Setting Endotoxin Acceptance Criteria for Biologics Intravenous (IV) and Subcutaneous (SC) Mono- and Combination Therapies

Friedrich von Wintzingerode
Senior Manager, Global Analytical Science and Technology, Roche-Genentech

Sara Parker
Technical Development Manager, Roche-Genentech

Richard Seipert
Technical Development Senior Scientist, Roche-Genentech

Tony Chen
Principal Quality Product Leader, Roche-Genentech

Michael Knight
Associate Director QC Microbiology and Environmental Monitoring, Roche-Genentech

Christian Gianinazzi
Planning and Coordination Leader Endotoxin Lab, Roche-Genentech

Farnaz Nowroozi
QC Scientist/Lab Manager, Global Analytical Science and Technology, Roche-Genentech

Note: All authors contributed equally to this article.

Introduction

Health Authority regulations require that parenteral drug products must be non-pyrogenic.

Endotoxins or lipopolysaccharides (LPS) are the most likely source of pyrogenic drug product contamination due to their ubiquity and high pyrogenic potency compared to other microbial pathogen-associated molecular patterns (PAMP).

If the presence of non-endotoxin pyrogens in drug product can be ruled out (e.g. by testing an appropriate number of batches using the compendial Rabbit Pyrogen Test (RPT) or Monocyte Activation Test (MAT)), the compendial Bacterial Endotoxins Test (BET) can be used to waive the pyrogen test requirement.

Drug product endotoxin acceptance criteria should be set such that the amount of endotoxin administered does not exceed the pyrogenic threshold (eg. 5 EU/kg/hr for intravenous administration or 0.2 EU/kg/hour for intrathecal administration).

Prior to 2012, calculation of drug product endotoxin acceptance criteria was largely harmonized among the leading Pharmacopeia (USP/EP/JP/ChP) and Health Authorities (FDA, EMA, PMDA, SFDA) by using the formula K/M, where K is the threshold pyrogenic dose of endotoxin per kg of body mass and M is the maximum recommended bolus dose of product per kg of body mass.

This formula is still in use. However, in 2012 FDA published its *"Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers"*, which states that a final endotoxin acceptance criteria should consider the maximum theoretical contribution from the primary packaging components, formulation excipients, water-for-injection, and bulk drug substance to ensure that theoretical endotoxin limits are not exceeded.

A similar guidance was later given by EP 8.8 chapter 5.1.10., which states *"the endotoxin limit must take into consideration any theoretical bacterial endotoxin load introduced by any other components used for reconstitution and/or dilution of the product (e.g. water for injection) or introduced by starting materials and/or raw materials".*

In addition, EP 5.1.10. states that *"the capability of the process to reduce or remove bacterial endotoxins during manufacture might result in lower endotoxin limits for certain processes".*

These additional requirements resulted in a complex regulatory landscape because USP and ChP have not aligned with FDA and EP yet.

The following paper describes an approach for setting endotoxin acceptance criteria for Biologics intravenous (IV) and subcutaneous (SC) mono and combination therapies, which meets current regulatory requirements.

Combination Therapies

Combination therapies are defined as the use of two or more drugs to treat a single disease in an individual patient. Using a combination of drugs directed at multiple therapeutic targets can have synergistic effects toward the treatment of the disease. Due to the high therapeutic potential of

combination therapies there has been a large increase in the number of combination therapy clinical trials with over 10,000 trials registered in the US alone [1].

Many of these therapies were originally designed to be given as an individual therapy and as a result their control systems were designed to meet required impurity limits as a monotherapy. To ensure patient safety the complete combination therapy should be evaluated as a whole. In order to evaluate the combination therapy for impurities the mode of administration needs to be considered. In principle combination therapy dosing can be achieved in many different ways e.g. by co-formulation of different APIs, mixture of different drug products prior to administration (e.g. in an IV bag or via a device) or by separate or simultaneous individual administrations.

General Considerations When Calculating Endotoxin Acceptance Criteria

When setting endotoxin acceptance criteria the following general rules are applied.

- In cases where the compendial calculation results in an acceptance criteria >6.0 EU/mL, set the acceptance criteria at a maximim of 6.0 EU/mL or comparable units unless otherwise justified.

  Rationale: EP chapter 5.1.10. requires that DP endotoxin acceptance criteria consider both safety aspects (pyrogenic threshold) and manufacturing processes capability. Safety based calculated endotoxin acceptance criteria are often too high to reflect state-of-the-art process capability of Biologics (e.g. mammalian cell based monoclonal antibody processes often show endotoxin values below the limit of quantification (LOQ)). Per recent publication [2] a standard endotoxin in-process action limit of > 3.0 EU/mL was established for mammalian cell based and E. coli-based processes. The defined maximum standard DP endotoxin specification of 6.0 EU/mL is twice the standard action limit for in-process steps. The increase is justified because the product disposition requirements are different for action limit violations and acceptance criterion failures.

- For lyophilized or solid DP the endotoxin specification is reported in EU/mg (the nominal value is used for calculation).
- For DS with no specified protein concentration range the endotoxin acceptance criterion is reported in EU/mg (the measured value is used for calculation).
- For liquid DS or DP with a specified protein concentration range the endotoxin acceptance criterion is reported in EU/mL.
- For some products specific reporting is required (e.g. EU/vial or EU/IU) per compendia or Health Authority request.
- When calculating endotoxin acceptance criteria intermediate rounding is not allowed and the calculated final value is truncated.
- For endotoxin acceptance criteria reported in EU/mL, the numerical value is given with one decimal place. (e.g. ≤ 2.0 EU/mL).

- For endotoxin acceptance criteria reported in EU/mg, the numerical value is given with two decimal places (e.g. ≤ 0.05 EU/mg). The nominal protein content of the Drug Product (DP) is used for calculation.
- For simplification endotoxin acceptance criteria for DS are back calculated from the DP, if there is no significant difference in formulation. Units are adjusted if necessary.
- A body weight of 60 kg is used for products intended for the standard adult population to align with the most conservative compendial requirement (ChP). Other body weights must be used for products intended for other patient populations (e.g. pediatric, malnourished or cachectic patient populations).
- Many Biologics are applied as infusions. Infusion time plays an important role in calculation of safety based endotoxin specification because of the endotoxin detoxification capability of the human liver (5 EU per kg bodyweight can be detoxified within an hour). The following rules apply for infusion times.
  - Bolus dose or infusion times < 1 hour are rounded to a full hour

    Examples: An infusion over 20 minutes is rounded to 1 hour and an infusion over 70 minutes is rounded to 2 hours.
  - Infusions at frequent intervals are considered as bolus dose or infusion within 1 hour if breaks between infusions are >= 1 hour.

    Example: Infusion of 10 mg DP over 20 minutes at 8 am followed by 10 mg DP over 30 minutes at 10 am, and 10 mg DP over 10 minutes at 12 am is considered as 10 mg DP per kg body weight per hour.
  - Injections at frequent intervals within 1 hour are summed up.

    Example: Injection of 10 mg DP at 8 am followed by 10 mg DP at 8:30 am followed by 10 mg DP at 8:45 am is considered as 30 mg DP per kg body weight per hour.

Setting Endotoxin Acceptance Criteria for Biologics IV and SC DP, Monotherapies

The following formula is used for calculation of DS/DP endotoxin acceptance criteria for monotherapies.

$$\frac{K - EU_D\ ^a}{M} = \text{EU/mg x protein concentration} = \text{EU/mL}$$

K: threshold pyrogenic dose of endotoxin per kg of body mass (5 EU/kg for IV and SC applications).

M: maximum clinical dose of product per kg of body mass.

Note: For lyophilized DP, the cake volume is not considered unless necessary to achieve an acceptance criterion within the method capability. The endotoxin contribution from the reconstitution fluid/diluent is considered when setting acceptance criteria. The contribution is determined from the compendial monograph (if available).

Example #1:

Maximum clinical dose of product X is 250 mg per patient for pediatric application infused over 1 hour. A body weight of 3 kg is assumed. Specified DP protein concentration is 170 mg/mL.

15 mL of saline are used as diluent for infusion. The maximum allowable endotoxin load per compendial monograph is 0.5 EU/mL.

Maximum dose of DP: 250 mg/3 kg per hour = 83 mg/kg per hour

Maximum endotoxin load of diluent: (15 mL x 0.5 EU/mL) / 3 kg per hour = 2.5 EU/kg per hour.

K – EU_D: 5 EU/kg per hour – 2.5 EU/kg per hour = 2.5 EU/kg per hour

M: 83 mg/kg per hour

DP endotoxin acceptance criterion: (K – diluent)/M = 2.5 EU/kg per hour / 83 mg/kg per hour x protein concentration = 0.03 EU/mg x 170 mg/mL = 5.1 EU/mL

▸An endotoxin acceptance criterion of <= 5.1 EU/mL is proposed for DS and DP of product X.

Example #2:

Maximum clinical dose of product Y is 250 mg per patient (adults) infused over 1 hour. A body weight of 60 kg is assumed. Specified DP protein content is 170 mg/mL.

15 mL of saline are used as diluent for infusion. The maximum allowable endotoxin load per compendial monograph is 0.5 EU/mL.

Maximum dose of DP: 250 mg/60 kg per hour = 4.2 mg/kg per hour

Maximum endotoxin load of diluent: (15 mL x 0.5 EU/mL) / 60 kg per hour = 0.125 EU/kg per hour.

K – diluent: 5 EU/kg per hour – 0.125 EU/kg per hour = 4.875 EU/kg per hour

M: 4.2 mg/kg per hour

DP endotoxin specification: (K – diluent)/M = 4.875 EU/kg per hour : 4.2 mg/kg per hour x protein concentration = 1.17 EU/mg x 170 mg/mL = 198.9 EU/mL

Calculated endotoxin acceptance criterion is clearly above the maximum allowable value of 6.0 EU/mL.

▸An endotoxin acceptance criterion of <= 6.0 EU/mL is proposed for DS and DP of product Y.

Setting Endotoxin Acceptance Criteria for Biologics IV and SC DP, Combination Therapies

When setting endotoxin acceptance criteria for combination therapies the maximum allowable endotoxin level (EL) is calculated based on existing endotoxin acceptance criteria of single products (calculated for monotherapies). There are two potential outcomes of EL calculation:

1. If EL is below the pyrogenic threshold (5 EU/kg body weight per hour), there is no impact on the control strategy of the combination therapy.
2. If EL is above the pyrogenic threshold, the control strategy of the combination therapy is impacted and a phase approach is recommended.

a. EU_D: Endotoxin contribution due to diluent or reconstitution fluid. The diluent volume resulting from the maximum drug dosage is taken into consideration.

Guidance for EL calculation

Endotoxin acceptance criterion is expressed in EU/mg

$$EL\ (EU/kg) = \frac{(EAC \times max.\ clinical\ dose\ Biologics\ 1) + (EAC \times max.\ clinical\ dose\ Biologics\ 2)}{average\ patient\ weight}$$

Endotoxin acceptance criterion is expressed in EU/mL

$$EL\ (EU/kg) = \frac{(EAC \times max.\ clinical\ dose\ Biologics\ 1 / protein\ conc) + (EAC \times max.\ clinical\ dose\ Biologics\ 2 / protein\ conc)}{average\ patient\ weight}$$

Example #1:

Product X and product Y applied as simultaneous – fixed-dose-combination (FDC)

Product X: maximum clinical dose = 1200 mg per patient per hour, endotoxin acceptance criterion is <= 0.15 EU/mg

Product Y: maximum clinical dose = 600 mg per patient per hour, endotoxin acceptance criterion <= 0.09 EU/mg

Average patient weight: 60 kg

$$EL = \frac{(0.15\ EU/mg \times 1200\ mg/hour) + (0.09\ EU/mg \times 600\ mg/hour)}{60\ kg} = 3.9\ EU/kg\ per\ hour$$

▹No impact on the control strategy of the combination therapy. The maximum Endotoxin Level of 3.9 EU/kg/h is below the pyrogenic threshold for IV or SC administrations of 5 EU/kg/h.

Example #2:

Product A and Product B combination study including pre-medication, separate consecutive IV administration with various durations and with no saline or diluent required.

Product	Max dose per patient (mg)	Endotoxin acceptance criterion	Treatment duration	Average body weight
Pre-medication	N/A	5 EU/kg body weight per hour (worst case assumption)	30 min	60
A	4500	0.03 EU/mg per hour	90 min	60
B	70	0.75 EU/mg per hour	30 min	60

Special considerations on pre-medication:

Administration of pre-medications for combination therapies can vary between oral dosage forms and injectables. The following scenarios apply when calculating endotoxin acceptance criteria for combination therapies:

1. pre-medication is an injectable and either a vendor-specific or compendial specification is applicable. In such a case the respective specification is used for calculation.
2. pre-medication is an injectable and neither a vendor-specific nor compendial specification is applicable. In such a case a worst case assumption is made (endotoxin load equals pyrogenic threshold of 5 EU/kg bodyweight per hour).
3. Pre-medication is an oral dosage form. In such a case no potential endotoxin load is considered.
4. Potential endotoxin load coming from pre-medication is only considered if the time period between two administrations is less than one hour.

$$EL = \frac{(0.03\ EU/mg \times 4500\ mg/hour) + (0.75\ EU/mg \times 70\ mg/hour) + (5\ EU/kg \times 60\ kg)}{60\ kg \times 3\ h^{b}} = 2.7\ EU/kg\ per\ hour$$

▹No impact on the control strategy of the combination therapy. The maximum Endotoxin Level of 2.7 EU/kg/h is below the pyrogenic threshold for IV or SC administrations of 5 EU/kg/h.

Example #3:

Product 1/product 2/product 3 combination study including separate - consecutive IV administration of product 2 and product 3 (minimum dosing time 2 hours) and a separate IV administration of product 1 (with ≥ 1 hour break).

Product 1: c = 25 mg/mL; max dose 50 mg

Product 2: c = 25 mg/mL; max dose 900 mg; EAC = 2.0 EU/mL; IV bag: 100 mL

Product 3: c = 60 mg/mL; max dose 840 mg; EAC = 6.0 EU/mL; IV bag: 250 mL

Saline in IV bag: EAC <= 0.5 EU/mL

The full volume of the saline in all IV bags or diluent used to administer medications must be considered when assessing the potential endotoxin load of the combination therapy.

Average patient weight: 60 kg

Worst case (minimum) dosing time: 2 hours

$$EL = \frac{(2.0\ EU/mL / 25\ mg/mL \times 900\ mg + 0.5\ EU/mL \times 100\ mL) + (6.0\ EU/mL / 60mg/mL \times 1200\ mg + (0.5\ EU/mL \times 250mL)}{60\ kg \times 2\ h} = 3.06\ EU/kg/h$$

▹No impact on the control strategy of the combination therapy. The maximum Endotoxin Level of 3.06 EU/kg/h is below the pyrogenic threshold for IV or SC administrations of 5 EU/kg/h.

▹Product 1 contribution is not considered because of the administration break of more than 1 hour.

Summary

The presented concept provides a simple procedure to set endotoxin acceptance criteria for Biologics Intravenous (IV) and Subcutaneous (SC) Mono- and Combination Therapies that meet the requirements of major health authorities.

References

1. Anonymous. Nat Med. 2017. 23:1113. doi: 10.1038/nm.4426.
2. von Wintzingerode and Knight. American Pharmaceutical Review, Endotoxin Supplement 2017. Page 20 – 23.

b. Total administration time of 150 minutes is rounded to 3 hours.