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Endotoxin Levels Matter

The Importance of Quality Proteins for Research, Development, and Manufacturing

INTRODUCTION

The development of cell and gene therapy products, often referred to as advanced therapy medicinal products (ATMPs), brings hopeful treatments and cures for patients worldwide. However, successful production of ATMPs requires the developers to strategically avoid contamination of the final drug product from adventitious agents. The World Health Organization defines adventitious agents as "contaminating microorganisms of the cell culture or source materials including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy agents, and viruses that have been unintentionally introduced into the manufacturing process of a biological product".[1]

Bacteria are often a concern for sterility in the manufacturing process, but even products proven to be sterile by growth promotion assays (USP <71>) can still contain bacterial endotoxins – a type of pyrogen. Endotoxins are lipopolysaccharides that are released during bacterial cell death or lysis, and they can cause multiple levels of concern for the ATMP developers. More specifically, cell culture is particularly susceptible to endotoxin contamination and can cause an upregulation or downregulation in the expression of proteins, such as anti-microbial factors, growth factors, and cytokines.[2] This contamination impacts the consistency of the manufacturing process and reliability of achieving a therapy with the desired yield and/or potency. Because endotoxins are also pyrogens, they can trigger severe inflammatory responses, septic shock, and death when administered to a patient downstream.[3] As a result, endotoxin testing is a mandatory safety component for the development of these drugs.

ENDOTOXIN TESTING GUIDELINES

ATMP guidelines are available from different regulatory agencies. The European Pharmacopoeia (EP) offers information on both raw materials for ATMPs (chapter 2.6.14) and gene transfer medicinal products (chapter 5.14).[4] In addition, both the European Medical Agency (EMA) and the United States Food and Drug Administration (FDA) have published several guidelines on the topic, highlighting the importance of endotoxin testing.[5, 6, 7] While ATMP manufacturers usually know the mandatory end-product release testing, it is worth noting that regulatory bodies specifically state that additional testing should be done throughout the manufacturing process of cell and gene therapy raw materials, specifically USP <1043> and ISO 20399:2022.

When testing incoming raw materials, a low endotoxin level should be ensured, including cell culture media as well as materials for collection, selection, culture, or modification (genetic/phenotypic) of cells. It is particularly important to identify raw materials that are more likely to have contact with bacteria. This includes those that utilize bacteria in their production, such as recombinant nucleic acids and proteins.[8]

It is also important to remember that any recombinant proteins used in ATMP manufacturing will be in contact with the cells that form the final drug product. Therefore, therapy developers should perform a risk assessment to ensure that they are sourcing materials with the lowest residual endotoxin levels and are testing the source materials using validated compendia methods and equipment. Additionally, it is advisable to use endotoxin-free lab ware and equipment and avoid bacterial contamination during the preparation and storage of the media.

The endotoxin criteria for raw materials for cell culture media may vary depending on the type of media, the source of the materials, and the intended use of the cell culture. However, some general guidelines are:

- The endotoxin level of the water used to prepare the media should be no more than 0.25 EU/mL
- The endotoxin level of the commercially prepared media should be no more than 1 EU/mL
- The endotoxin level of the non-animal-derived materials, such as recombinant proteins or synthetic peptides, should be no more than 0.5 EU/mL

The preferred assay to determine endotoxin levels is the limulus amebocyte lystate (LAL), which is completely harmonized between the different pharmacopeia monographs (USP, EP, JP), according to the 2012 revision of ICH Q4B annex 14.[9] This method is accepted by the Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Pharmaceuticals and Medical Devices Agency (PMDA), among others.[10] Details of this method are found in the USP monograph <85>, EP chapter 2.6.14, and JP 4.01. Additional information can be found in EP chapter 5.1.10 and USP chapter <1085>.

TECHNICAL NOTE

FUJIFILM BIOSCIENCES SHENANDOAH CTGRADE GMP PRODUCTS

FUJIFILM Biosciences takes endotoxin assessment very seriously to ensure that our products meet the stringent demands of the ATMP manufacturing community. Since LAL is recognized as the most sensitive and specific method available for detecting endotoxins, FUJIFILM Biosciences incorporates this method for its Shenandoah CTGrade GMP Recombinant Proteins. The LAL method outlined in USP <85>/EP 2.6.14 to detect bacterial endotoxin and quantitatively report the results can be found on our products' CoA.

Comparing the endotoxin levels of CTGrade GMP and commercially-available products:

GMP Cytokines	CTGrade	Competitor 1	Competitor 2	Competitor 3	Competitor 4
FLT-3 Ligand	< 50 EU/mg	≤ 50 EU/mg	< 50 EU/mg	< 10 EU/mg	-
IL-2	< 10 EU/mg	≤ 25 EU/mg	< 100 EU/mg	< 10 EU/mg	< 25 EU/mg
IL-3	< 50 EU/mg	≤ 50 EU/mg	< 200 EU/mg	< 100 EU/mg	-
IL-6	< 50 EU/mg	≤ 50 EU/mg	< 1,000 EU/mg	< 100 EU/mg	-
IL-7	< 50 EU/mg	≤ 25 EU/mg	< 200 EU/mg	< 100 EU/mg	-
IL-10	< 50 EU/mg	≤ 50 EU/mg	-	-	-
IL-15	< 50 EU/mg	≤ 25 EU/mg	< 400 EU/mg	< 100 EU/mg	< 25 EU/mg
IL-21	< 50 EU/mg	≤ 25 EU/mg	< 200 EU/mg	-	< 25 EU/mg

Reported unit in product certificate of analysis (COA) is EU/ug

CONCLUSION

Developing and getting regulatory approval for a new ATMP is challenging. By selecting a recombinant protein supplier that can ensure low endotoxin levels, the final product will be one step closer to meeting the quality requirements.

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