BIOMÉRIEUX

APPLICATION OF THE ENDOZYME® II ASSAY RANGE FOR RADIOPHARMACEUTICALS

Rapid and Easy-to-Use Fluorescent Microplate and Microstrip Assays Based on Sustainable Recombinant Factor C

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PIONEERING DIAGNOSTICS

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Introduction

According to Directive CEE 89/343, a radiopharmaceutical is defined as any medicinal product that contains one or more radionuclides (radioactive isotopes) intended for a medicinal purpose. These compounds play a crucial role in both diagnostic and therapeutic procedures and can be used alone or conjugated with drugs or peptide molecules. Radiopharmaceuticals are subject to stringent regulations and quality control measures to ensure their safety and efficacy.

The European Pharmacopoeia (Ph. Eur.) provides guidelines for the production and testing of these compounds through specific monographs. The general monograph Ph. Eur. 0125 (Radiopharmaceutical Preparations) has been updated to include Chapter 5.1.13 on pyrogenicity. This chapter allows testing of new radiopharmaceutical preparations (in the absence of a specific product monograph) for endotoxins using either the Limulus Amebocyte Lysate (LAL) test or the recombinant Factor C (rFC) method.

rFC offers a sustainable, biotechnology-based alternative to the animal-derived LAL test and was included in the European Pharmacopoeia in early 2021 (1). An additional advancement was the inclusion of rFC as Method G in Chapter 2.6.14 (2). This update is now in place, and the finalization of this implementation is expected by July 2026.

In this technical note, we demonstrate the applicability and advantages of the rapid and flexible rFC-based method **ENDOZYME® II GO Strips** for the commonly used fluorine-18 (18F) labeled radiopharmaceuticals: [^{18}F] Fluorodeoxyglucose ([^{18}F]FDG) and Prostate-Specific Membrane Antigen ([^{18}F]PSMA).

Materials and Methods

a. Reagents

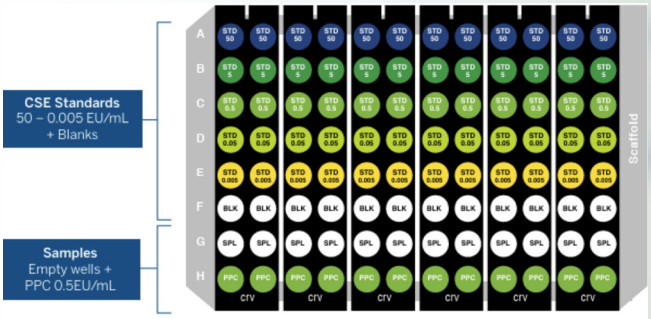
The ENDOZYME® II assay is part of the ENDONEXT™ portfolio of endotoxin tests. It is a enzymatic assay utilizing synthetic recombinant Factor C (rFC), derived from the LAL clotting cascade, along with a fluorogenic substrate. rFC is functionally identical to the Factor C enzyme used in the LAL/TAL assays but is produced synthetically rather than extracted from Limulus blood.

Once activated, rFC cleaves a fluorescent substrate directly, avoiding the multi-step enzymatic cascade of traditional LAL methods. This mechanism eliminates interference from β-glucans—one of the main advantages of rFC over LAL. Additionally, because the reagents consist of well-defined synthetic molecules, the lot-to-lot variability common with LAL is significantly reduced.

While ENDOZYME® II is a traditional format requiring manual preparation of control standard dilutions, **ENDOZYME® II GO Strips** offer a pre-filled plate format. These strips are pre-loaded with Control Standard Endotoxin (CSE) for standard curve generation and product control testing, thereby significantly reducing hands-on time by eliminating the need for vortexing, mixing, diluting, or CSE addition.

The strips are available in two formats: one for preparing the standard curve and another for testing up to four samples without the need for positive product control (PPC) preparation. The standard curve strip can also be used to test 1 sample with its respective PPC.

For the radiopharmaceutical samples tested in this study, a 20-minute assay protocol was used, offering a certified sensitivity of 0.05 EU/mL. Due to the short half-life of these products, rapid time to results is critical. Reduced sample handling and fewer preparatory steps further enhance operator safety.



b. Samples

Fluorine-labeled Prostate-Specific Membrane Antigen ([^{18}F]PSMA):

[^{18}F]PSMA is a radiopharmaceutical used in PET/CT imaging, targeting prostate-specific membrane antigen (PSMA) overexpressed in prostate cancer cells[3], used for the diagnosis and staging of prostate cancer after biochemical recurrence after initial treatment[4].

Fluorodeoxyglucose ([18F]FDG):

FDG is a glucose analogue rapidly taken up by most cancer cells due to their increased glucose transporter activity and enhanced glycolytic pathways. When labeled with fluorine-18, FDG serves as a radiotracer for positron emission tomography (PET) to detect malignant tissues[5].

Application Data and Discussion of Results

As with any product validation, a preliminary analysis was conducted to determine appropriate routine testing conditions. The first parameter assessed was the dosage (M), essential for determining the Endotoxin Limit (EL) and subsequently calculating the Maximum Valid Dilution (MVD).

According to Ph. Eur. 5.1.10, the endotoxin limit is defined as:
EL = K : M, where **K = 2.5 EU/kg** for radiopharmaceuticals and **M = dosage per kg of body weight[6].**

In USP <85>, the endotoxin limit (EL) for radiopharmaceutical products is calculated similarly to the approach in the Ph. Eur., although it is expressed as 175 EU/V, where V is the volume of injection[7]. Despite the different format, the final EL value is equivalent in both pharmacopeias.

For FDG, a typical batch volume of 10 mL

was used as the dosage reference. It has been used an M = 10 ml also for F-PSMA even if while the administered dose is approximately 2 mL and the manufacturing process typically yields around 24ml. 10 ml for FPSMA remains a worst case.

Thus, the calculated EL for both [^{18}F]FDG and [^{18}F]PSMA was:
EL = 2.5 EU/kg/h : (10 mL / 70 kg) = 17.5 EU/mL

Using a test sensitivity of 0.05 EU/mL, the **Maximum Valid Dilution** was:
MVD = EL : test sensitivity = 17.5 EU/ml : 0.05 EU/ml = 350

Samples were tested at various dilutions ranging from neat (undiluted) to the MVD. The table below summarizes the results.

Table 1: *Quantified Endotoxin (EU/mL) and PPC Recovery (%) for Radiopharmaceuticals at Different Dilutions*

ID	Sample	Dilution Factor	PPC Recovery (%)	EU/mL
1	[^{18}F]FDG	1	0	<0.05
2	[^{18}F]FDG	10	135	<0.05
3	[^{18}F]FDG	25	112	<0.05
4	[^{18}F]FDG	50	106	<0.05
5	[^{18}F]FDG	100	99	<0.05
6	[^{18}F]FDG	350	97	<0.05
7	[^{18}F]PSMA	1	0	<0.05
8	[^{18}F]PSMA	10	74	<0.05
9	[^{18}F]PSMA	25	90	<0.05
10	[^{18}F]PSMA	50	109	<0.05
11	[^{18}F]PSMA	100	114	<0.05
12	[^{18}F]PSMA	350	103	<0.05
13	WFI Control	N/A	109	<0.05

Note: PPC acceptance range is 50–200% as per Ph. Eur. guidelines.

The samples were simply diluted in Water for BET and analyzed. The 1:10 dilution, proposed for routine testing, met the required acceptance criteria. All tested dilutions—except the neat product—yielded acceptable PPC recovery values, indicating minimal interference. A single-step 1:10 dilution was sufficient to eliminate matrix effects in all cases.

Conclusions

Radiopharmaceuticals are essential tools in nuclear medicine for both diagnosis and therapy using different radioisotopes for these purposes. As the field advances, the need for rapid, reliable, and interference-free endotoxin testing grows, particularly given the short half-lives of these compounds.

The ENDOZYME® II assay range provides a fast, flexible solution with the needed sensitivity (0.05 EU/mL) and reduced time-to-results, making it particularly suitable for radiopharmaceutical applications. This method simplifies testing workflows and enhances operator safety by minimizing preparation steps.

bioMérieux supports the nuclear medicine sector with innovative solutions that align with evolving quality control requirements and patient safety standards.

Acknowledgements

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References:

(1) Ph. Eur. 2.6.32 – Test for Bacterial Endotoxins Using Recombinant Factor C

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(3) Huang, Siyu, et al, Comparison of 18F-based PSMA radiotracers with [68Ga]Ga-PSMA-11 in PET/CT imaging of prostate cancer—a systematic review and meta-analysis, in Prostate Cancer and Prostatic Diseases 27, pages 654–664 (2024).

(4) U.S. Food and Drug Administration, FDA Approves First PSMA-Targeted PET Imaging Drug for Men with Prostate Cancer, FDA News Release December 01, 2020 (2020).

(5)Fluorodeoxyglucose, an Overview, in Medicine and Dentistry, https://www.sciencedirect.com/topics/medicine-and-dentistry/fluorodeoxyglucose#:~:text=Fluorodeoxyglucose%20(FDG)%20is%20a%20structural%20analog%20of%202-deoxyglucose,%20which,%20like (December 17, 2024)

(6) Ph. Eur. 5.1.10 – Guidelines for Using the Test for Bacterial Endotoxins

(7) USP<85> Bacterial Endotoxin TestS

Authors:

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