

## Virulence Studies of *Clostridium difficile*

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**[Abstract]** *Clostridium difficile* (a Gram-positive, spore-forming, strict anaerobe) can colonize antibiotic-treated hosts (McFarland, 2008). Antibiotics alter the composition of the normal, benign microbial flora which leads to loss of colonization resistance (Wilson and Perini, 1988; Antonopoulos *et al.*, 2009). *C. difficile* spores germinate to actively growing bacteria which secrete toxins that damage the colonic epithelium (Voth and Ballard, 2005). The use of animal models of *C. difficile* disease have allowed the identification of mechanisms of colonization and virulence factors (Lyras *et al.*, 2009; Kuehne *et al.*, 2010; Francis *et al.*, 2013; Aubry *et al.*, 2012; Carter *et al.*, 2011). This protocol describes virulence studies of *C. difficile* in the hamster model of *C. difficile* infection (Bartlett *et al.*, 1978; Sambol *et al.*, 2001).

### **Materials and Reagents**

1. Institutional Animal Care and Use Committee (IACUC)-approved animal use protocol
2. *C. difficile* spores
3. Female Syrian golden hamsters (80 g–120 g)
4. Clindamycin Injection, USP (150 mg/ml) (Hospira, catalog number: 0409-4052)
5. Dulbecco's Modified Eagle Medium (DMEM)

### **Equipment**

1. Animal feeding needles (gavage needles)
2. 1 ml syringe
3. Dedicated BSL2 animal facility
4. Scale

### **Procedure**

1. Weigh each hamster.
2. Gavage 100  $\mu$ l clindamycin to each hamster (30 mg clindamycin / kg body weight).  
*Note: Dilute the stock clindamycin to the appropriate concentration using DMEM.*

3. After 5 days, gavage 100  $\mu$ l *C. difficile* spores.

*Notes:*

- a. Depending on the strain of *C. difficile* used, the infectious dose can vary. However, in several strains, inoculation with approximately 100 spores will result in lethal disease.
  - b. There are several methods to ensure reproducible infectious doses [e.g. suspending spores in phosphate buffered saline (PBS)]. Our laboratory uses Teflon-coated tubes to reduce spore adherence. Other laboratories can get reproducible infectious doses in water.
4. Closely monitor animals for signs of infection including: wet tail, poor fur coat, lethargy, loss of 15% body weight.  
*Note: Signs of disease are commonly observed in 2 – 4 days post infection. Be sure to closely monitor the animals for the above symptoms. Moribund animals can succumb to disease quickly.*
  5. Humanly euthanize moribund animals, in accordance with your protocol that was approved by your Institutional Care and Animal Use Committee (IACUC). Score the time required to reach a moribund state.

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