Pharma&Biotech

Testing Oil Solutions with PYROGENT™ Gel Clot LAL Assay

Technical Tips

By Scientific Support, U.S.

Oil-based parenterals have always been a challenge in endotoxin detection assays. Below, a liquid-liquid extraction procedure is described that can be used to remove the endotoxin from the oil into an aqueous phase, which can then be tested in a conventional manner. Water alone is usually not sufficient for this extraction. Endotoxins, due to their lipid character, tend to remain associated with oils and do not readily enter the aqueous phase of water-oil mixtures. The use of PYROSPERSE™, a dispersing agent, allows for dissociation of the endotoxin from the oils and for subsequent detection of endotoxin in the aqueous phase by Limulus Amebocyte Lysate (LAL).
The procedure has to be validated and, if necessary, adapted for different oily samples.

Materials

- PYROGENT™ Gel Clot LAL Assay
- PYROSPERSE™ Dispersing Agent
- LAL Reagent Water (LRW) – LAL Reagent Water is equivalent to Water for Bacterial Endotoxins Test (BET)

Test Procedure

1. Place 5 ml of oil sample into a 16 x 150 mm screw cap depyrogenated culture tube or similar glass container that can be sealed.
2. To the oil, add 5 ml of LRW and 0.1 ml of PYROSPERSE™ Dispersing Agent.
3. Shake the tube vigorously in a back and forth motion until the solutions form a homogenous mixture (approximately 30 minutes). Then, before the phases have had a chance to start separating, vortex the tube for 30 seconds to allow the solutions to extract thoroughly.
4. The two layers can now be allowed to separate on their own or they can be centrifuged. After the layers have separated, remove a sample of the aqueous phase and perform the PYROGENT™ Gel Clot LAL Assay as described in the Package Insert.

Controls

An acceptable qualification procedure is outlined below.

1. Prepare the endotoxin dilution series in the oil in the same manner as which the control standard endotoxin series is made.
2. Extract the 1.0 EU/ml, 0.5 EU/ml, 0.25 EU/ml, 0.125 EU/ml and 0.06 EU/ml endotoxin-oil solutions with LRW and PYROSPERSE™ Dispersing Agent as outlined above.
3. Test the oil extracts with lysate and compare the oil extract endpoints with the endpoints obtained from the water control dilution series. If the endpoints do not differ by more than a two-fold dilution and are between 2 λ and 0.5 λ, the oil can be tested with lysate per this procedure.

www.lonza.com/pharmabiotech
www.lonza.com/gelclot

Contact Information

North America
Customer Service: 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

Lonza Walkersville, Inc. – Walkersville, MD 21793

TS-PyrogentOil 05/13 RT-DS008