

FDA PERSPECTIVE ON RECOMBINANT ENDOTOXIN DETECTION SYSTEMS

**2021 USP Virtual Open Forum
Alternatives to Compendial Reagents used in the
Bacterial Endotoxins Test**

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Pharmaceutical Quality



A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Drugs are no different.

A close-up photograph showing a hand holding an orange pill bottle, pouring three white, oval-shaped pills into the palm of another hand. The background is softly blurred, focusing attention on the action of dispensing medication.

**Patients expect safe and effective
medicine with every dose they take.**

A close-up photograph of a person's hands. The left hand is holding an orange plastic pill bottle, tilted to pour three white, oval-shaped pills into the palm of the right hand. The background is softly blurred, showing a white bowl. The text is overlaid on the image in a clean, white, sans-serif font.

Pharmaceutical quality is
assuring *every* dose is safe and
effective, free of contamination
and defects.



**It is what gives patients confidence
in their *next* dose of medicine.**



DISCLAIMER

This presentation reflects the views of the presenter and should not be construed to represent FDA's views or policies.

OUTLINE

- Regulations
- Compendial and alternative tests
- FDA position on the use of non-compendial pyrogen detection tests
- Recombinant Factor C
 - FDA Case Studies
- Monocyte Activation Test
- Conclusions

REGULATIONS

CODE OF FEDERAL REGULATIONS

21 CFR 211.167(a)

“For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements.”

ADDITIONAL REGULATIONS FOR BIOLOGICAL PRODUCTS

21 CFR 610.13(b)

“Test for pyrogenic substances. Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbit...”

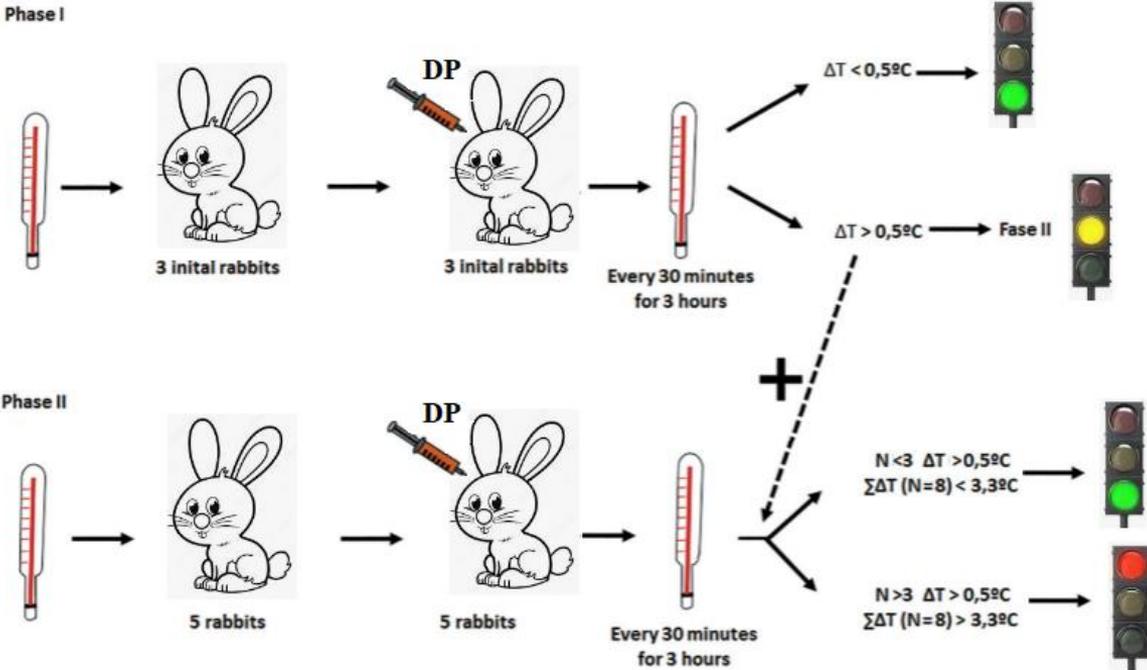
TESTING FOR PYROGENS IN BIOLOGICAL PRODUCTS



- Due to the potential of intrinsic pyrogenicity of biological products, the RPT is still required as a one-time characterization test.
- The LAL-BET is generally used for release testing to ensure that the product is not contaminated with endotoxin.
- Some biological products interfere with endotoxin recovery over time (endotoxin masking). If masked endotoxin contaminating the product is pyrogenic in rabbits, the RPT may be used as an interim release test until a new detection method is developed.

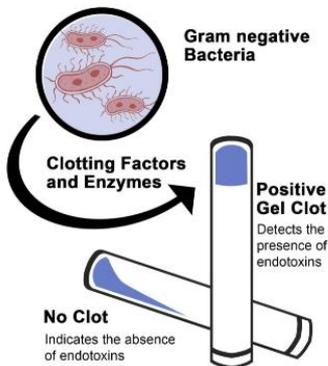
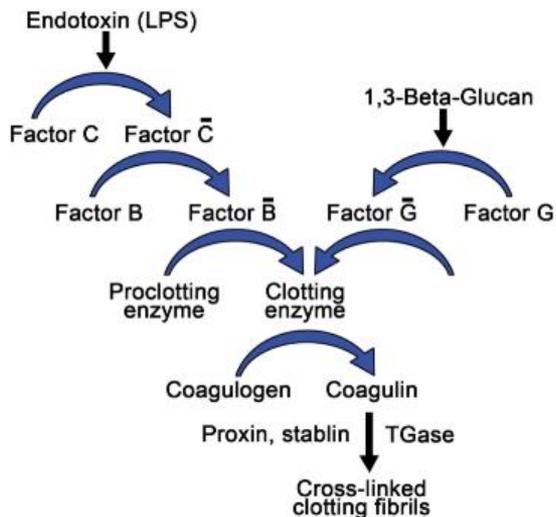
COMPENDIAL AND ALTERNATIVE TESTS

COMPENDIAL TESTS



Test for pyrogenic substances (21CFR610.13(b)); Pyrogen test (USP <151>)

COMPENDIAL TESTS



Bacterial Endotoxins Test (USP <85>)

Uses a lyophilized product from the amoebocyte lysate of horseshoe crabs (*Limulus polyphemus* or *Tachypleus tridentatus*)

From Tinker-Kulberg *et al.* Front. Mar. Sci., 01 April 2020

LIMITATIONS TO THE COMPENDIAL TESTS

- **(Rabbit) Pyrogen test (USP <151>)**
 - Low sensitivity: limit of detection in rabbits (50% rabbits developing fever) between 5 and 10 EU/Kg (endotoxin safety threshold in humans in 5 EU/Kg)
 - Not quantitative: difficult to implement endotoxin specification
 - Different sensitivity among rabbits
 - Involves animal husbandry, long reaction times, and personnel commitment
- **Bacterial Endotoxins Test (LAL-BET) (USP <85>)**
 - Batch to batch variability due to differences in the horseshoe crab used for the lysate (species, age, location...)
 - Non-specificity: false positives (beta-glucans detected by Factor G)
 - Potential for shortage of horseshoe crabs (mortality after harvest, reduced populations, climate change , etc.) may risk availability of the critical reagent.

ALTERNATIVE PYROGEN DETECTION TESTS

- **Monocyte Activation Test (MAT):** In 2009, general chapter 2.6.30 “Monocyte-activation test” was added to the European Pharmacopeia as an *in vitro* alternative to the rabbit pyrogen test for detection of both endotoxin and non-endotoxin pyrogens.
- **Recombinant Factor C (rFC):** In 2020, general chapter 2.6.32 “Test for bacterial endotoxins using recombinant factor C” was added to the European Pharmacopeia as an alternative to the (LAL)-based methods for the quantification of endotoxins from gram-negative bacteria.

ALTERNATIVE ENDOTOXIN DETECTION TESTS IN THE US



- In September 2019, the USP Microbiology Expert Committee proposed the inclusion of recombinant factors for endotoxin testing in an existing chapter of the USP-NF harmonized across Europe, Japan and the US (<85> Bacterial Endotoxins).
- The Expert Committee canceled the proposal of a revised chapter with all the endotoxin assays in the same chapter.
- In May 2020, USP published a Compendial Notice and Prospectus, reinforcing USP's commitment to the introduction of recombinant Factor C (rFC) into the official text of the USP-NF. Proposed new title was <1085.1> Use of Recombinant Reagents in the Bacterial Endotoxins Test - Photometric and Fluorometric Methods Using Recombinantly Derived Reagents.

FDA POSITION ON THE USE OF NON- COMPENDIAL PYROGEN DETECTION TESTS

FDA POSITION

- Currently, the new methods are not compendial and require full validation.
- To determine the validation required in an application to the FDA, FDA follows:
 - Data and information provided,
 - Current regulations,
 - FDA guidance, and
 - US pharmacopeia.

CODE OF FEDERAL REGULATIONS

21 CFR 610.09(a)

Equivalent methods and processes

“Modification of any particular test method ... shall be permitted only under the following conditions: The applicant presents evidence ... demonstrating that the modification will provide assurances of the safety, purity, potency, and effectiveness of the biological product equal to or greater than the assurances provided by the method or process specified in the general standards or additional standards for the biological product.”

FDA GUIDANCE

2012 FDA Guidance for Industry: Pyrogen and Endotoxins Testing Q&A

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

Yes, if they provide advantages

Such methods should be validated as per USP <1225> and should be shown to achieve better or equivalent results.

- Recombinant Horseshoe Crab Factor C Assay
 - Validated as per USP <85> and USP <1225>
- Monocyte Activation Type Pyrogen Test
 - Product-specific validation
 - Interference testing
 - Accurate detection of pyrogens in individual test samples

US PHARMACOPEIA

USP General Notices and Requirements 6.30

- Alternative methods and/or procedures may be used if they provide advantages.
- Such alternative procedures and methods shall be validated as described in the general chapter “Validation of Compendial Procedures” (1225) and must be shown to give equivalent or better results.
- Alternative procedures should be submitted to USP for evaluation as a potential replacement or addition to the standard.

RECOMBINANT FACTOR C

FDA POSITION

The Recombinant Factor C method has the same mechanism of action as the compendial BET, similar type of assay, execution of the method has low complexity, and there are multiple reagent sources and extensive data available comparing both methods.

FDA provided recommendations to the USP <1085.1> draft including:

- The alternative methods should be first validated without specific products to demonstrate non-inferiority to the compendial methods and once this has been demonstrated, method suitability using the specific product should be performed as in USP <85>,
- Eliminate references to USP <1223>,
- Eliminate references to autochthonous endotoxin sources,
- Streamline responsibilities of the supplier and the user.

VALIDATION + METHOD SUITABILITY

The alternative methods should be first validated without specific products to demonstrate non-inferiority to the compendial methods and once this has been demonstrated, method suitability using the specific product should be performed as in USP <85> (FDA comments to USP <1085.1>):

- “*Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the **performance characteristics of the procedure meet the requirements for the intended analytical applications.***” (USP <1225>).
- Method validation typically includes accuracy, precision, specificity, Detection limit/quantitation limit, linearity, range, and robustness (USP <1225>; ICHQ2)
- “*The suitability of all testing methods used shall be verified under the actual conditions of use.*” (21CFR211.194(a)(2)).

REFERENCES TO USP <1223>

Eliminate references to USP <1223>:

- USP <1223> “Validation of alternative microbiology methods” *“provides guidance on the selection, evaluation, and use of microbiological methods as alternatives to compendial methods.”*
- Microbial methods include qualitative methods to demonstrate presence or absence of microorganism and quantitative methods that yields a numerical result in terms of microbial content (USP <1223>).
- *“The endotoxin test methods described in USP <85> are biological test methods, not microbial test methods. Therefore, reference to USP <1223> Validation of Alternative Microbiological Methods in this chapter could confuse stakeholders.”* (FDA comments to USP <1085.1>).
- *“Alternative procedures and methods should be validated as described in the USP General Chapter <1225>, Validation of Compendial Procedures.”* (Guidance for Industry; Pyrogen and Endotoxins Testing: Questions and Answers; June 2012).

AUTOCHTHONOUS ENDOTOXIN SOURCES

Eliminate references to autochthonous endotoxin sources:

- *“We strongly recommend removing reference to “autochthonous endotoxin” and revising the paragraph to read as follows: “Samples should be “spiked” with the current USP Endotoxin RS (RSE) or commercially prepared control standard endotoxins (CSE) during the Test for Interfering Factors.” (FDA comments to USP <1085.1>).*
- *“FDA relies on the use of RSE and CSE for these studies instead of unknown sources of endotoxins (e.g., “naturally occurring endotoxins” or “autochthonous endotoxins”). The suggestion to use unknown sources of endotoxins for comparability studies appears to revert what the FDA has recommended for decades. The inclusion of this paragraph will cause confusion and lead to sponsors having to repeat studies using the appropriate standards at the FDA’s request.” (FDA comments to USP <1085.1>).*
- *“It would not be reasonable to expect firms to have a panel of endotoxin contaminated samples from a manufacturing environment.” (FDA comments to USP <1085.1>).*

AUTOCHTHONOUS ENDOTOXIN SOURCES (cont.)

Eliminate references to autochthonous endotoxin sources:

- “*Preparing endotoxins from autochthonous manufacturing sources is not scientifically justified. It is not clear why autochthonous manufacturing sources must be used and where would these be obtained. The same applicant could use different manufacturing facilities containing different microbial flora, which raises the question of whether their equivalence studies would need to be redone periodically and/or redone when the flora changes.*” (FDA comments to USP <1085.1>).
- CHO derived biotech processes are microbially controlled and are not expected to be contaminated with detectable levels of endotoxin. *E.coli* derived recombinant protein processes contain high levels of *E. coli* derived endotoxin which must be cleared during purification. RSE and CSE are *E. coli* derived standards and are appropriate as spiking agents.

AUTOCHTHONOUS ENDOTOXIN SOURCES (cont.)

Eliminate references to autochthonous endotoxin sources:

- The term “autochthonous” is unclear and confusing; the chapter uses it for non-purified water samples (autochthonous definition is “belonging to” as opposed to introduced; it does not apply to endotoxin in water samples as these are introduced by Gram- bacteria).
- Proposed “autochthonous samples” are uncharacterized samples. The use of uncharacterized reagents in the comparability study would result in additional variables. **A controlled experiment works with one variable at a time.** If several variables are changed at the same time, it is difficult to deduce the causal relationship to a particular attribute (which variable is responsible for the observed results).
- The Guidance for Industry; Pyrogen and Endotoxins Testing: Questions and Answers; June 2012 suggests to use “a battery of field samples of product found to be positive”. There are multiple publications comparing the compendial and recombinant methods using environmental samples and pharmaceutical products covering synthetic drugs, specified biological products and vaccines.
- Unsterile water samples containing unknown contaminations including (autochthonous) endotoxins and beta-glucans are not appropriate (beta-glucan blockers are not always 100% effective).

RESPONSIBILITIES OF SUPPLIER AND USER

Streamline responsibilities of the supplier and the user:

- Points to consider on supply quality appears to be out of the scope of the USP and it is not specific for recombinant reagents.
- LAL has been FDA licensed because of its animal nature and intrinsic variability. A request for designation was filed to the FDA in 2002 to determine the regulatory status of the rFC product. FDA determined that the product did not require premarket submission to CBER or CDRH because it was not intended to qualify blood or blood products and it was not intended for use in man, animals, clinical diagnosis or patient management.
- *“The supplier is responsible for characterizing and qualifying the reagent, while the user is responsible for using a reagent that meets quality standards (e.g., specific activity) and demonstrating that the reagent is fit for its intended use.”* (FDA comments to USP <1085.1>).

FDA CASE-STUDIES (DETAILED INFORMATION IN THE BACK-UP SLIDES)

FDA CASE-STUDY 1: RECOMBINANT FACTOR C AS THE PRIMARY METHOD FOR ENDOTOXIN DETECTION

FDA CASE-STUDY 1

BLA with rFC as the primary DS and DP release test as an alternative to the LAL-BET

- Liquid phase fluorescence end-point as the detection method
- Method validation approach:
 - Non-product specific validation (provided by the vendor):
 - Linearity (also part of method suitability AC in each run): 0.01 to 10 EU/mL
 - Range: 0.01 to 10 EU/mL
 - Limit of Quantification: 0.01 EU/mL
 - Accuracy
 - Precision
 - Specificity
 - Product-specific validation (provided by the applicant):
 - Accuracy
 - Precision
 - Method suitability

FDA CASE-STUDY 1

- The combination of non-product specific and product-specific validation was deemed acceptable to demonstrate that the rFC method was equal or better than the compendial method to detect endotoxin and no additional validation was requested.
- The rFC method was approved in 2018 as the primary endotoxin detection method for DS and DP release.
- A second biological product from the same applicant using the rFC method for release sample endotoxin testing was approved in 2020 using the same approach.

FDA CASE STUDY-2: RECOMBINANT FACTOR C AS AN ENDOTOXIN DETECTION METHOD DUE TO LOW ENDOTOXIN RECOVERY IN THE DRUG PRODUCT

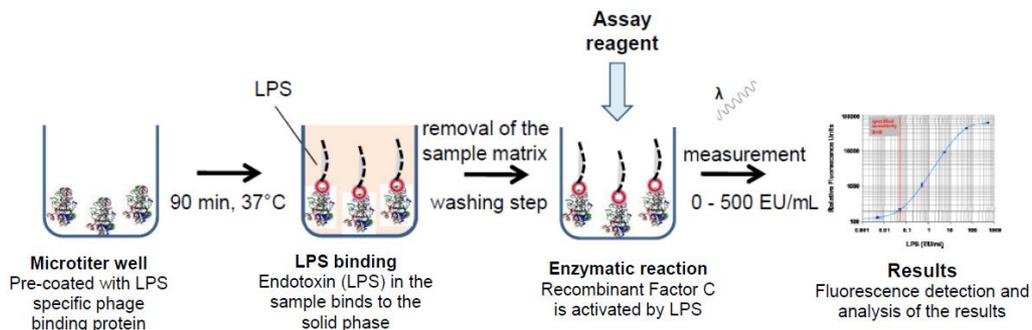
FDA CASE STUDY-2

BACKGROUND

- BLA for a monoclonal antibody formulated with polysorbate 20 and sodium citrate.
- Low endotoxin recovery in the presence of DP with the BET-LAL in the original BLA:
 - 50% of the nominal spike at time zero
 - 10% after 7 hours of incubation.
- Endotoxin in the presence of DP cause fever in rabbits.
- **Endotoxin present in the DP could not be detected by the BET-LAL method but caused a pyrogenic response in rabbits.**
- The BLA was approved using an INTERIM rabbit pyrogen test for detection of endotoxin.
- Post-marketing commitment: to develop and validate a new endotoxin detection method to replace the interim RPT.

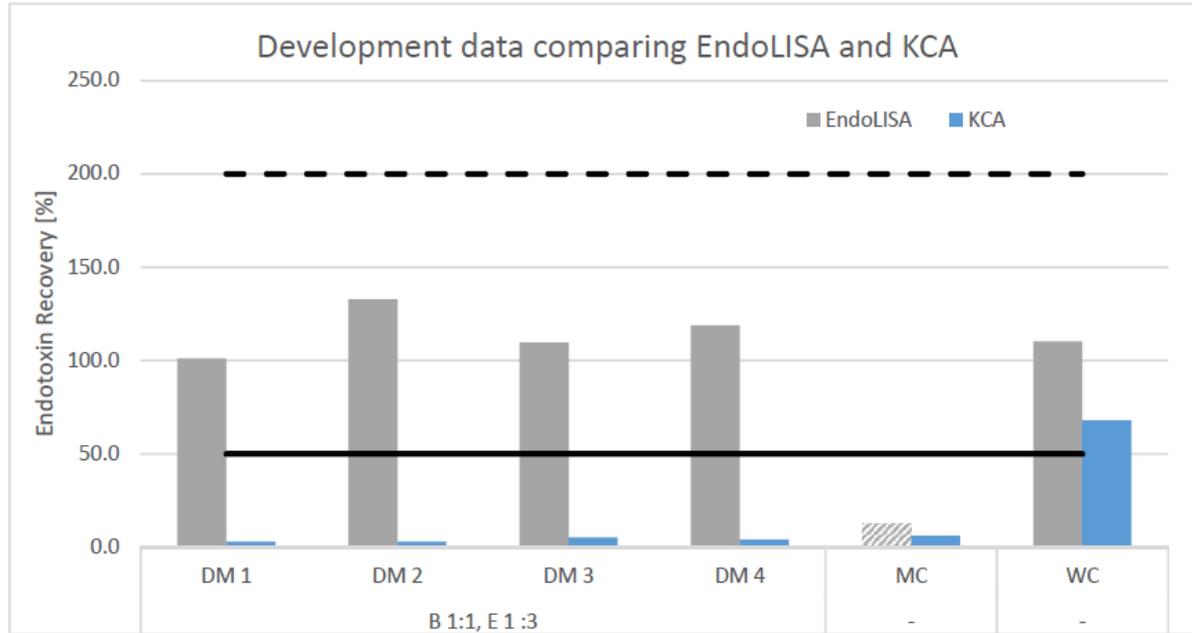
FDA CASE STUDY-2

- Alternative method included treating samples with a demasking agent (to destabilize the masked-endotoxin complex and form detectable endotoxin aggregates) followed by endotoxin detection by a solid-phase rFC-based matrix.
- Solvents and other interfering substances are eliminated after a washing step.
- Reaction mix (rFC + synthetic fluorogenic substrate) is added to the matrix.
- If endotoxins are bound to the matrix, rFC is activated and the fluorogenic substrate is cleaved releasing a fluorescent molecule.



From ENDOLISA package insert, bioMérieux

FDA CASE-STUDY 2



Endotoxin detection in samples tested using ENDOLISA and the LAL-BET

KCA: Kinetic Chromogenic Assay; **DM:** demasked samples; **MC:** non-demasked samples; **WC** water control

FDA CASE-STUDY 2

Method validation approach:

- Product-specific and non-product specific validation was conducted by the applicant
- Validation was conducted for the detection method alone and the combination of demasking and detection method. Only validation data using the combined demasking and detection methods were reviewed for the application.
 - Specificity
 - Accuracy
 - Precision
 - Limit of quantification; 6 EU/mL
 - Linearity: 6 to 12 EU/mL
 - Range: 6 to 50 EU/mL
 - Robustness

FDA CASE-STUDY 2

- FDA requested additional information from the applicant.
- Additional information could not be provided within the review cycle and the method could not be approved without the required information.
- **The combined method** presents an improvement over the currently approved methods (LAL-KCM and RPT) **for the specific product**.
- From a regulatory perspective it was important to implement the method as soon as possible to eliminate the RPT, provided the requested information was acceptable.
- An extension to the supplement was granted for the applicant to gather data to respond to FDA's concerns.

FDA CASE-STUDY 2

- The review is still ongoing.
- The rFC method was reviewed in the context of demasking.
- The method appears to present an improvement over the currently used method for this specific product.
- Potential approval would be for the combination of the demasking and rFC detection method for the specific product.

RECOMBINANT FACTOR C

COMPARISON BETWEEN THE 2 CASES

- FDA case-study 1:
 - Multiple data available from the vendor, including most of the non-product specific information.
 - Applicant data included product-specific information.
- FDA case-study 2:
 - The applicant did not provide any information from the vendor.
 - All data included in the submission was from the applicant.
- User access to the information produced by the vendor may facilitate the validation exercise.

MONOCYTE ACTIVATION TEST

MAT IN BLA SUBMISSIONS

- The Monocyte Activation Test (MAT) is not generally used as a replacement to the LAL endotoxin test, but to the rabbit pyrogen test.
- The Monocyte Activation Test (MAT) is not compendial in the US and requires full validation.
- Masked endotoxin not detected with the LAL-BET method was also not detected using the MAT (in 2 applications).
- MAT as an alternative to the rabbit pyrogen test:
 - In a proposal for a BLA (Type B meeting in the IND stage).
 - In a BLA submitted to the FDA.
- The MAT has not been approved for any CDER biological product as an alternative to the rabbit pyrogen test.

UPDATES ON MAT

- The FDA supports the use of the MAT test as long as equivalency is demonstrated as per 21 CFR 610.9.
- An Emergent Technology Team (ETT) meeting was held between FDA and a consortium of industry and academic experts.
- The team proposed a multi-integrant study to address deficiencies to the MAT validation:
 - MAT method proposal to be used by all participants.
 - Comparison between the MAT and RPT.
 - Use non-endotoxin pyrogen panel.
 - Use compendial standards.
- The results would be compiled and published.

CONCLUSIONS

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.

ACKNOWLEDGEMENTS

- Bo Chi, PhD
- Patricia Hughes, PhD



THANK YOU!

BACK-UP SLIDES

FDA CASE-STUDY 1: RECOMBINANT FACTOR C AS THE PRIMARY METHOD FOR ENDOTOXIN DETECTION

FDA CASE-STUDY 1

BLA proposing the rFC as the primary test method for endotoxin detection at DS and DP release as an alternative to the LAL-BET

- Liquid phase fluorescence end-point as the detection method (Pyrogene, Lonza)
- Method validation approach:
 - Non-product specific validation (provided by the vendor):
 - Linearity (also part of method suitability AC in each run)
 - Range
 - Limit of Quantification
 - Accuracy
 - Precision
 - Specificity
 - Product-specific validation (provided by the applicant):
 - Accuracy
 - Precision
 - Method suitability

FDA CASE-STUDY 1

Non-product specific validation:

- Linearity: Standard curves (18 total) with 4 standards (0.01, 0.1, 1, and 10 EU/mL) and 3 replicates with a **correlation (R) of 0.996**
- Range: **0.01 EU/mL to 10 EU/mL**
- Limit of Quantification: **0.01 EU/mL** with 3 lots of rFC in 3 separate assays (average recovery of 0.009EU/mL; SD 0.0007 EU/mL)
- Precision: Comparison of rFC with the LAL-BET in 3 test centers with low, medium, and high endotoxin concentrations (0.0316, 0.316, and 3.16 EU/mL); **similar results, lower %CV than the LAL-BET**
- Accuracy: 9 analysts on 3 different days (from precision study). **Similar results than for the LAL-BET; lower SD and higher % of results within 25% of nominal concentration than the LAL-BET**

FDA CASE-STUDY 1

Non-product specific validation (continue):

- Specificity:
 - Multicenter study (6 centers, 3 analysts per center) using 10 pharmaceutical products:
 - Testing methods rFC and LAL-KCM (Kinetic Chromogenic Method)
 - Spike of 0.1 EU/mL
 - Product dilution at MVD, MVD/2, and MVD/10
 - No endotoxin recovered from non-spiked samples
 - All results within 50 to 200% recovery except for Human Albumin, which inhibits the LAL test method
 - 87.5% rFC within target (25% of nominal spike); 75% KCM within target
 - 4 endotoxin sources (RSE, *E. coli* O55:B5, *P. aeruginosa* F-D type 1, and *S. Minnesota* R595) tested with rFC, KCM, and Kinetic Turbidimetric Method (KTM):
 - rFC recognized endotoxin from all sources tested
 - Higher differences in recovery between the LAL-KTC and LAL-KCM than between the rFC method and any of the other two LAL methods

FDA CASE-STUDY 1

Product-specific validation:

- Accuracy was assessed using 3 DP lots and high and low endotoxin spike concentrations: % recoveries 92 to 134% (AC: 50 to 200%)
- Precision from the study above: inter-assay CV of 12 to 13%, (AC: CV ≤ 25%)
- Inhibition/Enhancement spiking at mid-point of curve: % recoveries 91 to 120% (AC: 50 to 200%)
- pH suitability: pH of samples when combined with the rFC reagents was 7.73-7.91

FDA CASE-STUDY 1

- The combination of non-product specific and product-specific validation was deemed acceptable to demonstrate that the rFC method was equal or better than the compendial method to detect endotoxin and no additional validation was requested.
- The rFC method was approved in 2018 as the primary endotoxin detection method for DS and DP release.
- A second biological product from the same applicant using the rFC method for release sample endotoxin testing was approved in 2020 using the same approach.

FDA CASE STUDY-2: RECOMBINANT FACTOR C AS AN ENDOTOXIN DETECTION METHOD DUE TO LOW ENDOTOXIN RECOVERY IN THE DRUG PRODUCT

FDA CASE STUDY-2

BACKGROUND

- BLA for a monoclonal antibody formulated with polysorbate 20 and sodium citrate.
- Endotoxin recovery studies in the original BLA showed endotoxin detection in the presence of DP to be 50% of the nominal spike at time zero and 10% after 7 hours of incubation when using the BET-LAL Kinetic Chromogenic Method (LAL-KCM).
- Endotoxin (RSE, 35 to 40 EU/Kg) injected to rabbits in the presence of DP resulted in a pyrogenic reaction.
- **Endotoxin present in the DP could not be detected by the LAL-KCM method but caused a pyrogenic response in rabbits.**
- The BLA was approved using an INTERIM rabbit pyrogen test for detection of endotoxin.
- Post-marketing commitment: to develop and validate a new endotoxin detection method to replace the interim RPT.

FDA CASE STUDY-2

- To overcome low-endotoxin recovery, the applicant treated samples with a proprietary commercially available demasking agent (EndoRS, bioMerieux).
- The demasking agent is intended to be used with a solid-phase endotoxin detection kit (ENDOLISA, bioMerieux) due to the presence of solvents that may interfere with the LAL reagents; the ENDOLISA plate contains bacteriophage receptor binding proteins that bind specifically to the endotoxin lipid A. The use of EndoRS with the BET-LAL system requires additional method optimization.
- After treatment, demasked samples were added to the ENDOLISA plate; demasked endotoxins potentially present in the product bind to the matrix.
- Solvents and other Interfering substances are eliminated after a washing step.
- A reaction mix (rFC + synthetic fluorogenic substrate) is added to the matrix.
- If endotoxins are bound to the matrix, rFC is activated and the fluorogenic substrate is cleaved releasing a fluorescent molecule.

FDA CASE-STUDY 2

Applicant **Acceptance Criteria** for the test included:

- Each measurement:
 - Temperature 36 to 38°C
 - Standard curve coefficient of regression: $R \geq 0.980$
 - Back-calculations for the standards 50 to 0.05 EU/mL: 60 to 150%
 - Negative control (RFU of blank < RFU of lowest standard (0.05 EU/mL)
 - CV of standard duplicates $\leq 30\%$
- Sample AC (to be fulfilled in NLT 4 out of 6 replicates)
 - Sample CV of replicates $\leq 30\%$
 - PPC CV of replicates $\leq 30\%$
 - PPC recovery 50 to 200%

FDA CASE-STUDY 2

	Recovery to theoretic value at five time points				
	0 h	24 h	48 h	72 h	168 h
	Recovery [%]	Recovery [%]	Recovery [%]	Recovery [%]	Recovery [%]
WC	114	128	135	129	157
MC	< 13	< 13	< 13	< 13	< 13
DM replicate 1	133	87	154	110	152
DM replicate 2	125	96	128	93	148
DM replicate 3	110	74*	106	85	95
DM replicate 4	132	86	126	105	126
DM replicate 5	118	86	142	102	136
DM replicate 6	113	82	124	78	135
Mean recovery	122	87	130	96	132
CV [%]	8	6	13	13	15

* Invalid recovery due to invalid PPC

Endotoxin recovery using demasking agents/solid phase detection with rFC

Samples were spiked with 6 EU/mL RSE; **WC**: water control; **MC**: masked control (without demasking reagent)

FDA CASE-STUDY 2

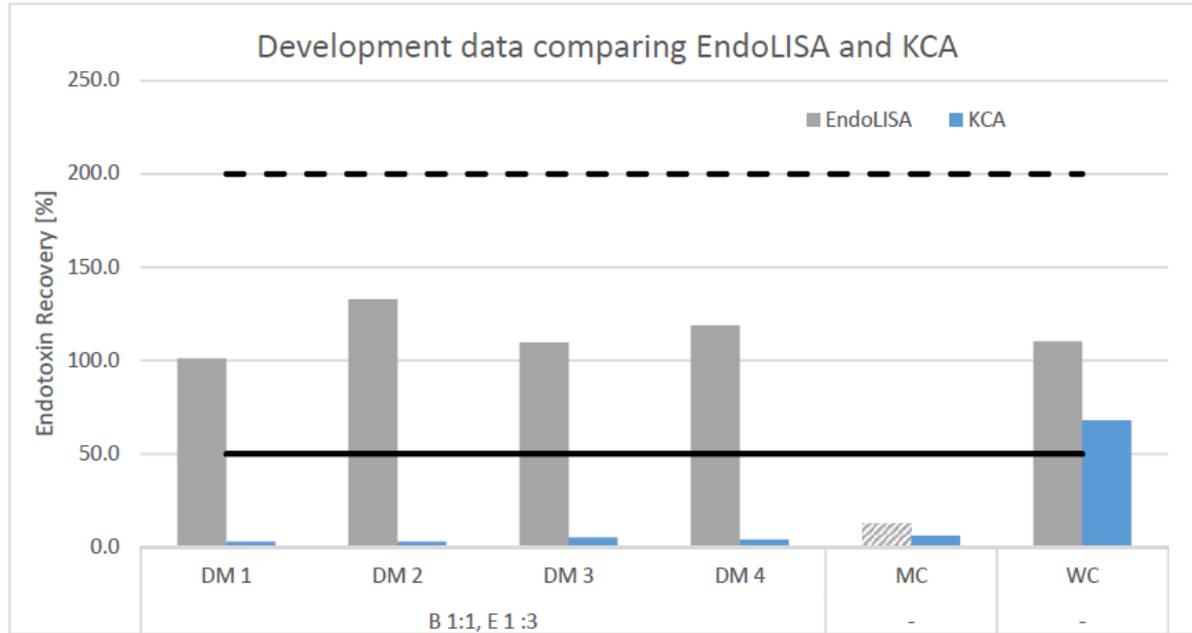
DP Lot A	Recovery to theoretic value at five time points									
	0 h		24 h		48 h		72 h		168 h	
	Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]	
WC		155		168		161		155		191
MC		15	<	13	<	13	<	13	<	13
Mean recovery	138		109		166		168		157	
CV [%]	7		13		8		6		15	

DP Lot B	Recovery to theoretic value at five time points									
	0 h		24 h		48 h		72 h		168 h	
	Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]	
WC		161		136		168		160		181
MC	<	13	<	13		23**	<	13	<	13
Mean recovery	138		108		160		145		133	
CV [%]	7		14		13		7		11	

DP Lot C	Recovery to theoretic value at five time points									
	0 h		24 h		48 h		72 h		168 h	
	Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]		Recovery [%]	
WC		114		128		135		129		157
MC	<	13	<	13	<	13	<	13	<	13
Mean recovery	122		87		130		96		132	
CV [%]	8		6		13		13		15	

Endotoxin recovery using demasking agents/solid phase detection with rFC

FDA CASE-STUDY 2



Endotoxin detection in samples tested using ENDOLISA and the LAL-BET

KCA: Kinetic Chromogenic Assay; **DM:** demasked samples; **MC:** non-demasked samples; **WC** water control

FDA CASE-STUDY 2

Method validation approach:

- Product-specific and non-product specific validation was conducted by the applicant
- Validation was conducted for the detection method alone and the combination of demasking and detection method. Only validation data using the combined demasking and detection methods were reviewed for the application.
 - Specificity
 - Accuracy
 - Precision
 - Limit of quantification
 - Linearity
 - Range
 - Robustness

FDA CASE-STUDY 2

Validation conducted for **product-specific** demasking + rFC detection method:

- Specificity: **no endotoxin recovered from non-spiked samples**
- Accuracy and linearity between **6 EU/mL and 12 EU/mL** using 6 replicates
- Precision: CV of **7 to 29% for the 6 replicates** ($AC \leq 30\%$) and inter-assay precision (5 assays by 2 operators) **CV per assay of 6 to 12%, overall CV of 27%** ($AC \leq 35\%$); all samples within **58 to 169% of the nominal spike**.
- Limit of quantification: **6 EU/mL**
- Range: **6 to 50 EU/mL** (the package only includes data from 6 to 12 EU/mL)
- Robustness: 3 lots of product and 3 lots of detection kit: **66 to 198 %** recovery ($AC: 50$ to 200%) and **%CV of 5 to 15%** ($AC \leq 35\%$)

FDA CASE-STUDY 2

Validation conducted for **non-product specific** rFC detection method (this information was not part of the application review):

- Specificity and Robustness: Referred to specificity of the combined method
- Accuracy and linearity between **0.05 EU/mL and 50 EU/mL (RSE, results compared to CSE standard curve)** using 5 concentrations and 6 replicates; recovery 106% to 141%;
- Precision: intra-assay CV of **3 to 9%** for the 5 concentrations, 6 replicates (AC ≤ 30%) and inter-assay precision (6 assays by 2 operators) **overall CV of 11%** (AC ≤ 35%); all samples within **107 to 148% of the nominal spike**.
- Limit of quantification: **0.05 EU/mL**
- Range: **0.5 to 50 EU/mL**

FDA CASE-STUDY 2

FDA questions to the applicant:

- Justification for the sample AC fulfillment of 4 out of 6 replicates.
- To provide data to substantiate the range of 6 to 50 EU/mL.
- To provide impact of demasking on water controls (this information will help understanding the high limit of quantification of the method using product).

FDA CASE-STUDY 2

- Additional information could not be provided within the review cycle.
- The method could not be approved without understanding the information requested.
- **The combined method** presents an improvement over the currently approved methods (LAL-KCM and RPT) **for the specific product**.
- From a regulatory perspective it was important to implement the method as soon as possible to eliminate the RPT, provided the requested information was acceptable.
- An extension to the supplement was granted for the applicant to gather data to respond to FDA's concerns.

FDA CASE-STUDY 2

- The review is still ongoing.
- The rFC method was reviewed in the context of demasking.
- The method appears to present an improvement over the currently used method for this specific product.
- Potential approval would be for the combination of the demasking and rFC detection method for the specific product.