



## SLP's Use in the Pharmaceutical World *Introduction to Silkworm Larvae Plasma (SLP)*

The silkworm, *Bombyx mori*, has an interesting defense against bacterial and fungal invasion. This defense is found in the hemolymph and cuticle of the insect, as well as various other insect species as well. The defense mechanism involves a chemical cascade, the pro-phenol oxidase (proPO) cascade, that ultimately results in melanin formation around the foreign matter within the insect's body. When one thinks of melanin, the first thing that comes to mind is the tanning process in basal skin cells in response to ultraviolet light from the sun. This response is not simply aesthetic – the formation of melanin in human cells is a protective mechanism guarding the cells' DNA against the ionization dangers of the radiation. In a similar manner, melanin is formed in the insects' bodies to contain foreign matter.<sup>1</sup>

The proPO cascade can be activated by one of two unique receptor proteins. One is activated by peptidoglycan (PG), and the other is activated by (1, 3)- $\beta$ -D-glucans (BG). These two molecules have three unique aspects that will be highlighted. First, these molecules are each signature components of different microorganisms. PG is a component of bacterial cell walls, and BG is a component of fungal cell walls. As a result, the proPO cascade is a defense against a wide variety of microorganisms. Second, this defense mechanism is not specific to living organisms. Any piece of bacterial or fungal matter containing PG or BG activates the proPO cascade. Third, these molecules themselves are pyrogens, fever producing substances. These three principles result in a means of detecting the presence of bacteria and fungi rapidly with no need of live cultures or the detection of an immune assay. Collection and formulation of the SLP has resulted in a rapid BG and PG detection method.<sup>1</sup>

### Exogenous Pyrogens

By definition, a pyrogen is any substance that initiates a human immune febrile response. Exogenous pyrogens are therefore foreign substances that initiate a febrile response in the human body. By far, the most problematic pyrogen is bacterial endotoxin – the LPS molecule of the outer membrane of gram-negative bacteria. This pyrogen's potency as well as resilience to sterilization techniques has prompted the pharmacopeial standard that all parenteral substances need be tested for bacterial endotoxin (USP 85). However, endotoxin is not the only form of pyrogen produced by microorganisms.<sup>2</sup>

PG and BG are both exogenous pyrogens. BG is easily detected long before the level of pyrogenicity is reached by the LAL assay – the same test method used to detect bacterial endotoxin. PG, however, is not detected by any pharmacopeial test. Problems with PG have arisen. Most notably, PG contamination of Icodextrin used in peritoneal dialysate led to negative patient effects. Since PG is typically not detectable apart from the detection of the living bacteria themselves, residual PG in a product after sterilization has eliminated the living producers of the PG can

remain both problematic and undetectable. The SLP assay can fill this need for both icodextrin production as well as any product that has the potential to lead to a human immune response due to the presence of undetected PG.<sup>3</sup>

### SLP's Potential in Bacteria Detection and Identification

The SLP assay's unique features has led to the exploration of several novel applications of the assay. One area that has seen much research in the past two decades has been the potential as a diagnostic tool to provide rapid detection and broad identification of bacterial infections. Meningitis can be bacterial or viral. Studies have indicated that the rate of gram-positive bacteria causing meningitis are on the rise, and the treatment of the disease is specific to the cause of the meningitis.<sup>4</sup> The use of the SLP assay along with the LAL assay can be used to detect the presence of bacteria as well as provide the broad classification of gram-positive or gram-negative, all within an hour. Since PG is found in both gram-positive and gram-negative bacteria, the SLP test can be seen as a broad test for the presence of bacteria in general. The application of the LAL test can then be used to judge between the presence of gram-positive and gram-negative bacteria since the LPS molecules are only found in gram-negative bacteria. Studies have shown accuracy rates of around 90% of the SLP in detecting the PG of gram-positive bacteria in this situation.<sup>4</sup>

However, caution is raised since this test is an indirect means of detecting gram-positive bacteria. Specifically, the presence of peptidoglycan does not perfectly directly align to gram-positive bacteria counts of the sample. Because of this much research is still needed in the potential of the SLP assay in conjunction with the LAL assay to provide rapid, valuable data on the nature of a bacterial infection.<sup>5</sup>

### SLP's potential in peptidoglycan detection

SLP, as mentioned above, already has a proven track record in fulfilling the need of PG detection in the manufacture of icodextrin for peritoneal dialysis solutions. SLP shows the same potential for any parenteral product that is at risk of harmful effects from PG contamination. In similar manner to the adoption of LAL reagents to fill the need for the test of bacterial endotoxins, the need has already risen for one product, and may arise for other products, for the need to test for PG as an exogenous pyrogen. The risk of undetected PG, although less in magnitude than the risk of endotoxin contamination, is present as a member of the category of exogenous pyrogens.

### Potential users of SLP

The potential uses of SLP lead to many areas of manufacturing that may benefit from the use of the SLP assay to ensure that their product contains no peptidoglycan. Potential users could fall in one of three categories. First, manufacturers of products that need a test for bacteria may benefit from the use of the SLP reagent. As a product of bacteria, a test for peptidoglycan may provide a sensitive, rapid test for the presence of bacteria.

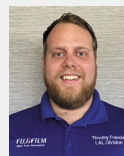


Second, as an exogenous pyrogen, manufacturers will want to ensure that their product remains peptidoglycan free as well as endotoxin free. A third potential user of SLP may be research institutes that may wish to explore the potential of the SLP reagent to detect peptidoglycan in a diagnostic setting. The manufacturers and distributors of the SLP reagent, FUJIFILM Wako Pure Chemicals in Japan and FUJIFILM Wako Chemicals U.S.A., are willing to assist and provide samples to any users that may potentially benefit from the adoption of the SLP reagent for the detection of peptidoglycan.<sup>6</sup>

## REFERENCES

1. <https://academic.oup.com/femspd/article/28/1/49/486267>
2. [https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q06\\_current\\_webpage\\_stage\\_6\\_monograph\\_23\\_nov\\_2011.pdf](https://www.usp.org/sites/default/files/usp/document/harmonization/gen-method/q06_current_webpage_stage_6_monograph_23_nov_2011.pdf)
3. [https://www.researchgate.net/publication/9021064\\_Exposure\\_to\\_the\\_Peptidoglycan\\_Contaminant\\_in\\_Icodextrin\\_May\\_Cause\\_Sensitization\\_of\\_the\\_Patient\\_Maintained\\_on\\_Peritoneal\\_Dialysis](https://www.researchgate.net/publication/9021064_Exposure_to_the_Peptidoglycan_Contaminant_in_Icodextrin_May_Cause_Sensitization_of_the_Patient_Maintained_on_Peritoneal_Dialysis)
4. <https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1348-0421.2003.tb03439.x>

5. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5195888/>
6. <https://www.wakopyrostar.com/lal-product-line/peptidoglycan-and-v-glucan-detection-reagent/slp-hs-single-reagent-set/>



## Timothy Francis

Timothy Francis is the Technical Specialist for the LAL Division of FUJIFILM Wako Chemicals U.S.A. Corporation. He comes into the Technical Specialist role with 5 years of experience teaching the natural sciences at a college level. He is proficient at taking the complex, technical aspects of a topic and breaking them down into clear, understandable pieces that all connect back to the big picture. He draws upon this experience to provide professional technical support and training for the PYROSTAR™ line and to help you with your technical needs. Outside of the work environment, he enjoys backyard astronomy, disc golf, kayaking, and enjoying time with his wife as they care for their pets.