Protocol 503_Dirty plate ELISA

ELISA FOR IgG against K. pneumoniae (9E) cytosolic protein extract

Reagents:

- ELISA coating buffer: 0.1M NaHCO3 (3.18g Na_2CO_3 + 5.88g $NaHCO_3$ made up to 1000ml with water).
- ELISA blocking buffer (PBS, 2% BSA – NB NO AZIDE – really inhibits HRP!!!!)
- ELISA wash buffer (PBS, 1:2000 Tween-20)
- ELISA substrate buffer: 0.1M NaH2PO4, pH 4 (13.8g NaH_2PO_4 made up to 1000ml)
- KP lysate
- HRP-anti-human IgG (Bethyl A80-104P)

Method:

Thaw 1 vial of K. pneumoniae lysate (see below for preparation)
Dilute 1:1000 in ELISA coating buffer and plate 50µl per well

Incubate overnight at 4°C

Wash 3x with ELISA wash buffer

Block for 3hrs with blocking buffer. Flick out blocking buffer.

Add titrated plasma samples (starting at a dilution of 1:10 in Blocking buffer and diluting 8 times 3-fold down the plate).

Incubate overnight at 4°C

Wash 6x with ELISA wash buffer

HRP-anti-Rat IgG 1:1000 in blocking buffer
Incubate 1hr at rm tempterature

Wash 6x with ELISA wash buffer

Add 100µl per well of ELISA substrate

Read OD 415

Supplementary method:
Preparation of K. pneumoniae lysate

Grow up overnight culture of Klebsiella 9E shaking overnight in 10ml BHI

In the morning, subculture 5ml into 100ml BHI and grow to a density of 8 x 10^8 cells per ml (0.1 reading at 1:8 dilution on ELISA reader). Approx 2.5hrs.

Centrifuge and resuspend pellet in 25ml PBS. Add 2.5ml FASTlyse solution (Promega)

Shake on rotary shaker for 10mins at rm temp

Take aliquots to 2ml centrifuge tubes and spin at 30000g for 15mins to pellet cell walls and debris

Remove supernatant and pool into a clean 50ml Falcon and aliquot for use

Take whole lysate and freeze in 2000µl aliquots