CIAL | How to Calculate Endotoxin Limits?

Effective January 1, 2025, CIAL requires **Limit Specifications** for *all* Endotoxin (END / ENDV) testing.

If a limit is not provided on the Sample Submission form, a **default limit** of **350 EU/mL** will be applied. This default limit established is based on a 1 mL max dose, 70 kg patient, parenteral administration.

Should a limit not be established for your formulation, CIAL recommends calculating Endotoxin Unit (EU) limits per the following criteria:

1. The max dosage the patient would receive
2. The patient's weight
3. Route of administration (parenteral or intrathecal)

Currently, 5 EU/kg is the average threshold for pyrogenic effects in humans and is listed in USP <85> and <1085>. However, we want to improve our safety and quality testing of your products as some specific USP monographs can conflict with this calculation and appropriate patient dosing.

A couple examples to illustrate how this 5 EU/kg limit and USP monograph limits can cause confusion and potential inconsistencies are detailed below:

> *Example 1*: The USP monograph for a Hydroxocobalamin lists 0.4 EU/µg or 400 EU/mg for an injectable solution. Oftentimes, at least 1 to 2 mg/mL all the way up to 50 mg/mL is used. Strict adherence to the USP monograph would have the lowest limit of 400-800 EU/mL, up to 20,000 EU/mL. You can see how these limits pose safety issues and quickly get out of hand.
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> *Example 2*: We receive many GLP-1 agonists which are formulated with various B-vitamins. While the two most common GLP-1 agonists we receive (i.e., Semaglutide and Tirzepatide) do not have monographs, many of the additional additives (e.g., B-vitamins) do. One of these B-vitamins is Pyridoxine, which has a listing of 0.4 EU/mg for an injectable solution. This is where we run into the opposite issue where limits may be too strict. A GLP-1 formulation with 2 mg/mL of Pyridoxine would issue a limit of 0.8 EU/mL, which is very strict and can be difficult to assay properly.

Based on historical data, this change should not affect pass/fail results for the vast majority of products and may not be relevant to your particular formulations, but want to be transparent in making you aware.

We believe this requirement allows CIAL to more accurately assay your products with patient safety at top of mind. For the most appropriate patient dosing information, please provide us with EU limits based on in-house risk assessment or general patient dosing information with your sample submissions (i.e. average dose, average patient weight, and route of administration).