Novel endotoxin assay by adsorption method with polycation-immobilized cellulose beads and limulus amoebocyte Lysate

（ポリカチオン修飾セルファインとリムルス試薬を用いた吸着法によるエンドトキシン定量）

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We developed a new endotoxin (lipopolysaccharide; LPS) assay using poly(ε-lysine)-immobilized cellulose beads (PL-Cellufine)[1] and Limulus amoebocyte lysate (LAL) reagent. In this study, we describe the optimal conditions of LPS adsorption on the beads and the LAL reaction of the LPS adsorbed on the beads. We also compare this adsorption method with a standard solution method for LPS assay.

First, LPS (*Escherichia coli* UKT-B, Wako) was selectively adsorbed on the beads in a solution containing various LAL-inhibiting or LAL-enhancing compounds (NaCl, amino acids, EtOH, proteins) and the LPS adsorbed on the beads was separated from the compounds by centrifugation. Second, the LPS adsorbed on the beads directly reacted with the LAL reagent (Limulus II single test Wako), and LPS concentration was determined by turbidimetric time assay using Toxinometer ET-2000 (Wako).

The relationship between LPS concentration and LAL gelation time was investigated by adsorption method using PL-Cellufine. As a shown in Fig.1, each calibration (n = 5) curve obtained between 0.0056 and 5.6 EU/mL was well correlated. Table 1 shows a comparison of the adsorption method with the standard method. In the adsorption method, the LPS concentration in a sample solution showed more approximate values (apparent LPS recovery 88-120%) to the original LPS concentration. By contrast, the apparent LPS recovery by a common standard method was unsatisfactory (the recovery 55-60%). This adsorption method could be used widely as a means of assaying LPS in solutions containing LAL-inhibiting or enhancing substances.

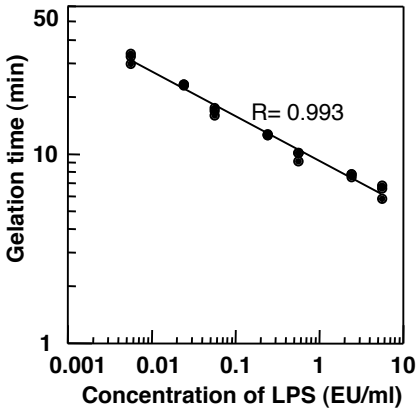

Fig. 1. Standard curve of LPS by the adsorption method using PL-Cellufine described in the text. Purified LPS (*E. coli* UKT-B) was added to LPS-free water. The LPS solution (0.0056-5.6 EU/ml) was used as a sample. Gelation times for LPS adsorbed on PL-Cellufine are shown as the total data of five measurements.

1) M. Todokoro, M. Sakata, et al., *J. Liq. Chrom. & Rel. Technol,*. **25**, 601 (2002).

Table 1 Recovery of LPS from the compounds by the adsorption method and the standard method

Sample			Apparent recovery of LPS by LAL test	
Compound		Concentration of LPS added	Adsorption method	Standard method
Name	Condition [a]	EU/mL	%	%
NaCl	0.2 M in H_2O (μ=0.2)	0.5	100	55
NaCl	0.4 M in H_2O (μ=0.4)	0.5	105	40
Methionine	5 wt% in H_2O	0.5	105	60
Phenylalanine	5 wt% in 0.02 M-PBS (pH 7, μ=0.05) [a]	1	120	44
Vitamin K	1 mg/mL of 95 vol% EtOH aq.	0.2	88	No gelation

To LPS-free solutions containing various compounds, 0.2-1.0 EU/mL of *E. coli*UKT-B was added. LPS concentration in the sample was assayed by the adsorption or standard method. Apparent recovery of LPS was estimated from the standard curve (LPS in water).

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