

## Analytical Validation of LAL Kinetic Assay for Detection and Quantification of Endotoxins in Measles's Vaccine Diluents

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### ABSTRACT

*Method validation is the process used to confirm that the analytical procedures employed for a specific test is able to produce reliable and replicable results. An analytical method should be conducted in order to demonstrate that it is suitable for its intended use [1]. The aim of this present work is to demonstrate and evaluate the suitability of method validation. The analytical validation discussed herein was conducted by prescribed protocol using 3 different batches of measles's vaccine diluents. All of the stipulated validation parameters: linearity, repeatability, reproducibility (intermediate precision) and accuracy were met. Additionally, this study demonstrated the method's capability for the determining endotoxin levels in measles's vaccine diluents and its further use in apyrogenic water samples.*

**Key words:** Bioanalytical method, endotoxin, validation

### INTRODUCTION

The *Limulus* amoebocyte lysate (LAL) kinetic assay is a test that is used to detect and quantify gram negative bacteria endotoxins (aka; lipopolysaccharides [LPS] or endogenous pyrogens) that may be present in biotechnological products. Once bacterial endotoxin is determined to be a contaminant, its levels must be measured, as this dramatically affects the results.

A sample is mixed with the LAL/substrate reagent, placed in an incubating plate reader, and monitored over time for the appearance of a turbidity (identified by a yellowish color). This may occur quite quickly if there are high levels of endotoxin present in it.

The time required to attain a turbid state is referred to as the Reaction Time and is inversely proportional to the amount of endotoxin present, and thus the concentration of endotoxin in unknown samples can be calculated from a standard curve.

The present method, described in the United States Pharmacopeia - USP[2], was validated according to parameters set by the U.S. Food and Drug Administration - FDA [3] plus precision parameters cited in the Guia para Qualidade em Química Analítica - ANVISA [4].

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