Comparative study of three methods for detection of bacterial endotoxins in radiopharmaceuticals

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Aim. Endotoxins, released by gram-negative bacteria, can cause a response in humans ranging from mild fever to shock and even death. A reliable method of detecting bacterial endotoxins in radiopharmaceuticals is of vital importance to patients for *in vivo* use in humans.[1, 2] Before the Limulus amebocyte lysate (LAL) test, the only available means of pirogenicity testing for parenteral drug products and medical devices was the USP rabbit pyrogen test. Especially for radiopharmaceuticals, the LAL test is the elective way to determine bacterial endotoxin.[2] The aim of this work is to compare three methods for determination of bacterial endotoxins in radiopharmaceuticals: gel-clot, kinetic chromogenic and kinetic turbidimetric. **Methods.** Experiments were performed in four radiopharmaceuticals used for diagnostic in nuclear medicine: 67Ga-citrate, 99mTc-Phytate, 99mTc-GHA and 99mTc-SAH. For the gel-clot method, licensed LAL (sensitivity (Ï) 0.125 Endotoxin Units per milliliter) and matched Control Standard Endotoxin (CSE) from Endosafe, Inc.TM, Charleston, SC were used and samples were prepared according to the USP Bacterial Endotoxins Test (BET). Assays were performed in an EletrolabTM water bath.[3] A Portable Test System (PTS) from Endosafe, single polystyrene cartridges containing dry LAL-reagents, CSE and synthetic colour substrate were used for all kinetic chromogenic tests. Tecan Sunrise with Endoscan-V software (Endosafe), disposable 96 well plates, LAL-KTA and CSE were used for all kinetic turbidimetric assays. The temperature of the reaction was 37 ±1°C in all tests. For the gel-clot method, a standard dilution series was performed with a CSE from 0.5 to 0.03 EU mL-1. For the kinetic chromogenic method, results were interpolated from an archived standard curve (5.0; 0.5 and 0.05 EU mL-1). For the kinetic turbidimetric, a standard curve with three endotoxin concentrations (5.0; 0.5 and 0.05 EU mL-1) was used to quantify the sample endotoxin concentration. **Results.** For each method, interferent test conditions were determined and serial product dilutions were performed until the Maximum Valid Dilution (MVD) calculated for the products [4]. In kinetic chromogenic and kinetic turbidimetric methods it is allowed higher dilutions than in the gel-clot method. In quantitative methods, the endotoxin concentration in the products was lower than the lowest concentration of the standard curve and the parameters of coefficient of correlation, recovery of positive product control and coefficient variation were satisfied. The LAL claimed sensitivity was confirmed for ^{67}Ga-citrate and ^{99m}Tc-SAH in the gel-clot method. For ^{99m}Tc-GHA and ^{99m}Tc-Phytate, interference was observed even at MVD (200 and 400 respectively) in the gel-clot method but results were valid in the chromogenic and turbidimetric methods at lower dilution. These differences in endotoxin detection indicate that more interference studies need to be performed.

TABLE I.—*Comparative test results of the gel-clot, kinetic chromogenic and kinetic turbidimetric.*

Product	Gel-clot	Chromogenic	Turbidimetric
^{67}Ga-citrate	Valid*	Confirm	Confirm
^{99m}Tc-SAH	Valid*	Confirm	Confirm
^{99m}Tc-GHA	Not valid**	Valid*	Confirm
^{99m}Tc-Phytate	Not valid****	Valid***	Confirm

*- 1:100 dilution factor **- 1:200 dilution factor ***- 1:300 dilution factor ****- 1:400 dilution factor

TABLE II.—*Comparative study of the gel-clot, chromogenic and turbidimetric methods.*[1]

Gel-clot	Chromogenic	Turbidimetric
Limit test	Quantitative (OD - 405 nm)	Quantitative (OD - 340 nm)
Simple, rapid (60 minutes)	Simple, faster (15 - 20 minutes), portable	Rapid (60 minutes)
100 µL sample	25 µL sample	100 µL sample
Do not need specialized equipment (37°C bath)	Photometric (PTS)	photometric(TECAN SUNRISE)
For short-lived radiopharmaceuticals, for water, in process and final product analysis	For short-lived radiopharmaceuticals, for water, in process and final product analysis	For radiopharmaceuticals, few samples for a 96 well-plate
LAL sensitivity 0.25 – 0.015 EU mL^{-1}	LAL sensitivity 0.05 EU mL^{-1}	LAL sensitivity 0.05 EU mL^{-1}
5 standard endotoxin concentrations for the standard curve 0.50; 0.25; 0.125; 0.06; 0.03 EU mL^{-1}	Archived standard curve 5.0; 0.5; 0.05 EU mL^{-1}	3 standard endotoxin concentrations for the standard curve 5.0; 0.5; 0.05 EU mL^{-1}
Result: fail/pass	Coefficient of correlation ≤-0.980, recovery of positive product control(RPPC) 50 - 200% and Coefficient Variation (CV) < 25%	Coefficient of correlation ≤ -0.980, recovery of positive product control(RPPC) 50 -200% and Coefficient Variation (CV) < 10%
End-point gel-clot	Colour development	Turbidity development
Naked eye reading	Automated	Automated
Interferences	Limitation of turbid or coloured samples	Limitation of turbid or coloured samples

References

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