Pre-release determination of Endotoxin in short-lived Radiopharmaceuticals using an Endosafe- PTS

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Introduction

The Endosafe®-PTS™ (Charles River Laboratories, Boston, USA) is a handheld spectrophotometer (see Fig 1) that utilizes FDA-approved disposable cartridges for accurate and fast endotoxin testing, providing quantitative test results in approximately 15 minutes.

Figure 1: The Endosafe®-PTS™

The test is carried out using single use cartridges which enable duplicate measurements of the product dose and positive control (spike, Fig 2).

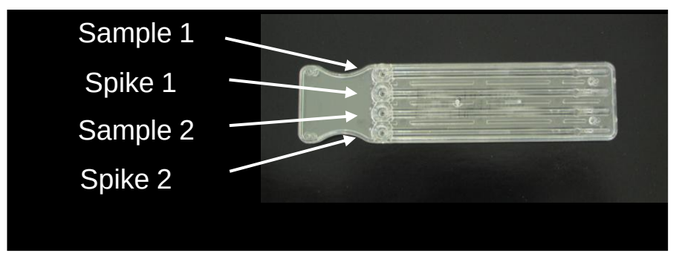

Figure 2: A single use PTS cartridge showing the 4 individual test channels

The PTS utilizes LAL kinetic chromogenic methodology to measure color intensity directly related to the endotoxin concentration in a sample (Fig 3).

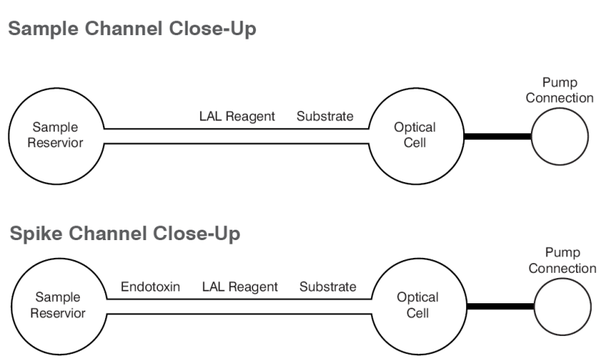

Figure 3: Schematic representation of the sample and spike channels of a PTS cartridge*

Objectives

The aim of this work was to validate endotoxin testing using the Endosafe®-PTS™ for several radiotracers formulated in a physiological saline solution containing approximately 10% ethanol (v/v).

*reproduced with permission from Charles-Rivers product brochure

Methods

Inhibition/Enhancement cartridges were used to determine the optimum dilution that gives recovery close to 100% and does not exceed the Maximum Valid Dilution (MVD), where:

$$MVD = \frac{(EndotoxinLimit)(ConcentrationOfSampleSolution)}{\lambda}$$

With:
- Endotoxin Limit = 175EU/V (Eur. Ph.)
- V = dose volume (11.1mL: 10mL saline + 1.1mL ethanol formulation)
- concentration = 1mL/mL (if endotoxin limit is in EU/mL)
- λ = confirmed label claim sensitivity of the gel clot lysate or lowest point on the reference standard curve.

E.g. for 10-0.1EU/mL cartridge sensitivity, the MVD is 15**7** and for 5-0.05 EU/mL cartridge sensitivity, the MVD is 315.

Dilutions of ^{11}C-labelled radiopharma-ceuticals for human use were prepared to determine the optimal dilution to avoid interference with the test.

MVD for each sensitivity cartridge were calculated for each tracer as formulations slightly differ in ethanol- saline proportion (from 9.1 to 18.2% ethanol in saline).

To validate the optimum dilution, three batches for each tracer must be run on the Endosafe PTS using the right sensitivity cartridge and pass the acceptance criteria:

Spike Recovery of 50-200%
Sample CV<25%
Spike CV <25%
CV being Coefficient of Variation

Results

The results for the optimal dilution for PET products manufactured at the GlaxoSmithKline Clinical Imaging Centre based on spike recovery can be seen in table 1.

TRACER	1:50	1:75	1:100	1:150	1:200	1:250	1:300
[^{11}C]DASB	63	58	94	61	-	-	-
[^{11}C]PHNO	84	61	71	70	98	84	88
[^{11}C]Raclopride	63	65	80	78	105	99	178
[^{11}C]GR205171	33	31	71	55	112	68	112
[^{11}C]PE2I	66	89	85	107	-	-	-
[^{11}C]GSK1034702	273	93	107	82	-	-	-
[^{11}C]Carfentanil	79	82	68	66	82	85	84

Table 1: Optimal dilutions for PET products based on spike recovery

The appropriate minimum cartridge sensitivity for the individual products is listed in table 2.

TRACER	ETHANOL (%) IN FORMULATION	OPTIMUM DILUTION	MINIMUM SENSITIVITY CARTRIDGE
[11C]PHNO	9.1	1:200	5-0.05EU/mL
[11C]GSK1034702	9.1	1:100	10-0.1EU/mL
[11C]Carfentanil	9.1	1:250	5-0.05EU/mL
[11C]DASB	9.9	1:100	10-0.1EU/mL
[11C]Raclopride	13.0	1:200	5-0.05EU/mL
[11C]PE2I	13.6	1:150	10-0.1EU/mL
[11C]GR205171	18.2	1:200	5-0.05EU/mL

Table 2: Minimal cartridge sensitivity as defined by the optimal dilution for the considered PET products

Table 3 shows the results for 3 consecutive measurements for each of the PET products under investigation.

TRACER	AVERAGE RECOVERY (%)	AVERAGE SAMPLE CV (%)	AVERAGE SPIKE CV (%)
[11C]DASB	97	0.0	3.7
[11C]PHNO	108	0.0	1.5
[11C]Raclopride	95	0.0	2.9
[11C]GR205171	111	0.0	3.9
[11C]PE2I	93	0.0	7.7
[11C]GSK1034702	99	0.0	6.1
[11C]Carfentanil	85	0.0	1.3

Table 3: Average values for recovery, Sample- and Spike Coefficient of Variation (CV)

Conclusions/Discussion

- The Endosafe®-PTS™ allows fast and reliable determination of endotoxin levels and may be used as a pre-release test for PET-labelled products.

- The optimum dilution depends on the ethanol contained in the formulation which is known to inhibit the test, not the radiopharmaceutical. The validated dilution for each tracer shows consistent results with recoveries close to 100% (acceptance criteria: 50-200%)

- LAL reagent present in the Endosafe cartridges is a biological reagent, not analytical, therefore inhibition / enhancement screen results are not strictly proportional to the ethanol content. As a general rule, the higher the ethanol content the more dilution is required.

References

- The Endosafe®-PTS™ Portable Test System User's Guide Version 7
- Eur Ph 2.6.14. Bacterial Endotoxins

Acknowledgements

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