

CHO Cell Tissue Culture Assay for Determination of *Clostridium difficile* Toxicity

REFERENCES:

M Ehrich, RL van Tassell, JM Libby and TD Wilkins (1980). Production of *Clostridium difficile* antitoxin. *Infection and Immunity*, **28**:1041-1043.

NM Sullivan, S Pellet and TD Wilkins (1982). Purification and characterization of toxins A and B of *Clostridium difficile*. *Infection and Immunity*, **35**:1032-1040.

METHOD:

1. Grow Chinese Hamster Ovary (CHO) cells to confluence in Iscove's Modified Dulbecco's Medium (IMDM) (Gibco-BRL) supplemented with 10% fetal bovine serum (FBS) and 100µg/ml gentamycin (Sigma) (IMDM-10% FBS) at 37°C and 5% CO₂ in a humidified incubator.
2. Trypsinize the cells, dilute into 20ml Hank's Balanced Salt Solution, wash by centrifugation at 400g for 10 minutes, and resuspend at 2 x 10⁵ cells/ml in IMDM-10% FBS.
3. Dispense 100µl aliquots containing 2 x 10⁴ cells/well into the wells of a 96-well tissue culture plate.
4. Incubate the cells at 37°C and 5% CO₂ in a humidified incubator overnight.
5. Prepare 2-fold dilutions of sample in IMDM-10% FBS and add 100µl volumes of each dilution to the wells.
6. Incubate the cells at 37°C and 5% CO₂ in a humidified incubator overnight.
7. Determine the endpoint as 50% CPE, which is the dilution which causes rounding of 40-60% of cells when compared with the control (no sample added).