METHOD STATEMENT

Determinand:
Endotoxin

Matrix:
Sample Type: Pure waters, renal fluids, medical devices and drugs.

Principle of Method:
The test is derived from the observations of Bang13.2. He observed the reaction of the blood of the Limulus Polyphemus (horse shoe crab) to the introduction of endotoxins, which resulted fatal intravascular coagulation.
The method described in this protocol uses a quantitative kinetic Limulus Amebocyte Lysate (LAL) assay. The sample is mixed with reconstituted LAL reagent, placed in a photometer, and monitored over time for the appearance of turbidity. The time required for the appearance of turbidity (reaction time) is inversely proportional to the amount of endotoxin present.

Sampling and Sample Preparation:
Samples should be collected in pyrogen-free containers and stored at 5 ± 3°C.
Analysis should start as soon as is practical. Samples should be stored at 5 ± 3°C until analysis is carried out to prevent microbial growth

Interferences
The degree of inhibition or enhancement of the LAL test will be dependent upon the concentration of interfering factors within the test sample. In order to determine the levels of inhibition/enhancement, a dilution should be carried out for the sample if a satisfactory spike recovery is not obtained.

Performance of Method:

Range of Application:
Endotoxins are reported in terms of Endotoxin Units per ml (EU/ml).

Limit of Detection:
The limit of detection for the endotoxin method is 0.01 EU/ml

Uncertainty of measurement:
The uncertainty has not been calculated for this method

References:
British Pharmacopoeia 2002.The Stationary Office.
Bang, F.B. (1956). A bacterial disease of Limulus polyphemus. Bull. John Hopkins Hosp. 98:325.
Limulus Amebocyte Lysate – Endosafe R KTA 2TM US License No 1197