

The Importance of Endotoxin Levels in Collagen Products

Introduction

Endotoxins unfortunately are often found as contaminants of protein products derived from bioprocesses and because of their strong biological effects at very low concentrations within the human body they are of extreme concern for clinicians and the pharmaceutical industry [1]. In patients even in small quantities endotoxins can result in fever, inflammation, sepsis, tissue damage and lead to death. For tissue and biomedical engineering endotoxins can trigger endotoxic shock, inflammation, or sepsis in animals and tissue culture.

Endotoxins, or lipopolysaccharides (LPS), are a heat-stable component of the outer layer of the cell wall that surrounds gram-negative bacteria, such as *Escherichia coli*. They are constantly released into the environment that the bacterial cells are growing in and when the cells die through natural or disinfection processes. The ubiquity of bacterial cells and by extension their endotoxins mean that it is of paramount importance to have processing and monitoring steps that ensure endotoxin levels are below a threshold within medical and pharmaceutical products.

Methods

ProColl's test for endotoxins is based on the Pierce Chromogenic Endotoxin Quant Kit [2] a Limulus amoebocyte lysate (LAL) assay relying on an extract from the blood of the Limulus polyphemus species of horseshoe crab. Currently the LAL-based method is the gold standard technique for detecting and monitoring the concentration of endotoxins in a biopharmaceutical product [3]. Indeed, most researchers also rely on the conventional LAL-based endotoxin detection method [4].

Product/Company	Endotoxin level	Method of determination	Processing
ProColl Bovine Collagen	< 0.2 EU/ml	LAL based	Sterile Filtered
ProColl Recombinant Human Collagen	< 0.2 EU/ml	LAL based	Sterile Filtered
Bovine Company α	< 0.5 EU/ml	Not given	Sterile Filtered
Bovine Company β	< 10 EU/ml	Not given	No Information

Results and Discussion

The table above presents the endotoxin levels determined using the LAL method for ProColl products in comparison with levels supplied for products offered by other companies. The production methods used by ProColl have ensured an endotoxin level much lower than that of similar products available in the market. The endotoxin levels of ProColl products (0.2 EU/ml) are significantly less than the industry norm of 0.5 EU/ml. Our review of the market unfortunately indicates that some manufacturers of collagen assume it is acceptable to supply collagen with high endotoxin levels when this indicator of purity and quality is so important for the subsequent clinical application of medical devices. The threshold level of endotoxin for intravenous applications is set to 5 endotoxin units (EU) per kg body weight per hour by all pharmacopoeias (European Pharmacopoeia, 1997). The term EU describes the biological activity of an endotoxin; 1 EU corresponds to 100 pg of endotoxin [5]. With the growing use of biomaterials such as collagen in tissue engineering applications, such as in 3D bioprinting, it is even more important that endotoxins are minimised as the quantity of collagen in medical devices increases.

References

1. Petsch D. and Anspach F.B. (2000) Endotoxin removal from protein solutions. *Journal of Biotechnology* 76: 97-119.
2. Thermo Scientific White Paper A highly sensitive assay for endotoxin detection and quantitation for a variety of sample types.
3. Schneier M., Razdan S., Miller A., Briceno M. and Barua S. (2020) Current technologies to endotoxin detection and removal for biopharmaceutical purification *Biotechnology and Bioengineering*. 117:2588–2609.
4. Dullah, EC and Ongkudon, CM (2017) Current trends in endotoxin detection and analysis of endotoxin-protein interaction *Critical Reviews in Biotechnology* 37: 251-261.
5. Kruger, D., 1989. Assessing the quality of medicinal products containing ingredients obtained by gene technology. *Drugs Made Germany* 32, 64–67.

If you are would like to hear more about our range of collagen products, please use the contact form on our website (www.procoll.co.uk) and one of the team will be in touch. ProColl's collagen is also available for bulk orders.