

Endotoxins in serum

Introduction

Endotoxins levels in serum for cell culture are important for pharmaceutical, veterinary and clinical manufacture and also in some research applications where cells are sensitive to the presence of endotoxins.

Serum collection

It is important that serum, specifically Foetal Bovine serum (FBS), is collected in such a way to ensure that the serum maintains low levels of endotoxin. Endotoxin may be an indicator of the care taken during the collection and processing of the material.

To maintain low levels of endotoxin it is important to maintain low temperatures throughout the production. Although the blood is usually collected into single-use sterile blood bags, the environment for the collection of the material is non-sterile giving rise to the potential for bacterial contamination. Once processed aseptically any bacteria contamination will be removed but between collection and processing it is important to maintain serum at low temperatures to minimise the potential for bacterial activity with the resulting formation of endotoxins.

Endotoxins

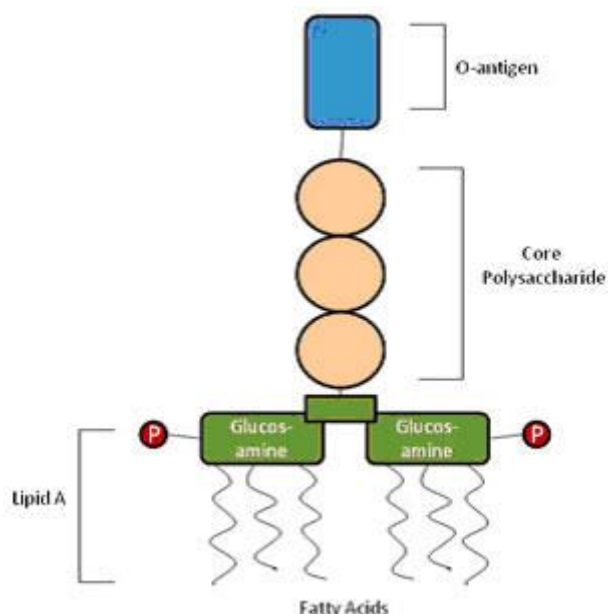


Figure 1. Diagram of an endotoxin 5

In mammals, endotoxins can cause fever and chills and in more extreme responses, septic shock. Their presence in media for cell culture can be a problem. An endotoxin is a lipopolysaccharide present in the outer membrane of most gram-negative bacteria, like *E.coli*. (A single *E.coli* contains about 2 million lipopolysaccharides.) While the cell is alive and intact, the endotoxins are mostly held in the cell membrane. Bacteria only shed small amounts of endotoxin into their surrounding environment when alive. However, they shed large amounts into their surroundings when they die. The process of filtration to remove the bacteria would then cause the release of endotoxin into the serum.

Other sources of endotoxins include laboratory glass and plasticware, as endotoxins can adhere strongly to glass and plastics, water (ultrapure water is a necessity) and humans, since endotoxins may be transmitted by direct contact.

Serum is a biological environment manufactured to support cell growth and it is therefore an ideal medium for bacterial growth, specifically those pathogens that infect warm-blooded animals and grow at body temperature. Such bacterial pathogens are mesophiles.

Bacterium	Generation Time (minutes)
<i>Escherichia coli</i>	17
<i>Bacillus megaterium</i>	25
<i>Streptococcus lactis</i>	26
<i>Streptococcus lactis</i>	48
<i>Staphylococcus aureus</i>	27-30
<i>Lactobacillus acidophilus</i>	66-87
<i>Rhizobium japonicum</i>	344-461
<i>Mycobacterium tuberculosis</i>	792-932
<i>Treponema pallidum</i>	1980

Figure 2. Table of generation doubling times for common bacteria under optimum growth conditions 1

Temperature is a major environmental factor in controlling bacterial growth. The range of temperature at which growth is possible varies very widely among bacteria. The diagram below displays the optimal temperature growth range for different categories of bacteria.

Classification of Microorganisms by Temperature Requirements

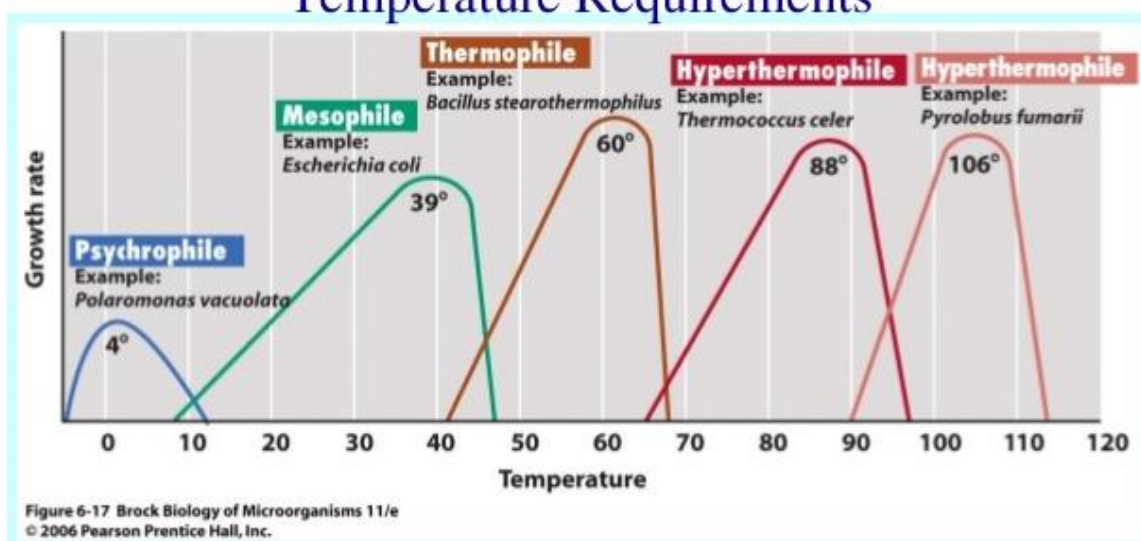
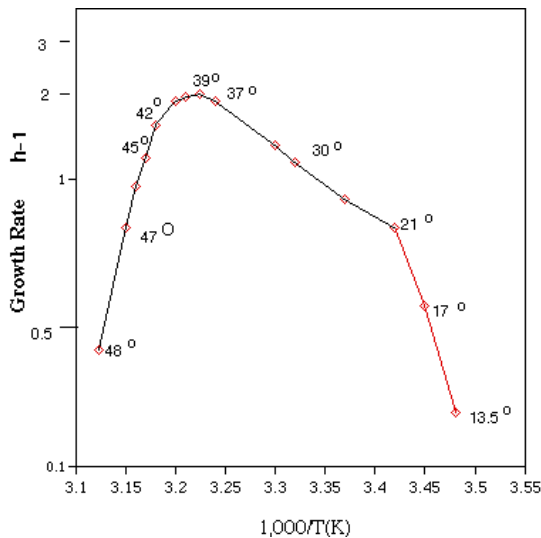


Figure 3. Temperature growth range for different microorganisms 2



As can be seen from Figure 3, the optimal growth temperature for a mesophile is 39°C. The growth rate declines below 39°C and drops sharply above it.

As can be seen in Figure 4, *E.coli* growth drops sharply below 13°C and above 48°C.

Figure 4. Graph depicting the effect of temperature on the growth rate of *E.coli* 3

The chemical and nutritional components in serum can be adversely affected by an increase in temperature. Processed serum should be stored at low temperatures, +4°C for short-term storage and -20°C for longer-term storage. Mesophiles are also growth inhibited below 8°C. It is important therefore that unprocessed serum are kept at -20°C (+/- 10°C). Defrosting of unprocessed serum is conducted at control temperatures to ensure that the serum does not achieve a temperature of above +4°C. The defrosting process recommends that a small remnant of ice remains in the bottle just before the pooling stage to ensure that the liquid maintains temperature.

Precipitation in serum

Multiple freeze/thaw cycles should be avoided as this will degrade the serum/plasma nutrients and could induce the formation of cryo-precipitates. Small amounts of cryo-precipitates are not uncommon and will not affect product performance. When thawed incorrectly, significant amounts of cryo-precipitates may form and these are usually insoluble and will require centrifugation or filtration to remove. Filtration of the serum may also lead to the loss of nutrients such as growth factors, mitogens and other proteins.

References

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