Validation of a method for the detection and quantification of bacterial endotoxins in sterile injectable preparations using a kinetic colorimetry technique.

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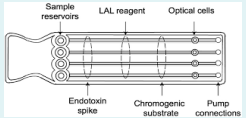
Objective

The release of **bacterial endotoxins** during **lysis of GRAM negative bacteria** must be checked for injectable preparations; their presence, in quantities above the accepted limit, can lead to **septic shock.**

➔ **Bacterial endotoxin assay (Ph.Eur. 2.6.14 & USP <85>)**
- Detection and/or quantification of endotoxins using a limulus amoebocyte lysate (LAL)
- 3 techniques : gel clot, turbidimetry and **colorimetry.**

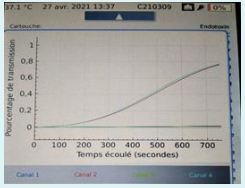
➔ CHU de Liège:
- Switch of technique: GEL CLOT → KINETIC COLORIMETRY **(Méthod D – Ph.Eur. 2.6.14)**
- Technical validation of kinetic colorimetry

Kinetic colorimetry principle – NEXGEN PTS (Charles River)
- Cartridge: 4 sample wells
  - ✓ 2,4: spiked channels;
  - ✓ 1,3: sample channels
- Dispensing: 25 µL
- Thermostating: 37°C
- Enzymatic reactions – initiated by the presence of endotoxins
- Detection – UV spectrophotometer



$$Proenzyme\ (LAL\ reagent) \xrightarrow{Endotoxins} Enzymes$$

$$\text{Chromogenic susbtrate} \xrightarrow{Enzymes} Peptide + p-nitroaniline\ (385-410\ nm)$$



Method

Equipment validation

→ **Use of the USP <85>/ Ph.Eur (2.6.14) Monographs.**

→ **Development of a validation form**

❖ **Products description**
- ✓ Product name, galenic formulation, qualitative and quantitative composition, route of administration

❖ **Method of administration**
- ✓ Dilution of the product, maximum single dose, maximum daily dose

❖ **1st Phase – Calculation**
- ✓ EL – Endotoxin limit

EL=K/M

K= Threshold pyrogenic dose of endotoxin per kg of body weight,
K= 5,0 UE/Kg (IV route) // K=0,2 UE/Kg (IT route)
M= Maximum recommended bolus dose of product per kg of body weight. When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.

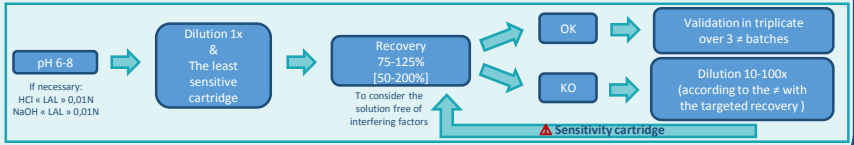
- ✓ MVD – Maximum valid dilution

MVD=EL/γ

γ = Refers to the lowest concentration in the standard curve.
The MVD will be determined for each type of cartridge. The more sensitive the cartridge is, the higher the MVD will be. The least sensitive cartridge should always be used to achieve the desired dilution.

Cartridge 10 → 0,1 EU/mL (γ=0,1)
Cartridge 5 → 0,05 EU/mL (γ=0,05)
Cartridge 1 → 0,01 EU/mL (γ=0,01)
Cartridge 0,5 → 0,005 EU/mL (γ=0,005)

❖ **2nd Phase – Determining the dilution to be validated**



→ **Drafting of the validation and use protocol for the equipment**

Results

- Validation of the equipment and the operating conditions (different for each preparation to be analyzed);
- Training for the manipulators to the use of this analytical tool in routine;
- 25 validated preparations;
- Gain in terms of:
  - Time: 15 minutes vs. 1 hour (gel clot);
  - Sample handling: semi-automated vs. manual ;
  - Accuracy: quantitative vs. qualitative/ semi-quantitative.

Discussion

As a pharmacist, we must guarantee the safety and quality of the preparations produced by the pharmacy.
The systematic endotoxins research in preparations intended for intravenous or intrathecal administration is an integral part of this permanent quality and safety objective. This is why the implementation of a quantitative method of endotoxins detection reinforces the safety and quality of the preparations made for our patients.