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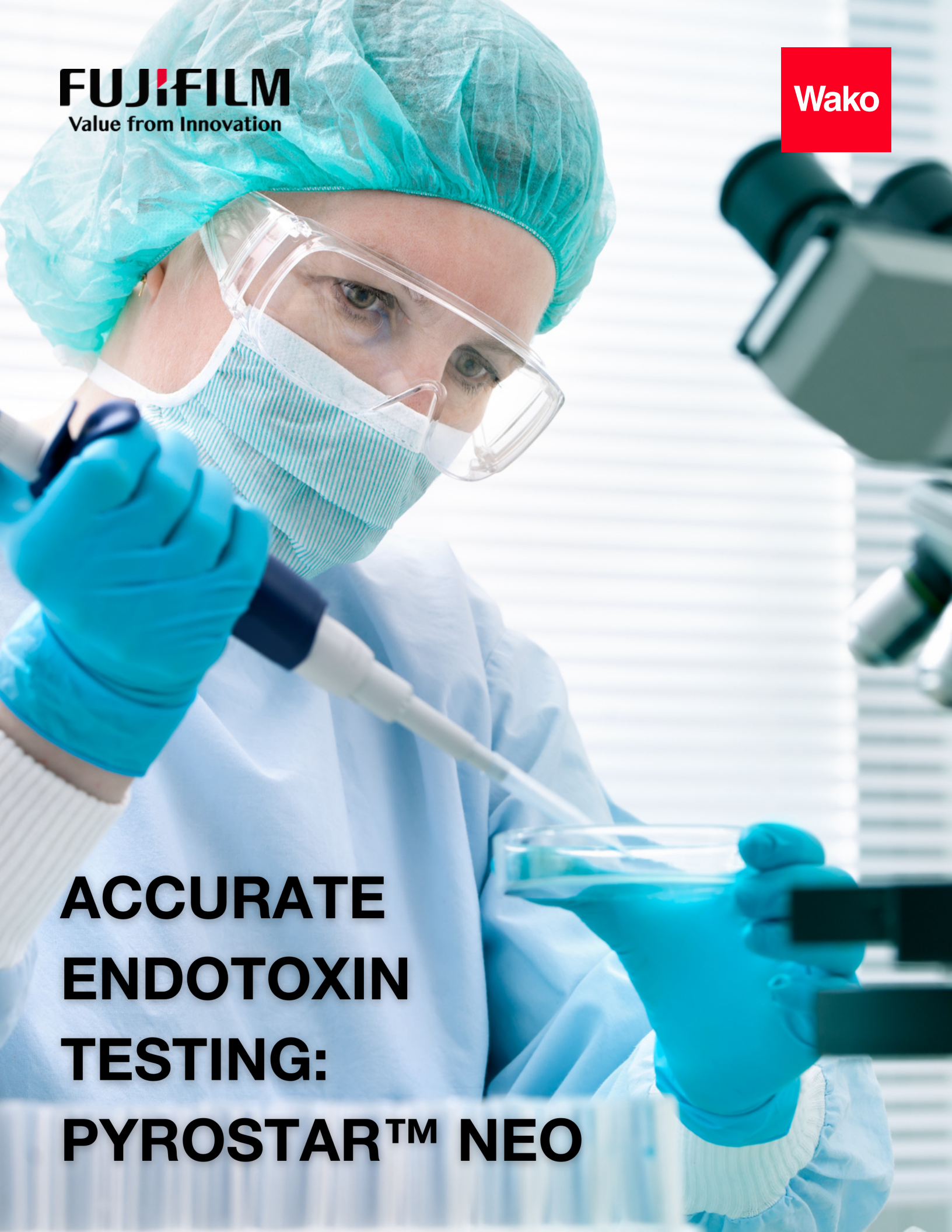

ACCURATE ENDOTOXIN TESTING: PYROSTAR™ NEO

INTRODUCTION

Endotoxins are a component of the outer membrane of gram-negative bacteria. They are released from bacteria primarily during cell lysis and are ubiquitous in the environment. Moreover, endotoxins are strong pyrogens, and, if they gain access to the bloodstream, may cause fever, inflammation, septic shock, and hemorrhage. Because of the iniquitousness and pronounced pyrogenic activities of endotoxins, injectable pharmaceuticals and medical devices are routinely tested for endotoxin contamination.

Historically, the in vivo rabbit pyrogen test was an early method for pyrogen testing, which also detected, but was not specific for, endotoxins. However, it has largely been replaced by the in vitro Limulus amebocyte lysate (LAL) assay, which is specific for endotoxins, and, in some instances, by the monocyte activation test, which detects all pyrogens (Suvarna et al., 2015).

The LAL assay has been established as the standard method for endotoxin testing, which is both sensitive and specific. It relies on the LAL reagent isolated from amebocytes of the Atlantic horseshoe crab (Limulus polyphemus). When the LAL reagent is incubated with endotoxin, the coagulation cascade is activated, and a clot is formed. Notably, the LAL assay can be performed as a qualitative gel-clot test or as a quantitative, chromogenic or turbidimetric test.

Despite the excellent sensitivity and specificity of the traditional LAL assay, it requires the use of horseshoe crab amebocytes. Therefore, there have been efforts to develop a synthetic version of the LAL test. An assay relying on recombinant factor C, which is the initial component of the horseshoe crab clotting cascade, was the first synthetic LAL method. However, FUJIFILM Wako focused on the development of a recombinant LAL assay that more closely reflects the effects of natural LAL and engineered recombinant proteins to recreate the entire coagulation cascade.

THE TECHNOLOGY OF PYROSTAR™ NEO

PYROSTAR™ NEO is a new endotoxin detection reagent that provides a horseshoe-crab free alternative to the Limulus Amoebocyte Lysate (LAL) Reagent. This reagent is an example of the groundbreaking alternatives produced in recent years in providing quantitative endotoxin testing by purely synthetic means.

PYROSTAR™ NEO has been engineered to mimic the natural activation pathway of LAL as closely as possible. LAL has a cascade pathway of four proteins that naturally creates a clotting effect in the presence of bacterial endotoxin (the LPS component of the outer membrane of Gram-negative bacteria). The activation of the fourth protein, coagulogen, is what produces coagulin, resulting in the traditional gel clot of the LAL assay. By measuring the increase in turbidity as the insoluble coagulin forms, kinetic techniques allow for the quantification of endotoxin to be determined.

Limitations with this method arise from the interference that many samples have to the natural production of this turbidity. To improve on the method, the compendial authorities have allowed the addition of peptidyl-pNA to produce a chromophore when activated. This colorimetric method allows for greater absorbance when activated and overcomes common interferences. These advantages allow for an improvement of the endotoxin detection of natural LAL.

The potential of synthetic LAL has been an appealing alternative to naturally-sourced LAL. As previously noted, the first synthetic LAL used was recombinant Factor C, the protein in the LAL cascade that binds to endotoxin. The recombinant protein would be used to cleave a fluorescent substrate; however, this method has little resemblance to the Bacterial Endotoxin Test with natural LAL. FUJIFILM Wako believes that a better method is to create recombinant LAL that most closely matches the actions of natural LAL and has therefore engineered the recombinant proteins to recreate the entire four-protein cascade. With the addition of the chromogenic substrate, the result is a reagent in PYROSTAR™ NEO that closely matches the compendial chromogenic LAL assay.

The one significant omission from natural LAL in PYROSTAR™ NEO is the Factor G protein that provides an alternative activation of coagulogen in response to β-1,3-glucans. Since β-1,3-glucans are not harmful pyrogens at these detection limits, the omission of this protein gives PYROSTAR™ NEO endotoxin specificity from unwanted glucan interference. This feature brings to the PYROSTAR™ NEO the endotoxin specificity that has been the signature of the PYROSTAR™ ES-F line of reagents.

THE ADVANTAGES OF PYROSTAR™ NEO

These features of the reagent's design allow PYROSTAR™ NEO several unique advantages among recombinant LAL reagents. The addition of the chromogenic substrate creates the need for testing conditions identical to chromogenic natural LAL. For both FUJIFILM Wako's Color-KY reagent and PYROSTAR™ NEO, measurements are taken at the same wavelengths of 405 nm (measurement) and 650 nm (reference). This provides an advantage over the rFC reagents which require fluorescent measurements in the UV range.

The formulation of PYROSTAR™ NEO allows the reagent to be delivered as a lyophilized powder. It is also the only recombinant LAL reagent that can be reconstituted by Lysate Reagent Water (LRW) in the same manner as the PYROSTAR™ ES-F reagent. These advantages result in preparation instructions that are nearly identical to the preparation of natural LAL.

The final advantage to replicating the complete LAL protein cascade is a reagent that closely matches the recovery rates of natural LAL. Although Factor C is the primary protein activated by endotoxin, much is still unknown about the interactions of natural sources of endotoxin with the proteins of LAL. Since the criteria for an alternative method to the compendial BET is the ability to generate equivalent results, the close replication to the function of natural LAL ensures that PYROSTAR™ NEO is a viable alternative to Bacterial Endotoxin Testing.

THE ADAPTATION OF PYROSTAR™ NEO

The regulatory and compendial requirements allow compendial methods to forgo a complete analytical methods validation in favor of an abbreviated suitability testing. However, as a non-compendial, alternative method, users of PYROSTAR™ NEO will need to have the test method undergo an analytical methods validation for end-process testing. This validation, outlined in USP <1225>, has already been performed by the manufacturer, and customers will receive a copy of those results. They can then follow the procedure outlined to replicate the manufacturer's study in performing the analytical methods validation.

The USP has made recent encouraging announcements that indicate that their focus is to add recombinant LAL methods to the pharmacopeia. If this result is achieved, then customers will be able to adopt PYROSTAR™ NEO with the same suitability testing required of natural LAL. Due to PYROSTAR™ NEO's close correspondence to FUJIFILM Wako's natural LAL, this will allow clients to switch with minimal effort in the near future.

Sources

1. United States Pharmacopeia, Chapter 85, Bacterial Endotoxin Test
2. United States Pharmacopeia, Chapter 1225, Validation of Compendial Methods
3. FUJIFILM Wako Pure Chemicals Corporation, News Release “FUJIFILM Wako Pure Chemicals Corporation launches recombinant protein reagent ‘PYROSTAR™ NEO’ for Endotoxin Detection.” Osaka Japan, 2021.
4. Suvarna, K. Endotoxin detection methods – Where are we now? American Pharmaceutical Review. 2015, August 25.