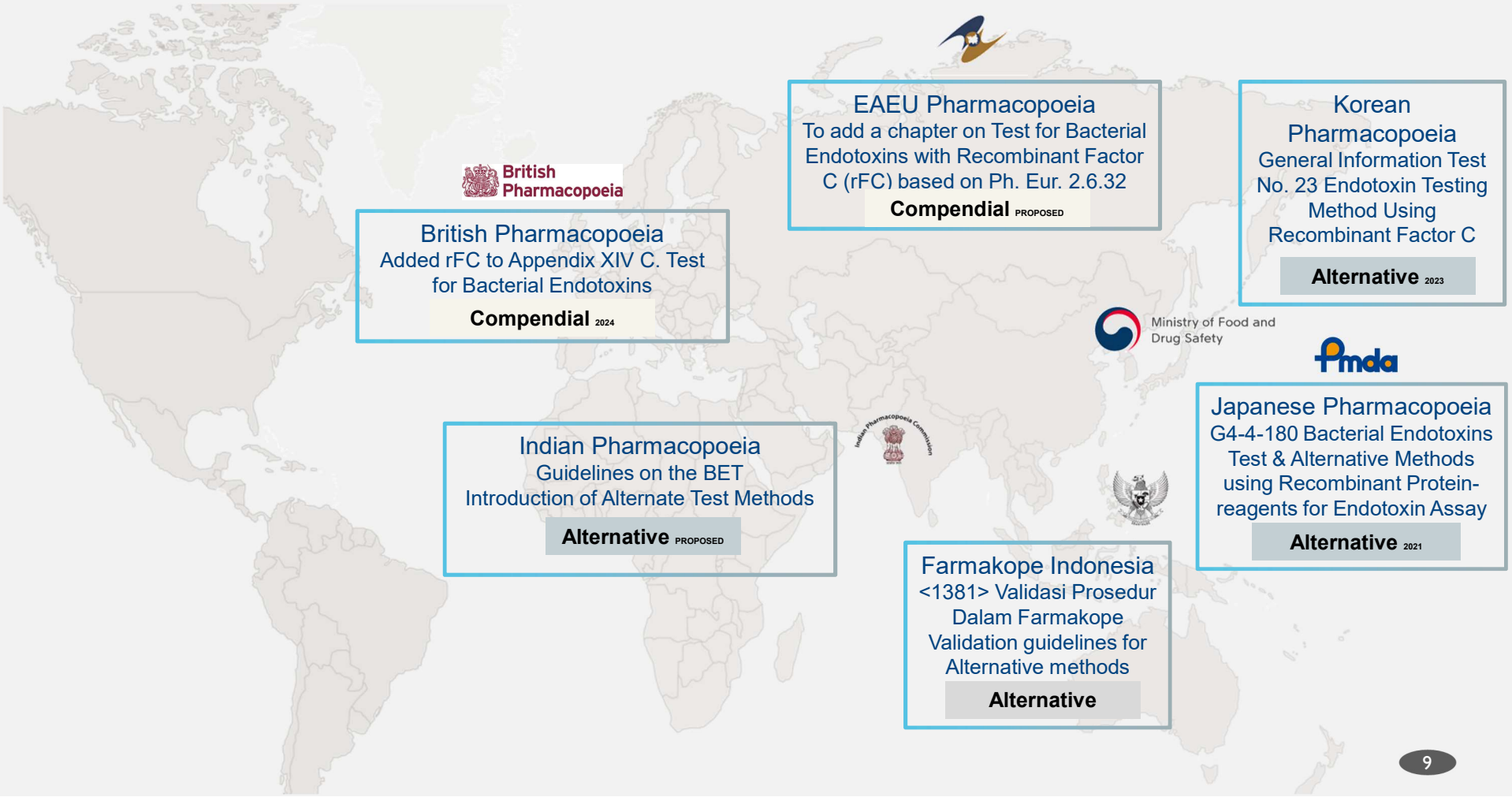


EAEU Pharmacopoeia
To add a chapter on Test for Bacterial Endotoxins with Recombinant Factor C (rFC) based on Ph. Eur. 2.6.32
Compendial PROPOSED

Korean Pharmacopoeia
General Information Test No. 23 Endotoxin Testing Method Using Recombinant Factor C
Alternative 2023

British Pharmacopoeia
Added rFC to Appendix XIV C. Test for Bacterial Endotoxins
Compendial 2024

Japanese Pharmacopoeia
G4-4-180 Bacterial Endotoxins Test & Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay
Alternative 2021

Indian Pharmacopoeia
Guidelines on the BET Introduction of Alternate Test Methods
Alternative PROPOSED

Farmakope Indonesia
<1381> Validasi Prosedur Dalam Farmakope Validation guidelines for Alternative methods
Alternative

重组C因子的全球验证策略

PDA

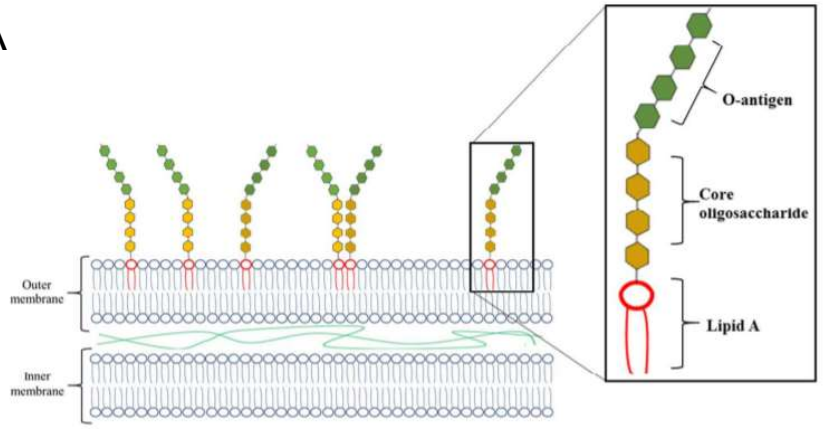
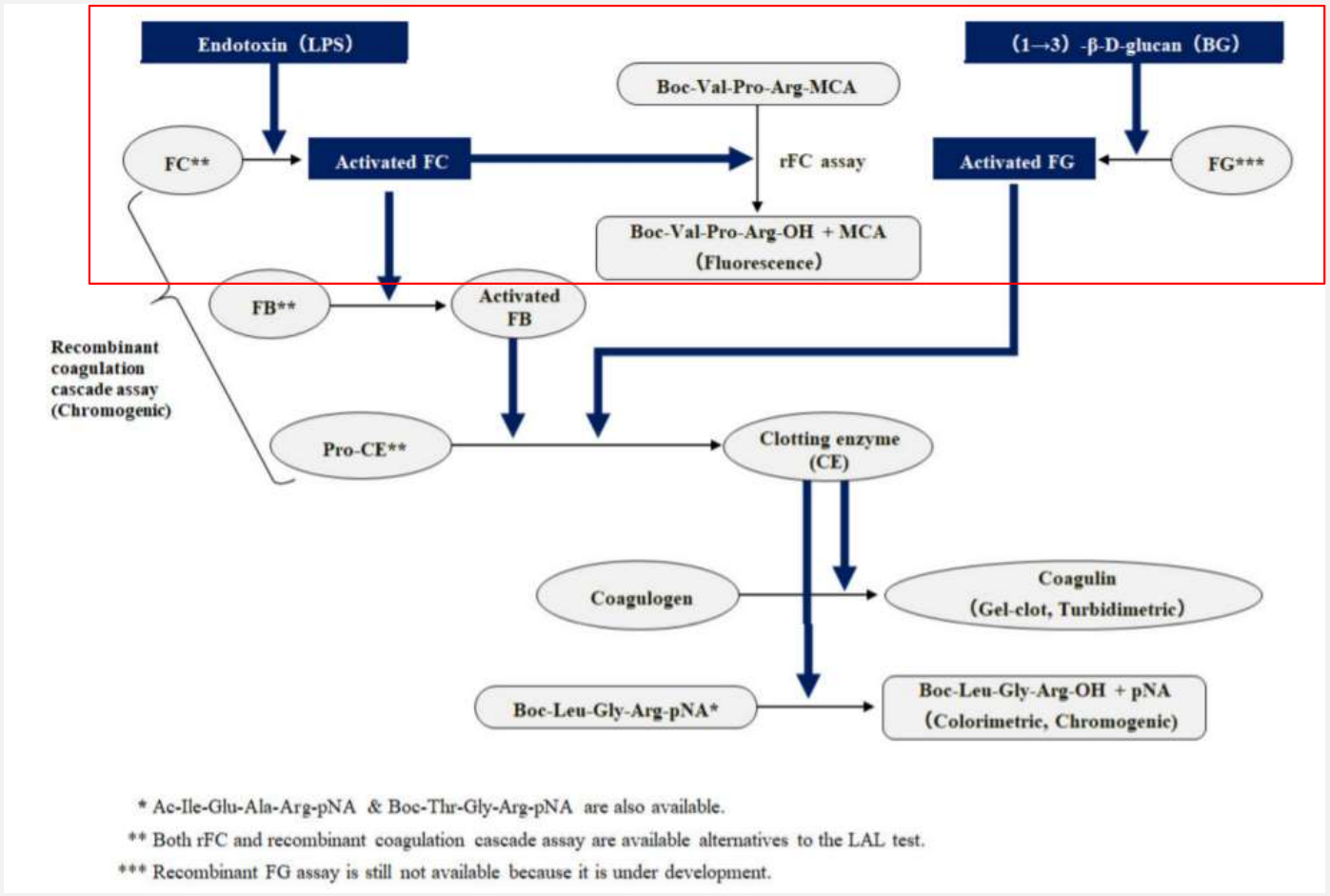


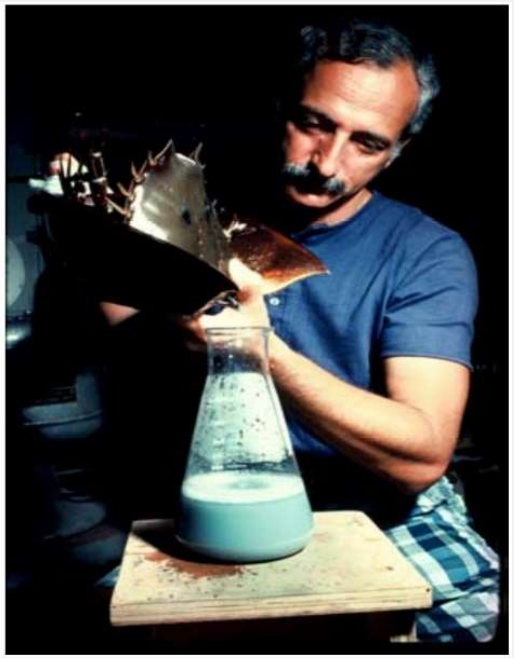
Figure 1. Structure of lipopolysaccharide (LPS).
LPS is found on the cell wall of gram-negative bacteria, such as Escherichia coli. The lipid A region, depicted in red, elicits the immune response.

《中国药典》1143 细菌内毒素检查法

- 本法系利用鲎试剂来检测或量化由革兰阴性菌产生的细菌内毒素，以判断供试品中细菌内毒素的限量是否符合规定的一种方法。

重组C因子的全球验证策略





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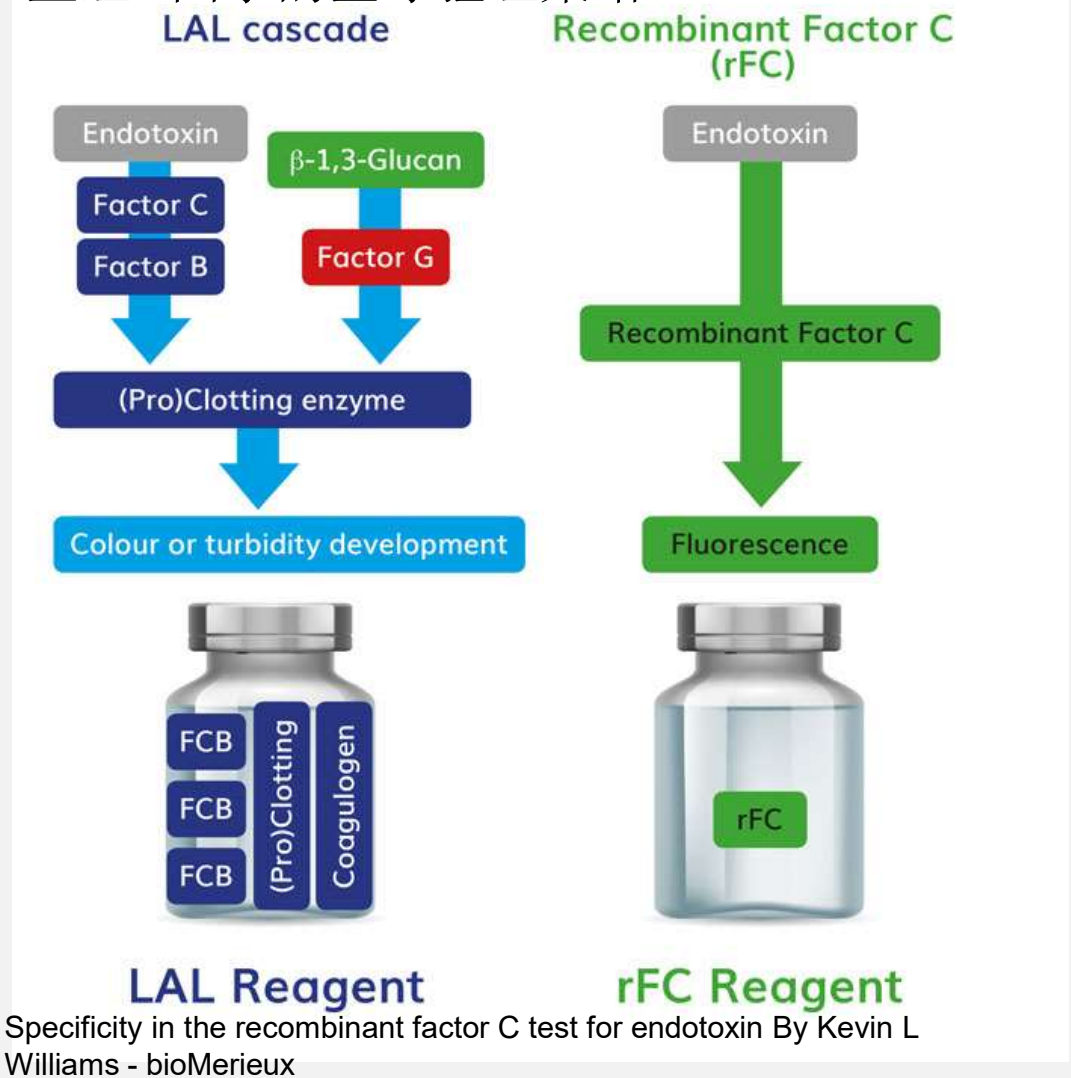
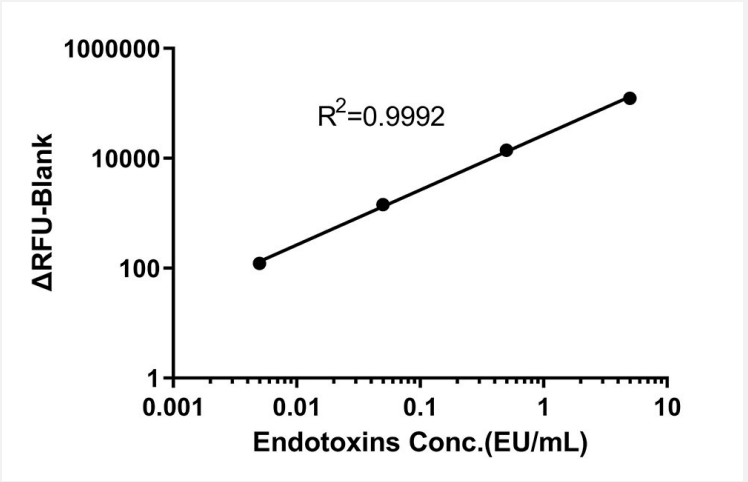




Specificity in the recombinant factor C test for endotoxin By Kevin L Williams - bioMerieux

重组C因子的全球验证策略

《中国药典》9251 细菌内毒素检查法应用指导原则

115 **重组 C 因子法**

116 C 因子是鲎试剂中对细菌内毒素敏感的蛋白，能够选择性识别内毒素。重
117 组 C 因子是一种人工合成的 C 因子，它被细菌内毒素活化后，可与荧光底物
118 作用产生与内毒素浓度成比例的荧光信号。

119 本法系依据反应混合物中的内毒素浓度和其孵育终止时的荧光值之间存
120 在量化关系来测定细菌内毒素的含量。本法为终点荧光法。依据检测原理，
121 本法不存在 G 因子旁路干扰，具有较高的专属性，因此适合于含有 β 葡聚糖
122 干扰的样品检测；本法所用试剂不含有 B 因子和凝固酶原、凝固蛋白原等，
123 因此含有对上述物质抑制或增强作用的样品适合使用重组 C 因子法。

124 重组 C 因子法试验需采用荧光酶标仪，其激发和发射波长等参数参照试
125 剂的使用说明书，激发/发射波长一般为：380nm/440nm，检测温度一般为 37℃
126 ±1℃。

127 仪器灵敏度（增益值）调节、重组 C 因子试剂的配制方法、保温时间等，
128 参照所用仪器和试剂的有关说明进行。

129 **标准曲线的可靠性试验、干扰试验、检查法以及结果判断** 参照细菌内毒

130 素检查法（通则 1143）中的~~方法 2~~ 光度~~法~~检测技术。

《中国药典》凡例

检验方法和限度

二十一、本版药典品种正文收载的所有品种，均应按规定的方法进行检验。采用药典规定的方法进行检验时，应对方法的适用性进行确认。如采用其他方法，应进行方法学验证，并与规定的方法比对，根据试验结果选择使用，但应以本版药典规定的方法为准。

重组C因子的全球验证策略

USP<1085>

Primary validation: Product independent + Suitability testing: Product specific

- ALTERNATE TEST METHODS
- The methods listed in ⟨85⟩ for the detection of bacterial endotoxins (gel-clot limits test, gel-clot assay, kinetic chromogenic, endpoint chromogenic, kinetic turbidimetric) are considered to be validated. However, a laboratory may choose to use an assay methodology that is not listed in ⟨85⟩. If such a choice is made, the alternate test for the detection of bacterial endotoxins must be fully validated to ensure that decisions made using the alternate methodology are equivalent to or better than decisions made using the validated USP methods and ultimately approved by the appropriate regulatory authority. Although endotoxin testing is not specifically cited, guidance on how to think about the validation of alternate methods can be found in Validation of Alternative Microbiological Methods ⟨1223⟩ and Validation of Compendial Procedures ⟨1225⟩.

⟨86⟩ Bacterial Endotoxins Test Using Recombinant Reagents. This proposed new test chapter provides additional techniques using nonanimal derived reagents to the *Bacterial Endotoxins Test* ⟨85⟩.

This general chapter is not currently being introduced into a specific monograph or listed in *General Notices*. It is the responsibility of the user to review the supplier's primary validation package and to verify product suitability for use in testing specific products or materials. This verification must include specific experiments to confirm that the method is suitable for its intended purpose under the conditions of use for the material, drug substance, and/or drug product. The selected verification experiments should be based on an assessment of the complexity of the material to which the method is being applied. The user should refer to *Verification of Compendial Procedures* ⟨1226⟩. Regulatory authorities may require supplemental data prior to acceptance. An example of supplemental data may include a comparative study of the material tested by techniques described in this chapter and those in ⟨85⟩.

重组C因子的全球验证策略

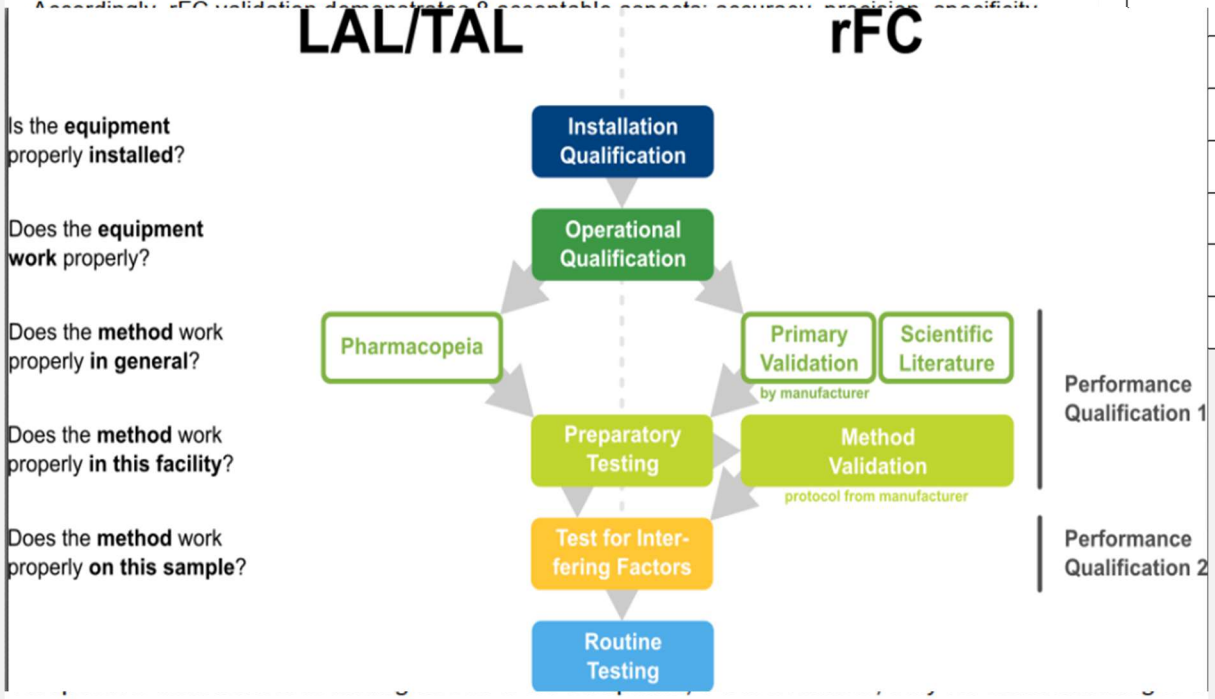
USP<1225>药典方法的验证

Table 2. Data Elements Required for Validation

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	[a]	[a]	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	[a]	Yes
Detection limit	No	No	Yes	[a]	No
Quantitation limit	No	Yes	No	[a]	No
Linearity	Yes	Yes	No	[a]	No
Range	Yes	Yes	[a]	[a]	No

[a] May be required, depending on the nature of the specific test.

What about the authorities? The FDA and the EDQM have both strongly recognized these advantages and equivalency. They both published guidance that specifically mentioned rFC assays for BET (6),(7). The FDA's 2012 Q&A guidance document refers to USP chapter <1225> Validation of Compendial Procedures (2) (ICH Q2(R1)(8)) to demonstrate the desired validation parameters.



Method Suitability on three lots of product, i.e. investigation of inhibition/enhancement (compendial Test for Interfering Factors).

With the aforementioned comparative studies and some Preparatory Testing, rFC can be quickly validated and working as intended in a given laboratory.

rFC manufacturers may support rFC users by supplying Primary Validation reports as well as ready-to-fill-out protocols and worksheets. Thus, Method Validation and Suitability testing becomes a straight-forward process (see Figure 1). Adding hardware and software Installation and Operational Qualification (IQ, OQ), Preparatory Testing and operator training – the same as required for LAL – rFC establishment can take just 5 days in the laboratory.

Table 1. Typical Analytical Characteristics Used in Method Validation

Accuracy
Precision
Specificity
Detection limit
Quantitation limit
Linearity
Range
Robustness

方法验证：

- 替代方法验证？药典方法确认？
- 验证参数（USP<1225>）？
- 不同类型内毒素的检测能力？
- 是否需要和LAL方法比对？
- 样品对检测结果的干扰？

重组C因子的全球验证策略

Validation Strategy for New Recombinant Factor C Users

Monday, February 28, 2022

Evelyn Der, Senior Scientist, Roche Genentech, QC Technology Innovation and Implementation Group, South San Francisco, CA, USA.

Carmen Marin, QC Scientist Microbiology, F. Hoffmann La Roche Ltd, Kaiseraugst, Switzerland.

Viviane Grunert da Fonseca, PhD, Non-clinical Statistician, Roche, MSAT (Manufacturing, Science and Technology), Penzberg, Germany.

Lindsey Silva, PhD, QC Director, Roche Genentech, Microbiology, South San Francisco, CA USA.

Introduction

This paper presents Roche's validation strategy of the recombinant factor C (rFC) endotoxin testing method. The validation approach uses statistical non-inferiority hypothesis testing based on spiked samples with different concentrations of commercial endotoxin standard. This approach is different from published literature studies while in accordance with the current regulatory framework and streamlines laboratory work. The validation activities are ongoing at the time of this publication at a Roche pilot laboratory. As the validation progresses, validation activities are to be continued at the other Roche global network QC operations and applied for water and product endotoxin testing.

重组C因子

Table 1A. POC: Recommended rFC Vendor Reagents

Evaluated endotoxin recovery results with LAL KCA assay and rFC vendor kits with vendor recommended plate readers.

- RSE 4-Point Standard Curve (0.005 to 5.0 EU/mL)
- 0.5 EU/mL RSE PPC
- Two testers

Two Testers (Tester 1/Tester 2)	LAL KCA Vendor A		Vendor A rFC Reagents		Vendor B rFC Reagents	
	% Endotoxin Recovery[2]	% PPC[2]	% Endotoxin Recovery[2]	% PPC[2]	% Endotoxin Recovery[2]	% PPC[2]
Monocloral Antibody #1	72/55	91/93	83/61	150/135	59/47[1]	88/105
Monocloral Antibody #2	102/93	97/113	135/130	171/153	82/82	90/103
Monocloral Antibody #3	96/86	90/98	138/104	156/149	86/65	85/94
LAL WATER	120/144	79/113	129/149	116/134	100/100	106/95

[1]Expected 50 to 200% PPC and spiked endotoxin recoveries met except for reading performed with Vendor B reader. Likely related to equipment set-up.
[2]First reported value is from Tester 1. Second reported value is from Tester 2.

Acceptable standard curves, spiked RSE PPC recoveries of 50-200%, and coefficient of variation (≤ 25%; </= 10%) were observed for samples tested by LAL and rFC test methods, respectively.

Table 1B. POC: Interchanged rFC Vendor Reagents and Readers

Evaluated endotoxin recovery results with LAL KCA assay and interchanged rFC vendor kits with vendor plate readers.

- Same instructions as in Table 1A, but switching vendor reagents on alternative vendor's readers. Readers settings were in accordance to reagent vendor's instructions but on alternative vendor's reader for emission wavelength.

Two Testers (Tester 1/Tester 2)	LAL KCA Vendor A		Vendor A rFC Reagents		Vendor B rFC Reagents	
	% Endotoxin Recovery[2]	% PPC[2]	% Endotoxin Recovery[2]	% PPC[2]	% Endotoxin Recovery[2]	% PPC[2]
Monocloral Antibody #1	73/73	105/93	54/56	96/103	43/43[1]	71/76
Monocloral Antibody #2	91/113	106/103	77/85	95/111	118/120	141/132
Monocloral Antibody #3	89/121	103/93	74/88	95/96	97/107	117/106
LAL WATER	124/158	101/106	101/117	95/115	111/124	90/93

[1]Expected 50 to 200% PPC and spiked endotoxin recoveries most except for 2 readings performed with Vendor B reader that were likely related to equipment set-up.
[2]First reported value is from Tester 1. Second reported value is from Tester 2.

Acceptable standard curves, spiked RSE PPC recoveries of 50-200%, and coefficient of variation (≤ 25%) were observed for samples tested by LAL and rFC test methods. Results from both rFC vendor kits on one vendor's fluorescence plate reader gave comparable results to the KCA LAL assay.

重组C因子的全球验证策略

Table 1C. POC: Spiked Endotoxin Recovery Over Time

Evaluated endotoxin recoveries from spiked RSE in biologic drug product using rFC reagents for masking during storage

- Determined non-inhibitory dilution of biologic drug product using rFC.
- Spike drug product and LAL Reagent Water control to concentration of 2 EU/mL with RSE.
- Test using rFC reagents from Vendor A at Time 0. Store spiked sample at 20-25°C. Repeat test using rFC at Days 1 and 5.

	Time Points (Days)	Dilution	Sample EU/mL	Sample Spike Recovery (%)	PPC Reserved EU/mL	% PPC
Spiked 2 EU/mL RSE Drug Product (T0)	0	100	1.88	94	0.567	110
	1		2.95	148	0.652	125
	5		2.74	137	0.702	135
Spiked 2 EU/mL RSE Water Control (T0)	0	100	2.05	103	0.467	89
	1		1.96	98	0.441	84
	5		1.85	93	0.478	92

Spiked endotoxin recoveries calculated against the theoretical spike of 2 EU/mL and corresponding %PPC met expected spike recovery of 50-200% at T0, T1, and T5 in the drug product and water control.

POC:

- rFC能够更好的克服beta-glucans引起的假阳性
- rFC未出现LER

重组C因子的全球验证策略

Table 2. General Validation Risk Assessment

Category	Example of potential risk identified	Risk reduction measure
Sample & reagent volume	Concentration of rFC Enzyme is not optimal	No gap identified
rFC Enzyme performance	rFC Enzyme cannot be activated by endotoxins	No gap identified
Sample & reagent handling	Reagents (fluorescence substrate, rFC enzyme, buffer) do not perform as expected after opening, storage at 2 - 8°C and mixing with freshly opened vials	Open rFC reagent hold time test
	Additional reagents used (e.g. Tris-buffer, Pyrosperse) are not compatible with rFC	Compatibility check with Tris-buffer
Fluorescence & standard curve	Sensitivity setting is not correct (varies over time)	No gap identified
Result precision	The plate reader affects obtained results	No gap identified
Result accuracy	False negative/positive results are obtained/recovery of spiked endotoxin is below 50% or above 200%	No gap identified
Other	Response to different endotoxin sources	No gap identified
	Response to beta glucans	No gap identified
	Single Source-Supply of rFC Reagents	Additional supplier to be validated in future. Traditional Bacterial Endotoxin Test using LAL-reagent will be used as backup.

方法验证的风险评估：

- 评估了rFC方法的超过40个风险因子
- 绝大多数都是低风险，无需采取风险缓解措施，极少数需要验证，如试剂开瓶有效期、检测用试剂的信号增强或抑制
- 方法适用性试验、PPC、实验室的来料检测（标准曲线可靠性）可以缓解这些风险

重组C因子的全球验证策略

Product Independent验证

Table 3. General rFC Method Validation (Performed at Roche)

Attribute	Evaluation/Risk Assessment	Required by	References
Linearity	These parameters were not evaluated as separate attributes in the validation because: • Risk Assessment: Low risk based on literature review • Standard curve is performed for each plate • Existing controls on incoming reagents	USP <1225> & <1223>	1, 3, 9
Range			1, 3
Limit of Quantitation			1, 3
Specificity	This parameter was not evaluated separately in the validation because this was assessed as low risk based on a literature review (different endotoxin sources & beta glucans and reported endotoxin data for accuracy and precision)	USP <1225> & <1223>	3, 5, 7, 8, 11, 12, 14
Robustness	Risk Assessment: Low to medium risks	USP <1225> & <1223>	2, 3, 6, 9, 10, 11, 12, 13, 14
Accuracy	Risk Assessment: Low risk based on Literature Review. The evaluation for all three attributes involved: • Methods: rFC & LAL • Sample Type: PW & WFI (endotoxin free) • Endotoxin Standard: RSE Data analysis: Statistical hypothesis test (i.e. confidence interval used for acceptance criteria)	USP <1225> & <1223>	2, 3, 6, 9, 12, 13, 15
Precision		USP <1225> & <1223>	2, 3, 9, 10, 11, 12
Equivalence [a]		FDA Guidance 2012 (& USP <1223>)	2, 4, 5

Parameters bolded were evaluated as separate attributes in the validation.
[a]Not verified on its own but rather with respect to parameters accuracy and precision.

数据来自于公开的数据

缺乏具有统计学代表性数据证明方法的一致性，且一致性不能单独评估

重组C因子的全球验证策略

Product Independent验证

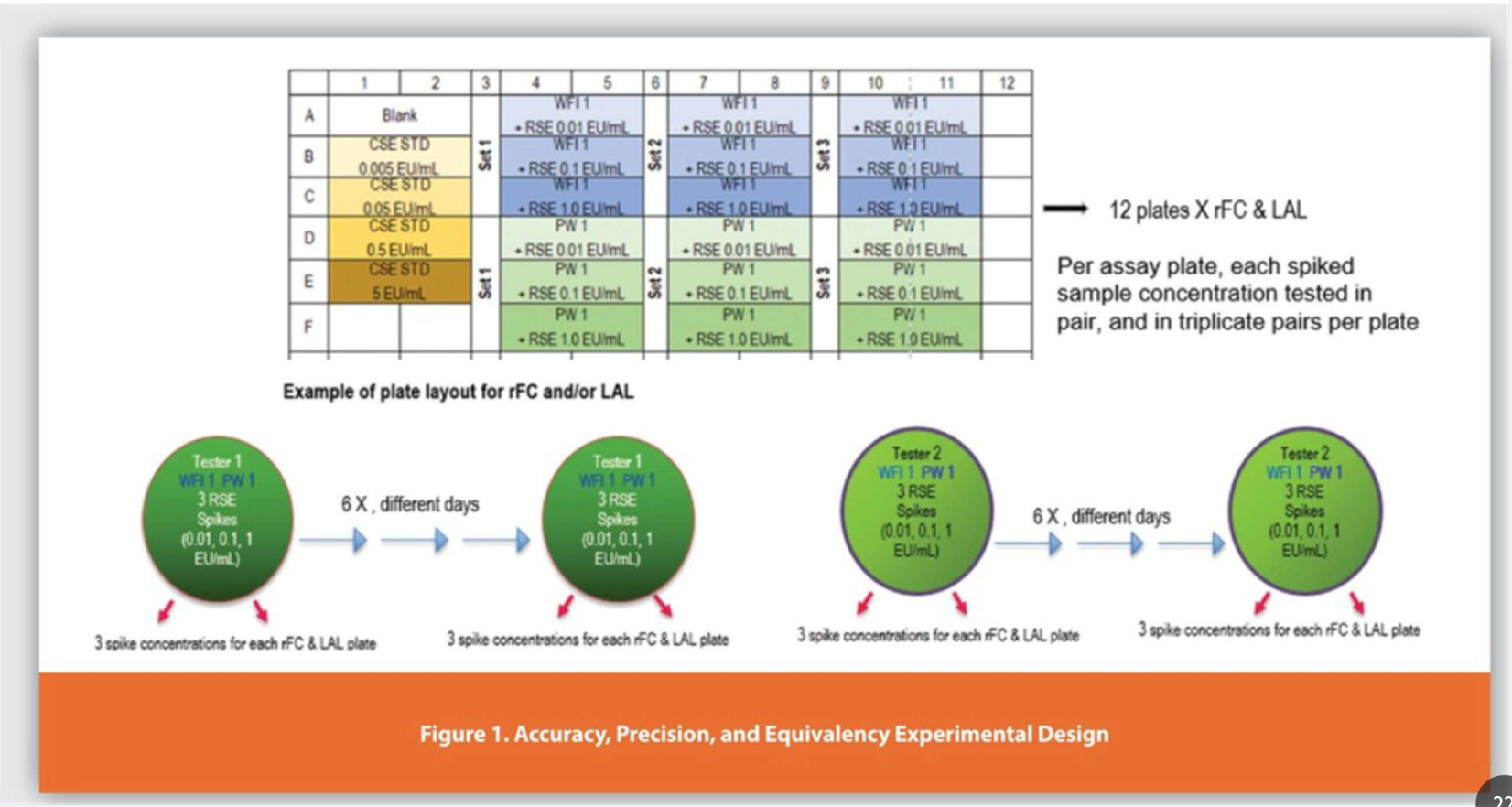


Figure 1. Accuracy, Precision, and Equivalency Experimental Design

重组C因子的全球验证策略

Product Independent验证

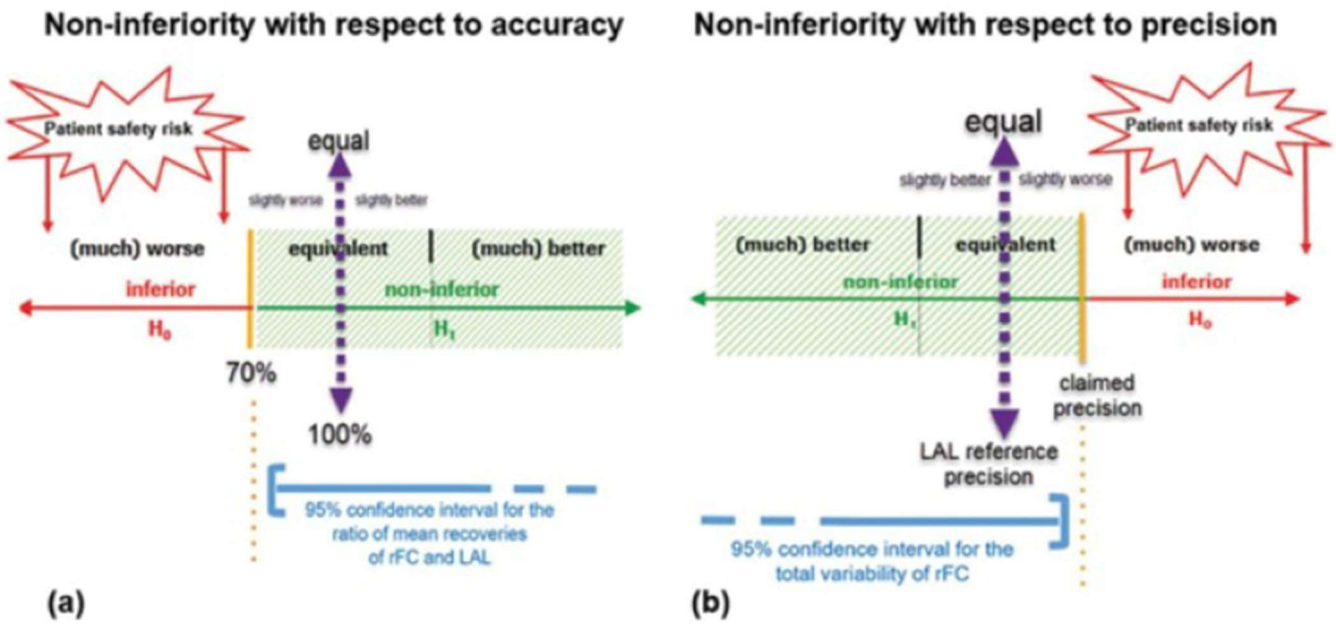


Figure 2. Illustrated Hypothesis Test Problems for Equivalence: Accuracy and Precision

重组C因子的全球验证策略

Product Specific验证 - MST

2.6.32. Test for bacterial endotoxins using recombinant factor C

Table 2.6.32.-1

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of replicates
A	None	Test solution	Not less than 2
B	Middle concentration of the standard curve	Test solution	Not less than 2
C	At least 3 concentrations (lowest concentration is designated λ)	Water for BET	Each concentration not less than 2
D	None	Water for BET	Not less than 2

The test is considered valid when the following conditions are met:

- the absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980;
- the result with solution D does not exceed the limit of the blank value required in the description of the reagent mixture employed, or it is less than the endotoxin detection limit of the rFC employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution (if any) (solution A, Table 2.6.32.-1) from that in the solution containing the added endotoxin (solution B, Table 2.6.32.-1).

The test solution is considered free of interfering factors if, under the conditions of the test, the measured concentration of the endotoxin added to the test solution is within 50-200 per cent of the known added endotoxin concentration, after subtraction of any endotoxin detected in the solution without added endotoxin.

谢谢！