COMPARISON OF ENDOTOXIN QUANTITATIVE TEST METHODS IN INTRAVENOUS PHARMACEUTICAL PRODUCTS

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ABSTRACT

Endotoxin is a biological pyrogen obtained from the lipid A-part of outer membrane lipopolysaccharide (LPS) in Gram-negative bacteria. Contaminated endotoxin in blood circulation can stimulate the immune system by releasing inflammatory cytokines leading to fever, multiple organ failure (MOF) and multiple organ dysfunction syndrome (MODS). Therefore, contaminated endotoxin in intravenous pharmaceutical products can increase the severity of the illness and the mortality rate of patients. To reduce this problem, quantification of endotoxin contamination in sterile pharmaceutical products is regulation in the quality control processes. For standard methods, endotoxin was quantified using Limulus amebocyte lysate (LAL) based assays: gel clot, turbidimetric and chromogenic methods. Recently, an alternative method, using a recombinant Factor C (rFC), has been available for measuring endotoxin. The aim of study was to comparatively evaluate the LAL kinetic turbidimetric and rFC endpoint fluorescence methods for measuring endotoxins in five intravenous pharmaceutical products, *i.e.*, Lidocaine, Ciprofloxacin, Levofloxacin, Ondansetron and Meropenem for Injections. Two groups of assayed samples used in this study included group A; spike standard endotoxin (0.495 EU/mL) into an appropriated dilution of samples and group B; spike standard endotoxin (0.495 EU/mL) into an appropriated dilution of samples with pre-added standard endotoxin (10 EU/mL). For group A, the Wilcoxon matched-pairs signed rank test ($P < 0.05$) showed that both methods for endotoxin detection were significantly different from all testing samples. The data points of endotoxin detected by rFC endpoint fluorescence methods were deviated less from the zero-line, which represented a different value from 0.495 EU/ml of standard endotoxin added to the appropriately diluted samples, when compared with the LAL kinetic turbidimetric method. Moreover, the accuracy (%recovery) of endotoxin detected by the rFC endpoint fluorescence methods showed a higher result than the kinetic turbidimetric method. Thus, the rFC endpoint fluorescence method provided a high accuracy for endotoxin detection as represented by %recovery which was closest to 100%. In addition, the statistical analysis for group B showed an insignificant difference between both methods compared by Wilcoxon matched-pairs signed rank test ($p<0.05$) for all testing samples. However, the deviation from the zero-line of endotoxin detection by the LAL kinetic turbidimetric method was less than the rFC method. Moreover, the accuracy (% recovery) of endotoxin detection by the LAL kinetic turbidimetric method provided a high accuracy for endotoxin detection as represented by %recovery, which was closest to 100%. It can be concluded that the rFC endpoint fluorescence method was appropriate for endotoxin detection, especially at low levels, and might be developed for to be the standard method of endotoxin or pyrogen detection in sterile pharmaceutical products, in the future.

KEY WORDS: ENDOTOXIN/ RECOMBINANT FACTOR C/LAL TURBIDIMETRIC/ INTRAVENOUS PHARMACEUTICAL

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