

**BURECO AG is a GMP and GLP certified bioanalytical service organization for the Life Science Industry worldwide. We offer biochemical, radiometric, immunological and cell based analytical technologies since 1992.**

## PYROGEN TESTING

### LIMULUS AMEBOCYTE LYSATE (LAL) TEST

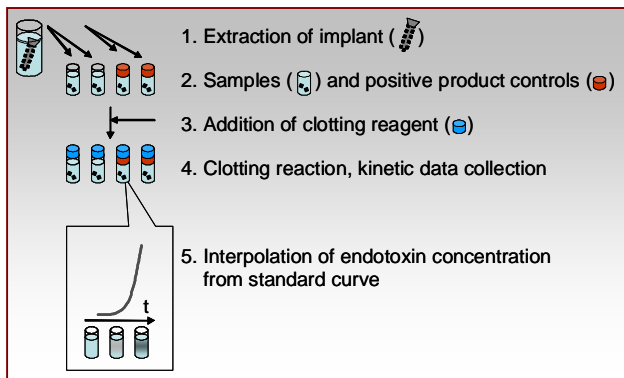
BURECO AG conducts turbidimetric or chromogenic kinetic Limulus Amebocyte Lysate (LAL) tests compliant with EU and U.S. GMP guidelines <sup>(1-4)</sup>. The test can be applied for release and in process control testing of pharmaceutical active ingredients and final products, as well as for environmental monitoring. A project specific validation of the LAL test may be conducted as needed according to the relevant guidelines by inhibition and enhancement testing.

### ENDOTOXIN EXTRACTION (MEDICAL DEVICES)

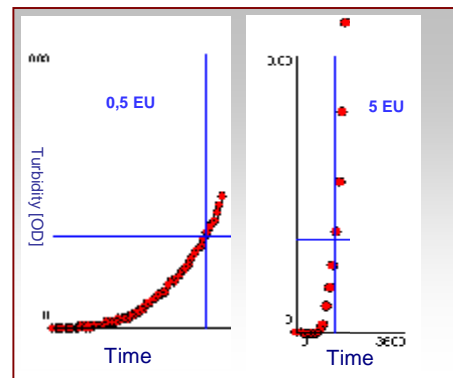
BURECO AG has established a proprietary extraction protocol to assess the endotoxin load of medical devices, e.g. implants. The method is able to detect concentrations of endotoxins below the requirement of 20 endotoxin units (EU) on the surface of metallic implants. This detection limit can usually not be reached by applying the standard LAL protocol for medical devices <sup>(2)</sup>. BURECO AG offers a project specific validation of the method in accordance with the listed guidelines <sup>(2)</sup> that enables testing of medical devices. Testing of samples can be conducted under GMP compliance after project specific validation.

### ASSAY TECHNOLOGY FOR ENDOTOXIN DETERMINATION

The quantification of the endotoxin contamination on implants by BURECO's LAL assay is based on a kinetic-turbidimetric measurement. The method is compliant with USP <161> Transfusion and Infusion Assemblies and Similar Medical Devices, the European Pharmacopoeia (2.6.14) and associated guidelines <sup>(1-4)</sup>. The assay principle is described in Fig. 1, and Fig. 2 shows examples of increasing turbidity of positive product controls (spiked sample). In order to detect even low amounts of endotoxin the method has been optimized for a working range spanning from 0.01 EU/mL to 5 EU/mL of standard endotoxin.



**Fig 1: Assay principle**

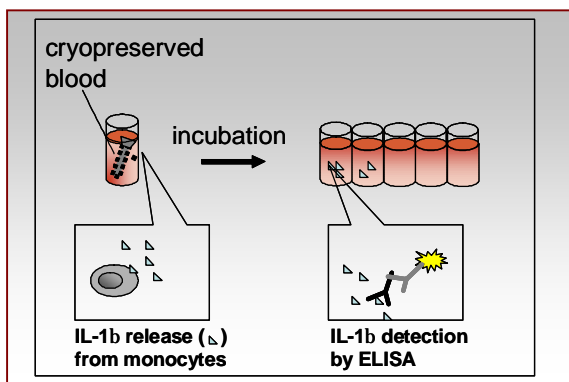


**Fig 2: Increasing turbidity with 0.5 EU/mL and 5 EU/mL**

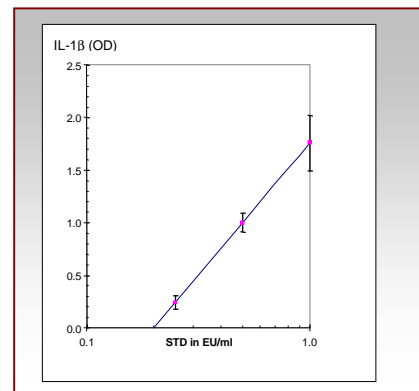
## IN VITRO PYROGEN TESTING (IPT)

Bacterial endotoxins (lipopolysaccharides) that can be detected using the LAL test are the pyrogens that pose the greatest safety risk for patients by causing fever, shock and death. There are other pyrogens, particularly associated with biologics or with medical devices, that cannot be detected by the LAL test. These non-endotoxin pyrogens have classically been detected using the rabbit pyrogen test. In recent years, different alternative pyrogen test methods have been developed that are based on the stimulation of cytokine release by human monocytes<sup>(5, 6)</sup>. These in vitro tests yield more accurate results, are faster and do not require animals. BURECO AG offers alternative pyrogen tests, e.g. the in vitro pyrogen test provided by Charles River Laboratories, which is based on the use of cryopreserved human blood and the release of interleukin-1 $\beta$ .

The assay principle is shown in Fig. 3 and an example of a standard curve is given in Fig 4.



**Fig 3: Assay principle**



**Fig 4: Standard curve (IL-1b)**

- 
- Ref. 1 European Pharmacopoeia 2.6.14, Bacterial Endotoxins  
Ref. 2 FDA Guideline on Validation of the Limulus Amebocyte Lysate Test as an End Product Endotoxin Test for Human and Animal Parental Drugs, Biological Products, and Medical Devices; December 1987  
Ref. 3 FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL Techniques; July 15, 1991  
Ref. 4 USP <85>, Bacterial Endotoxins Test  
Ref. 5 Hoffmann, S. et al. (2005), International Validation of Novel Pyrogen Tests Based on Human Monocytoid Cells; J. Immunol. Meth. 298, 161 – 173  
Ref. 6 Schindler, S. et al. (2006), International Validation of Pyrogen tests Based on Cryopreserved Human Primary Blood Cells; J. Immunol. Meth. 316, 42 – 51
-