3PC-019

Development of a method for the quantification of bacterial endotoxins in parenteral vitamin-lipid emulsion preparations using the recombinant C factor

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CONTEXT

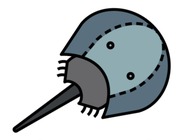
Medicines administered parenterally, such as intravenous lipid emulsions **(ELIV)** for nutrition in neonatal patients**, must be free** of **endotoxins.**

Bacterial endotoxin test **(BET)** is verified by European Pharmacopoeia (EP) methods :

- 2.6.14: Based on the reaction of horse-shoe crab (**HSC**) amoebocyte lysate (**LAL**)
- 2.6.32: Based on the cleavage of a fluorogenic peptide by recombinant factor C (**rFC**)

PROBLEMATICS

- **Interferences:** Lipids in the sample → opacity → reading disturbances



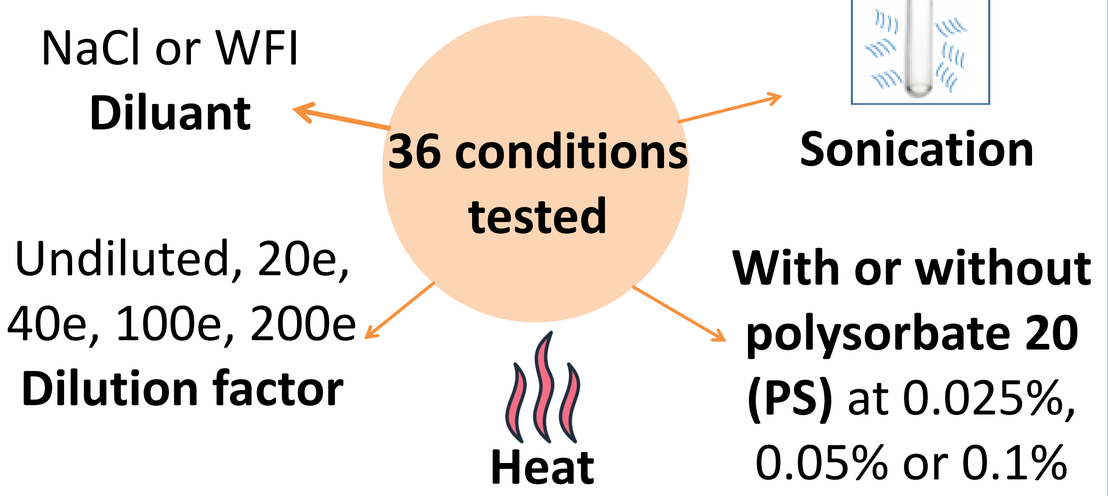
- LAL methods: **Ethical and environnemental impact:** HSC bleeding → 15-30% of mortality

OBJECTIVE

Develop a sample preparation method that would allow the quantification of BET in a lipid matrix using the rFC method

MATERIALS AND METHODS

Sample preparation

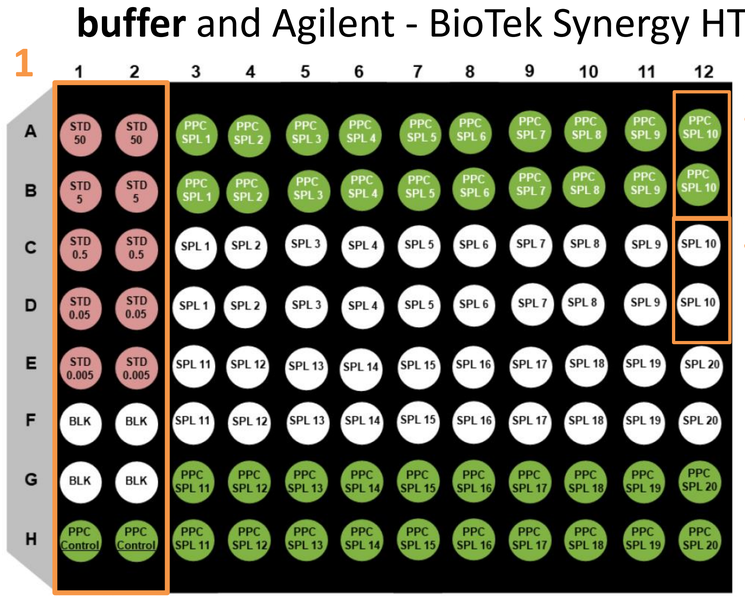


Validation with doped sample

ELIV were spiked at 0.01, 0.02, 0.03 and 0.05 EU/mL **to validate the absence of interference** (n=5)

Dosing and selection of optimal parameters

FLUORESCENCE MEASURED BY: ENDOZYME® II GO: rFC, fluorescent substrat, buffer and Agilent - BioTek Synergy HTX - ENDONEXT™ - bioMérieux



Specifications:

Spike recovery (SR): 50 - 200%

Coefficient of variation (CV): <25%

1 Calibration wells (0.005 to 5 EU/mL) with WFI

2 2 control wells (0.5 EU/mL) } with sample

3 2 sample wells

RESULTS

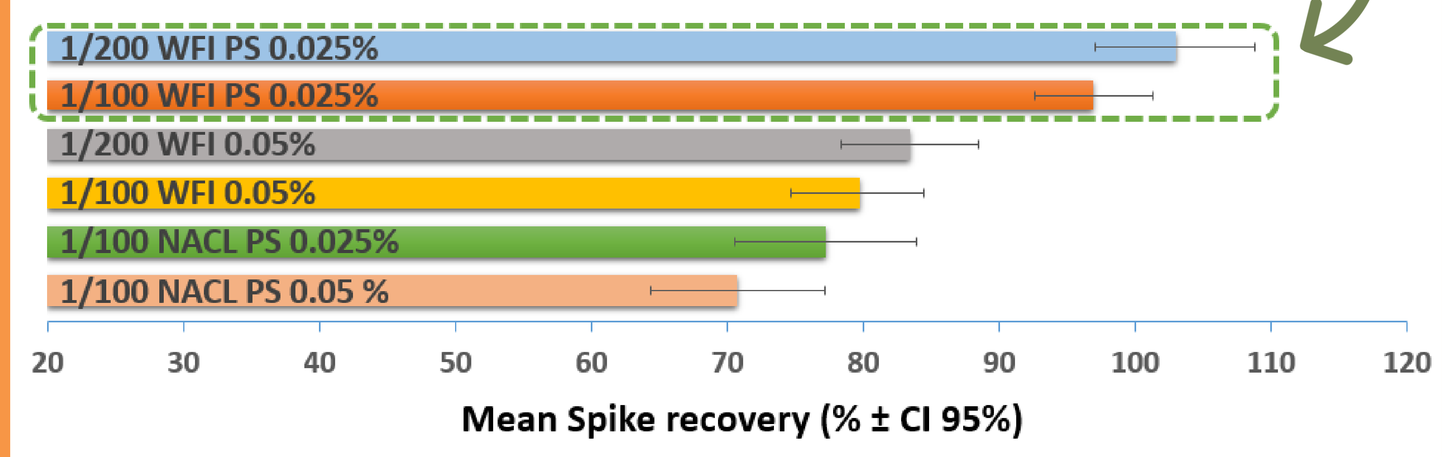
Sensitivity was set at 0.005 EU/mL in line with the **BET limit concentration** (3.3 EU/mL) and the **significant maximum dilution** based on 600 prescriptions for neonatal patients

36 conditions tested → **6 conditions complied to monograph 2.6.32 (with CV <25%)**

Selected parameters:

SR = 103% ± 6 **SR = 97% ± 4**

Average error percentage: 31% ± 6



DISCUSSION - CONCLUSION

BET quantification using the rFC method in ELIV was validated. Despite sensitivity-related underestimation, these dilutions meet BET-derived limits and **offer a more ethical and environemental friendly alternative to the LAL method.**

Limits : Greater handling time required than for conventional LAL equipment with Cartridge

Perspectives : This work could be adapted to other parenteral drugs such as magistral preparation for parenteral nutrition with various patient-adjusted intakes.