The Road to Next Generation LAL Technology

"We stand at the portals of a new age for the bacterial endotoxins test with amazing results of recombinant DNA technology"

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COLUMN ARTICLE

The Limulus Amoebocyte Lysate (LAL) test is the single most sensitive and practical technique for the detection of trace amounts of bacterial endotoxins (lipopolysaccharides, or LPS), and is the official method to replace the rabbit pyrogen test for evaluations of parenteral drugs, biological products, and medical devices. Since the late 1970s, these tests have been successfully formulated and commercialized by US and Japanese LAL manufacturers. Thereafter, it was demonstrated that an elaborate cascade pathway for endotoxin-induced LAL coagulation consists of three sequential activations of hemolymph serine protease zymogens (endotoxin sensitive Factor C, Factor B and proclotting enzyme) (left side of Figure). On the other hand, LAL was found to be also capable of sensitively reacting with (1→3)-β-D-glucan, a major fungal cell wall component (right side of Figure).

In the 1990s, Muta and Iwanaga successfully accomplished the cDNA cloning of Japanese horseshoe crab coagulation factors. Based on the findings, Ding et al. of National University of Singapore developed a new endotoxin-specific assay (PyroGene) introduced by Cambrex (now Lonza) using recombinant factor C (rFC) alone from Southeast Asian horseshoe crab (*Carcinoscorpius rotundicauda*). Recently, two German companies, Hyglos and Haemochrom Diagnostica GmbH, launched the rFC-based endotoxin assay kits comparable to PyroGene using different species of horseshoe crab. More recently, a novel chromogenic LAL reagent containing all of the recombinant factors from horseshoe crab and a chromogenic substrate, Boc-Leu-Gly-Arg-pNA has newly been developed, which may lead to the creation of a next generation LAL alternative. Thus, the new endotoxin assay kit 'PyroSmart' (Seikagaku Corporation) was released in December 2015 based on the collaboration between Seikagaku Corp. and Kyushu University. PyroSmart is the first reagent kit having very high sensitivity (0.001 EU/mL in 30-min reaction), which is prepared by recombining all the recombinant serine protease zymogens from horseshoe crab (*Tachypleus tridentatus*) involved in the coagulation cascade.

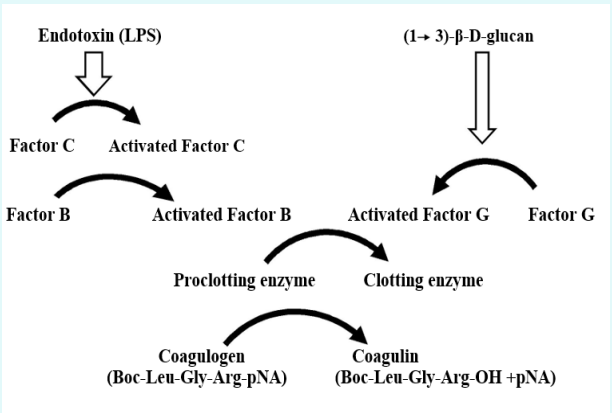

Figure: Scheme of two coagulation pathways in horseshoe crab amebocyte lysate and the principle of the LAL test.

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In addition, rFC-based assays have been introduced as a valid alternative method in the FDA “Guidance for Industry - Pyrogen and Endotoxins Testing: Questions and Answers”. It is stated that such alternative procedures and methods should be validated, as described in the USP General Chapter <1225> Validation of Compendial Procedures and should be shown to achieve equivalent or better results. Additionally, the revised chapter by the European Pharmacopoeia (EP, 5.1.10) is supposed to be effective this year. We need to focus on the amazing results of recombinant DNA technology and regulatory trends in animal-free LAL alternatives.

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