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ASSOCIATES OF CAPE COD INCORPORATED

Specialists in Endotoxin and Glucan Detection

Pyrosate®

Improved and Now FDA Licensed

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Letter From The Editor

We are very pleased to announce that our Pyrosate® product is now licensed by FDA. We recently received approve for a supplement that add the product to our Biologics license for LAL reagent. Pyrosate has been available for more than 10 years and has been well received for use in area in which a licensed reagent is not required. Now it can be used in any application, including the testing of raw and in-process materials and finished drug, biological and medical device products. This LAL Update is dedicated to a description of the product and it application. The package insert for the Pyrosate is available on our website.

On an unrelated matter, I would like to point out that the USP has published in Pharmacopeial Forum a planned new information chapter, <1228> Depyrogenation. I urge anyone who has an interest in this subject to review the chapter at http://www.usppf.com. It is in the March/April issue of Pharmacopeial Forum, Volume 40, number 2. The deadline for comments on the chapter is May 31, 2014. This is the first of a series of chapter that will address depyrogenation issues.

Finally, anyone interested in the hot topic of Low Endotoxin Recovery (LER) should consider attending the Bacterial Endotoxins Summit meeting in Philadelphia on May 15 and 16, 2014. See http://www.microbiologyforum.org/event_detail.asp?event=17 for details. I hope to see you there.

With best wishes,

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Introduction

Pyrosate kits manufactured by Associates of Cape Cod, Inc. (ACC), which have been available since 2003, are now licensed by the United States Food and Drug Administration (FDA). Also, kits are available in greater sensitivities and the shelf life of the product has been extended. Pyrosate is a version of a gel-clot *Limulus* Amebocyte Lysate (LAL) endotoxin detection reagent that is presented in single test vials. Key features of the product are:

1) ease of use

2) matched positive product controls (PPCs)

3) lot specific incubation times that are often less than the 60 minutes required for other gel-clot tests

4) stability at room temperature

5) a buffered formulation that includes Factor G Inhibitor, giving an endotoxin-specific reagent

While the original, simple test using Pyrosate can still be performed and is described in the product overview section below, the package insert now describes more sophisticated tests that comply with the USP Bacterial Endotoxin Test (BET) chapter[1]. These tests include additional controls and a test to quantify endotoxin concentrations that can be readily carried out.

When Pyrosate was first introduced by ACC, it was designed primarily for use in dialysis centers for testing water and dialysate. Since then, use in other areas in which licensed reagent is not required increased significantly. The new licensing allows Pyrosate to be used in an even greater range of applications, including quality control testing of pharmaceuticals and medical devices. Pyrosate is particularly well suited for the detection of endotoxin in water.

This article describes the recent improvements to Pyrosate and gives an overview of the product and its use. A historical summary of Pyrosate is also presented.

New Features of Pyrosate

ACC recently received approval from the Center for Biologics Evaluation and Research at FDA for a supplement that adds Pyrosate to our product license for LAL. This allows it to be used in applications requiring a reagent manufactured "according to the regulations of the competent authority," to use the wording of the USP BET chapter. The new applications include testing of injectable drug and biological products and non-pyrogenic medical devices, subject to the validation requirements that apply to all LAL test methods.

In response to the changing needs of our customers and to updates to standards (ISO 13959:2014[2] and ISO 11663:2014[3]) for testing water for dialysis and dialysate, Pyrosate reagent is now available with higher sensitivities. In addition to the previously offered sensitivity of 0.25 EU/mL, sensitivities of 0.125 and 0.03 EU/mL are now available, each with their own matching 2λ PPC (where λ is the labeled reagent sensitivity of the LAL reagent).

The test methods that can be performed with Pyrosate have been expanded and validated to include limit and quantitative assays that are compliant with USP BET[1]. This gives the flexibility to select from the following options, depending upon the application:

- Limit test using reference standard endotoxin (RSE)
- Limit test using PPC vials
- Quantitative test using RSE
- Simplified limit test using PPC vials (the original test)

Based on stability study results, the expiry date of Pyrosate has been increased from 2 years to 3 years. Hence, Pyrosate kits can be stored and used over a longer period of time than before.

Detailed instructions are included in the package insert with each kit in a similar format to that of other ACC licensed lysate products. Not only does the insert provide information on Pyrosate and instructions for both limit and quantitative tests, it also includes convenient Quick Guides for each of the tests. These Quick Guides show the test steps pictorially together with notes and descriptive text. These are useful visual aids when setting up and performing the tests. The package insert is available on the ACC website at http://www.acciusa.com/pdfs/accProduct/pisheets/PyrosateIFU_PN002546.pdf.

Product Overview

Pyrosate kits include two types of vial. One type is a blue capped vial containing the buffered lysate formulation and glucan inhibitor, which is designated as the SPL vial for the sample. The other type of vial is red capped and contains a control standard endotoxin (CSE) preparation for use as the PPC.

Pyrosate differs from other gel-clot products available from Associates of Capes Cod, Inc. in the following ways:

1) It is endotoxin-specific; it does not react with (1→3)-β-D-glucan[4], so use of a glucan blocker is not necessary

2) The formulation includes a buffer and there is no need to use a separate buffer

3) The lysate is supplied together with a matching PPC equivalent to twice the lysate sensitivity (2λ) for use as an interference control with the test

4) Pyrosate is very stable and is stored at room temperature

Pyrosate is available in sensitivities of 0.25 EU/mL, 0.125 EU/mL and 0.03 EU/mL and in two kit configurations. The 10-test kit contains one pack of 10 SPL vials and one pack of 10 PPC vials, together with disposable plastic pipettes. The 30-test kit configuration contains three 10 packs each of the SPL and PPC vials,

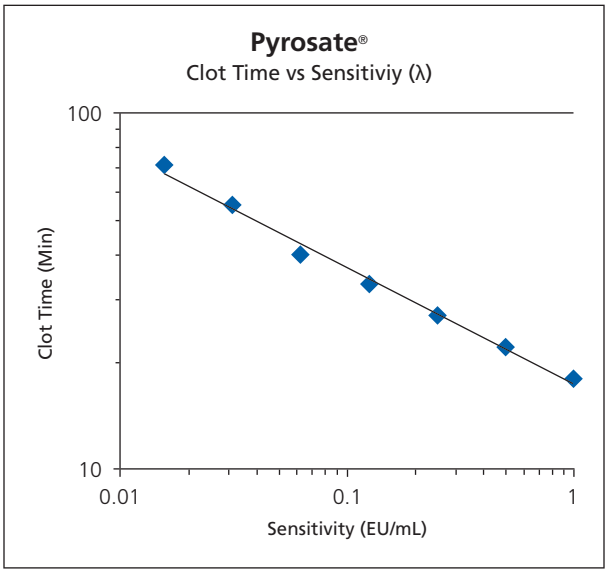
without pipettes. (Pipettes are available separately, ACC catalog number PPT50.) In every kit, the PPC is matched to the lysate reagent in the kit and must not be used with lysate from other kits. A Certificate of Compliance for each kit is available on the ACC website. Catalog numbers for Pyrosate kits are given in Table 1.

Table 1. Catalog Numbers for Pyrosate Kits

Sensitivity	Kit Size	Catalog Number
0.25 EU/mL	10 test	PSD250-10
	30 test	PSD250-30
0.125 EU/mL	10 test	PSD125-10
	30 test	PSD125-30
0.03 EU/mL	10 test	PSD030-10
	30 test	PSD030-30

The incubation period for Pyrosate is specific for each lot of product to give the stated sensitivity. The incubation time is given on the labels of the 10 vial packs of both SPL (lysate reagent) and PPC vials. The higher the minimum endotoxin concentration to be detected, the shorter the incubation time that is required. Consequently, the incubation time is significantly shorter for a sensitivity of 0.25 EU/mL than for 0.03 EU/mL. The incubation time for the 0.25 EU/mL sensitivity can be as short as 25 minutes, compared with about 60 minutes for the 0.03 EU/mL sensitivity. Figure 1 demonstrates the relationship between the clot time of Pyrosate and the endotoxin concentration.

Figure 1 - Typical Plot of Pyrosate Clot Times versus Lysate Sensitivity (Endotoxin Concentration)



In the simplest test, 0.5 mL of sample is added to the SPL vial and mixed to reconstitute the lysate, which is complete within about one minute. Half of the volume (0.25 mL) in the SPL vial is then transferred to the PPC vial and mixed. The endotoxin concentration of the PPC vial is 2λ, which is double the sensitivity of the lysate in the kit. The vials are then incubated at 37°C for the time given on the labels on the packs of SPL and PPC vials (also stated on the Certificate of Compliance). At the end of the incubation period the test is read by inverting each vial in turn by 180° (unless it is clear that the reaction mixture has not clotted), just like any other gel-clot test is read. If a firm clot has formed, the test is scored as positive. All other results are scored as negative, even if a gel forms but breaks upon inversion. The possible different results for the simplest test are summarized in Table 2.

Table 2. Possible Results and their Interpretation for a Simple Pyrosate Test

Result Scenario	Control (PPC)	Sample (SPL)	Interpretation
1.	Clot (+)	Clot (+)	Valid assay. Sample contains an endotoxin concentration of greater than or equal to the labeled reagent sensitivity
2.	Clot (+)	No clot (-)	Valid assay. Sample contains an endotoxin concentration of less than the labeled reagent sensitivity
3.	No clot (-)	Clot (+)	Assay is NOT valid Repeat the test
4.	No clot (-)	No clot (-)	Assay is NOT valid Repeat the test

In addition to the simplest form of the test, limit tests and assays described in the USP chapter for BET can be performed with Pyrosate. Clear directions for these tests are given in the package insert for the product.

Historical Summary

Pyrosate was first introduced to the market in 2003 and was primarily designed for determination of endotoxin in dialysate and water for dialysis. Given the endotoxin limits specified in AAMI guidelines at the time, two sensitivities, 1 EU/mL and 0.25 EU/mL, were available. (Note: Now that more stringent endotoxin limits for water for dialysis and for dialysis fluid have been implemented in ISO 13959 and ISO11663, the 1 EU/mL sensitivity has been discontinued.) An article on endotoxin testing in dialysis applications was published in Nephrology News & Issues[5] in 2004. It described the simple test method using Pyrosate and how the

sensitivity is based on time. It showed that a typical clot time for the 0.25 EU/mL sensitivity is 25-26 min, compared to 60 minutes for a traditional gel-clot procedure. A technical note[6] followed, in which the performance of Pyrosate was shown to be comparable to Pyrotell STV for the analysis of water and dialysate. Additionally, the preparation and performance of Pyrosate tests were shown to be much simpler than for the corresponding STV tests.

Conclusions

The global success of Pyrosate and customer demand for reagent that can be used in pharmaceutical and medical device applications led to the successful application for FDA licensing of Pyrosate and to the expansion of the product line with the addition of higher sensitivities. In addition to meeting the requirements of dialysis centers, the increased sensitivities also increase the flexibility of testing for hard-to-test samples, which may be encountered when testing pharmaceuticals and medical devices. Also, the increased shelf-life allows the kits to be stored and used over a longer period of time leading to cost savings.

The presentation of Pyrosate remains unchanged; the lysate reagent continues to be buffered and endotoxin-specific and supplied with matching 2λ PPC vials. The test methods given in USP BET chapter have been validated for Pyrosate, which can thus be used for both limit and quantitative tests. The new package insert includes Quick Guides for each of the tests described. In conclusion, the newly licensed Pyrosate offers a rapid test that is easy to use and has a wide range of applications.

References

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