Selective assay for endotoxin using poly(ε-lysine)-immobilized Cellufine and Limulus amoebocyte lysate (LAL)

（ポリリジン固定化セルファインとリムルス試薬を用いた
選択的エンドトキシン定量）

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We developed a selective endotoxin (lipopolysaccharide; LPS) assay using poly(ε-lysine)-immobilizedcellulose beads (PL-Cellufine) and Limulus amoebocyte lysate (LAL). First, LPS was selectively adsorbedon the beads in a solution containing various LAL-inhibiting or LAL-enhancing compounds (e.g., aminoacids, enzymes) 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, and the LPS concentrationwas determined by a turbidimetric time assay. As shown in Table 1, The accuracy of the adsorption method with PL-Cellufinewas high compared with that of a common solution method. Apparent recovery of LPS from compoundsolution was 88–120%.

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

Sample			Apparent recovery of LPS by LAL test	
Compound		Concentration of LPS added	Adsorption method	Standard method
Name	Condition [a]	EU/mL	%	%
NaCl	0.05 M in H_2O　(μ=0.05)	0.5	100	98
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]	1.0	120	44
Lysozyme	0.4 wt% in 0.02 M-PBS(pH 7, μ=0.05) [a]	0.5	110	210
Lysozyme	0.4 wt% in 0.02 M-PBS (pH 7, μ=0.2) [a]	0.5	90	150
Ethanol	20 vol% in H_2O	1.0	90	No gelation
Vitamin K	1 mg/mL of 95 vol% ethanol 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 solutions was assayed by the adsorption method or standard solution method. Apparent recovery of LPS was estimated from the standard curve (LPS in water) in Fig. 1b.
[a] Ionic strength (μ) of the phosphate buffered saline (PBS) was adjusted using NaCl.

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