METHOD STATEMENT

Determinand:
Endotoxins.

Matrix:
Water.

Principle of Method:
This method allows for the enumeration of low levels of endotoxin in pharmaceutical water samples. It is suitable for the enumeration of endotoxin concentration in pharmaceutical grade waters where 0.25 EU/ml is the acceptable limit, as stated in the European Pharmacopoeia.

The method is based on the observations that amebocytes derived from the blood of the horseshoe crab (*Limulus polyphemus*) produce enzymes, which clot in the presence of minute quantities of endotoxin. Levels of endotoxin present are determined by optically measuring the turbidity of the clot formed.

In the test a purified standardised lysate is mixed with a sample suspected of containing endotoxin, its subsequent turbidity compared to that of a standard series of known endotoxin concentrations.

Sampling and Sample Preparation:
Samples must be taken in accordance with customers' own specifications into depyrogenated, screw capped glass tubes. Sample containers should be filled approximately ⅔ full and once capped may be protected with parafilm.

Testing should commence as soon as possible to avoid bacterial contamination. Storage in a refrigerator is suitable if a delay in testing is anticipated.

Interferences:
pH can interfere with the assay. The optimum pH for a sample is 6.5 – 8. If the pH is thought to be a possible cause of interference, the pH of the sample must be tested in a 1:1 solution of lystate:sample as addition of lysate to the sample may lower the pH.

Performance of method:
Results are expressed in EU/ml within the standard curve or as >5.0EU/ml or <0.05EU/ml for levels detected outside the range of the standard curve.
The Limit of Detection has not been determined for this method.

References:
European Pharmacopoeia 5th edition, 2005

Charles River Endosafe. Kinetic Turbidmetric Assay product information.

Charles River Endosafe PTS User's Guide – Version 7