P201 A PORTABLE SYSTEM FOR BACTERIAL ENDOTOXIN QUANTIFICATION IN RADIOPHARMACEUTICALS BY THE KINETIC CHROMOGENIC METHOD

N. FUKUMORI*, D. CAMPOS, L. SILVA, A. FERNANDES, J. MENGATTI, C. SILVA and M. MATSUDA
IPEN-CNEN/SP, Radiopharmacy Directory, Sao Paulo, Brazil

Objectives: Pyrogens include any substance capable of eliciting a febrile response upon injection or infection. Endotoxin is a subset of pyrogens that are strictly of gram-negative origin, a natural complex of lipopolysaccharide ocurring in the outer layer of the bilayered gram-negative bacterial cell. From the circulating blood cells of Limulus polyphemus, called amebocytes, a clear lysate is obtained which forms an opaque gel in the presence of extremely small concentrations of bacterial endotoxins. A quantitative kinetic chromogenic test in an automated Portable Test System (PTS) has been developed for determination of bacterial endotoxins in water, in-process and end-products using the Limulus amebocyte lysate (LAL). The aim of this work was to validate the method for some radiopharmaceuticals with no interfering factors.

Methods: Experiments were performed in three consecutive batches of the radiopharmaceuticals ^{18}F-FDG, ^{99m}Tc, Methylenediphosphonic Acid (MDP) and Pyrophosphate (PYRO) produced at IPEN-CNEN/SP using the PTS from Endosafe, Inc. ™, Charleston, SC. Single polystyrene Endosafe cartridges containing dry LAL-reagents, Control Standard Endotoxin (CSE) and synthetic color substrate were used. The LAL sensitivity was 0.05 EU mL^{-1}. Serial dilutions (1:1; 1:5; 1:10; 1:20; 1:50; 1:100 and 1:200) were carried out. 25 μL samples were pipetted into the cartridge wells and the temperature of the reaction was 37 ±1°C. Results were obtained for the endotoxin concentration in samples by interpolation of an archived standard curve (5.0; 0.5 and 0.05 EU mL^{-1}) at 405 nm OD (Optical Density), after 20 minutes.

Results: The Maximum Valid Dilution (MVD) was calculated to establish the extent of dilution to avoid interfering test conditions (MVD=500). Despite product dilution in this method can be greater than in the gel clot method (MVD=200 for radiopharmaceuticals and 0.125 EU mL^{-1} LAL Reagent sensibility), it is necessary to perform validation experiments to determine the minimum interfering dilution. Experiments showed better results above 1:5 dilution factor for ^{18}F-FDG and ^{99m}Tc, above 1:20 for MDP and 1:100 for PYRO. It was observed that there is a specific dilution for every radiopharmaceutical and the profile for the %RPPC and dilution factor graph was similar in the three analyzed batches of each product. The parameters of coefficient correlation (R) ≤ 0.980, recovery of positive product control (RPPC) 50 - 200% and coefficient variation (CV) of the samples less than 25% were satisfied and the endotoxin concentration was lower than the lowest concentration of the standard curve (0.05 EU mL^{-1}), therefore less than the established limit in pharmacopoeias.

Conclusions: The PTS is a rapid, simple and accurate technique using the quantitative kinetic chromogenic method for bacterial endotoxin determination. For this reason, it is very practical in the pharmaceutical area and especially for short-lived radiopharmaceuticals, it trends to be the method of choice for the pyrogen test. For ^{18}F-FDG, ^{99m}Tc, MDP and PYRO, the validation was successfully performed.