Hes·so
Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
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Master of Science HES-SO in Life Sciences

Low Endotoxin Recovery

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CHEMICAL DEVELOPMENT & PRODUCTION

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DESCRIPTION

Endotoxin (ETs) are harmful to humans and they are dangerous contaminants in biopharmaceutical products. It follows that health authorities regulate the manufactures of pharmaceutical / biotechnological products for human application and consumption that the final product must be free of ET. The allowed ET limits for medical devices are at 20 Endotoxin (EU)/ devices or 0.5 EU /mL for products directly or indirectly in contact with the cardiovascular or lymphatic system.

Low Endotoxin Recovery (LER) is defined as the failure to detect a known amount of spiked endotoxin in an undiluted product. Especially the Limulus amoebocyte Lysate (LAL) test, the most used ET quantification assay, is concerned by it .

The Kdo-DMB-LC assay developed at HES-SO Valais-Wallis, uses quantitative ET hydrolysis at elevated temperatures to release the ET specific sugar acid Kdo to measure ET concentration while the conventional LAL assay uses biological enzymes to detect ET.

OBJECTIFS

•In the frame of the master thesis, the LER effect in different pharmaceutical formulations / products should be compared between the conventional LAL test and the HESSO novel ET specific chemical analytical assay

•The following points should be addressed:

- Objective A: Define in which conditions LPS recovery in high - matrix load samples are around 100% for BSA, IgG and Lysozyme.
- Objective B: Experiment with modified matrices using the LAL test and the HESSO assay. The matrices are modified by having different incubation time and different treatment methods (temperature, pH).

RESULTS

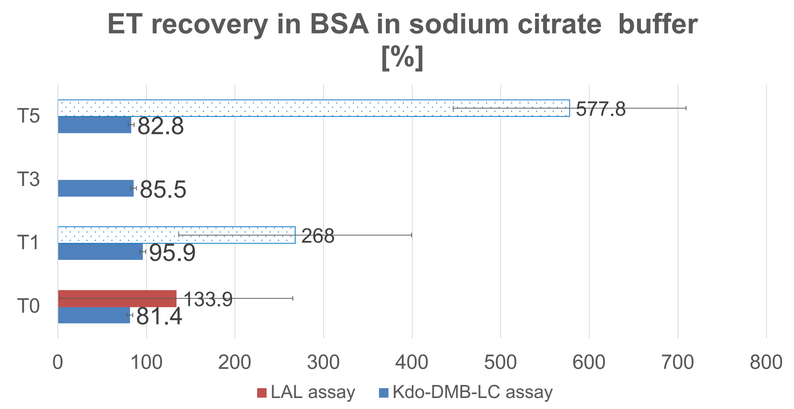

Figure 1 :Comparison of the LAL assay and the Kdo-DMB-LC assay ET recovery results for samples containing LPS from E.coli O55:B5 with c=500 ng per mL in BSA dissolved in sodium citrate on days 0,1,3,5

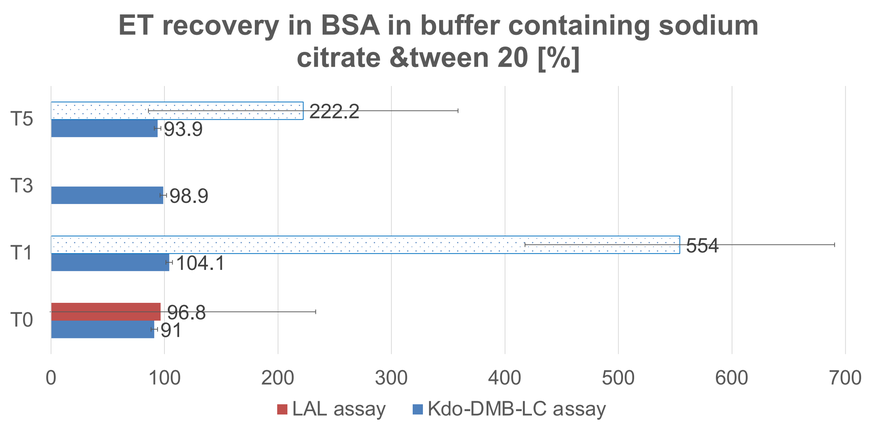

Figure 2:Comparison of the LAL assay and the Kdo-DMB-LC assay ET recovery results for samples containing LPS from E.coli O55:B5 with c=500 ng per mL in BSA dissolved in sodium citrate and tween 20 on days 0,1,3,5

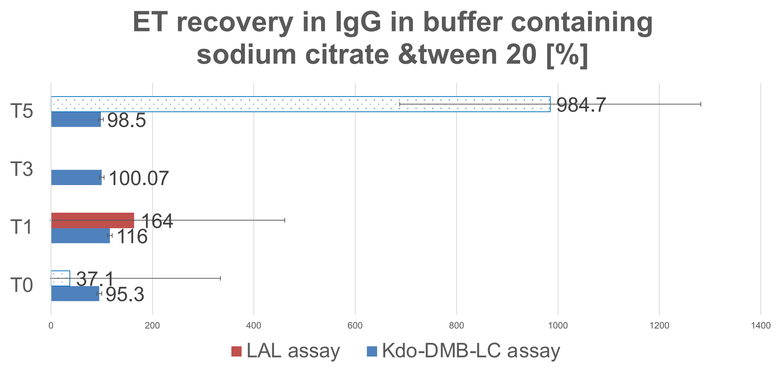

Figure 3:Comparison of the LAL assay and the Kdo-DMB-LC assay ET recovery results for samples containing LPS from E.coli O55:B5 with c=500 ng per mL in IgG dissolved in sodium citrate and tween 20 on days 0,1,3,5

CONCLUSION

- The Kdo-DMB-LC assay does not suffer from LER as it can detect ETs even after 5 days incubation. Moreover, the presence of surfactants in biopharmaceutical products helps the assay to better detect ET.
- The LAL assay suffers from LER as it cannot detect ETs even after 1 day of incubation. Longer incubation time tends to lead to worse recovery for the LAL assay
- The optimal hydrolysis condition for the Kdo-DMB-LC assay to maximize releasing Kdo are with 5µL acid acetic and either 120 min or 150 min of hydrolysis time.

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