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Pyrogenicity, Endotoxin and Monocyte Activation Testing

Pyrogen and endotoxin detection is offered as part of our process manufacturing support network and lot release testing services as a vital step before a product is released to the market. Charles River prides itself on providing you with services to advance your drug development efforts, which is why we offer a number of different testing methods to ensure that you are able to select the option most conducive to your pyrogen and endotoxin detection needs.

Services Available

- *In vivo* pyrogen testing
- *In vitro* bacterial endotoxin testing
- Monocyte activation testing

Pyrogenicity

With over 30 years of experience, Charles River offers *in vivo* pyrogen testing services as an essential part of the quality control process to clients. Our pyrogen testing is compliant with both USP <151> and EP 2.6.8 standards. Test items include protein-based material, non-protein material and blood products. Testing is performed in a dedicated Good Manufacturing Practice (GMP)-compliant facility using specific pathogen-free (SPF) rabbits. The rabbit pyrogen test remains a viable mammalian test model to use when testing for non-endotoxin pyrogens and a variety of products for which the LAL method is limited. One-time pyrogen testing may also be required by regulatory authorities to support routine use of endotoxin tests. Our pyrogen testing is supported by a team of experienced technical staff that ensures fast sample turnaround in a GMP-compliant facility.

Endotoxin

We also offer *in vitro* bacterial endotoxin testing. All tests are performed to meet all of the pharmacopoeia requirements, including gel-clot (qualitative), and turbidimetric kinetic and chromogenic (quantitative) methods. We provide preliminary screening and validation of products as well as a backup technical service to clients.

Monocyte Activation Testing

Charles River is committed to expanding our portfolio with *in vitro* alternatives to *in vivo* tests. We now offer the monocyte activation test (MAT) according to EP 2.6.30. The MAT works by predicting the human response to pyrogens on the basis of human fever rather than animal models. This test may be used as an alternative to the rabbit pyrogen test. The MAT can test for Gram-positive and Gram-negative organisms, other biological pyrogens (e.g., yeast) and parasitic and viral pyrogens.

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Expansion of the current services provides clients with the opportunity to work closely with our team of experienced staff for release of their product and to explore alternative methods to assess the pyrogenicity of their materials. Following a product-specific validation, the MAT may be used for products such as drugs that affect body temperature regulation (e.g., antipyretic drugs and steroids), drugs that cause immunological reactions (e.g., immunoglobulins), detergents and some blood-derived products (e.g., stem cells). The MAT can also be considered in place of other methods currently used to test products that are turbidimetric, strongly colored or interfere with clotting. The MAT quantifies substances that activate human monocytes or monocytic cell lines to release endogenous mediators, such as pro-inflammatory cytokines.

Charles River currently assesses pyrogenicity using the Biotest PyroDetect System. The kit uses cryopreserved human blood as the source of monocytes. These immune cells recognize pyrogens and respond by releasing fever-inducing signal molecules such as interleukin-1 beta (IL-1β). IL-1β is a molecule produced in response to Gram-negative, Gram-positive and other infectious/inflammatory challenges. It possesses a wide spectrum of immunologic and nonimmunologic activities including the capacity to induce fever. The released IL-1β is detected by ELISA. A spectrophotometer can then be used to measure the amounts of IL-1β produced. The assay may be made quantitative by using an endotoxin standard, or semi-quantitative results may be compared to a reference lot.

EP 2.6.30 allows the use of other approaches to the MAT and these can be developed in collaboration with Charles River. Human blood can be substituted with human monocytic cell lines and the release of other pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) or interleukin-6 (IL-6), are quantified by ELISA. A product-specific validation with the developed method is offered.

Pyrogen Detection Application Suitability

		Rabbit Pyrogen Test	Endotoxin (LAL)	MAT
		Principle of Test		
Detectable Pyrogens		Fever Reaction Mammal	Defense Mechanism Arthropoda	Fever Reaction Human
	Gram-negative	+	+	+
	Gram-positive	+	-	+
	Fungi	+	-	+
	Virus	+/-[1]	-	+
Applications	Pharmaceuticals	+	+	+
	Biologicals	+	+/-[2]	+
	Blood components	-	-	+
	Cellular products	-	+/-	+
	Air pollutants	+[3]	+/-[3]	+
	Medical devices	+[3]	+/-[3]	+

[1]Variable pyrogenic responses [2]Rabbit testing often required [3]Can only be tested indirectly by extracting device or filter with pyrogen free water or saline

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