Apr 03, 2025

Citrobacter rodentium Model of Enteropathogenic and Enterohemorrhagic (EPEC, EHEC) *Escherichia coli* Infection

In 4 collections

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpdej8gzp/v1

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Protocol Citation: Alexandra Kazanova, samantha.gruenheid 2025. Citrobacter rodentium Model of Enteropathogenic and Enterohemorrhagic (EPEC, EHEC) Escherichia coli Infection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpdej8gzp/v1

Protocol status: Working
We use this protocol and it's working

Created: March 11, 2025

Last Modified: April 03, 2025

Protocol Integer ID: 124537

Keywords: ASAPCRN, os citrobacter rodentium infection in mice, os citrobacter rodentium infection, rodentium model of enteropathogenic, rodentium model, enterohemorrhagic, coli, enteropathogenic, preparation of inoculum, infection purpose, epec, mice, inoculum, infection, escherichia

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000525

Abstract

Purpose: To outline preparation of inoculum and per os Citrobacter rodentium infection in mice.

Guidelines

Brief safety overview:

Citrobacter rodentium is a Gram-negative enteric bacterium and Bio-Safety Level (BSL) 2 pathogen. Containment level 2 practices, safety equipment, and facilities are recommended for work involving infectious or potentially infectious materials, animals, or cultures.

Abbreviations:
CFU: Colony forming Unit
LB: Luria-Bertani
PBS: phosphate-buffered saline
RPM: rotations per minute

Note

N.B. Diet has a large impact on infection, we wean mice onto Teklad 2918 and maintain them on this diet.

	A	B	C
	Mouse strains	Age	Outcome
	C57BL/6	4-8 weeks	resistant: peak bacterial burden at day 12 p.i. but mice recover

Materials

- Citrobacter rodentium strain DBS100 (chloramphenicol-resistant) used for CFU experiments
- 75% ethanol spray bottle
- Autoclaved 50% glycerol
- LB broth: Dissolve and mix 10 g Bacto tryptone (casein extract), 5 g yeast extract, and 10 g NaCl in 1 L dH_2O then autoclave.

	A	B
	Bacto tryptone (casein extract)	10 g
	Yeast extract	5 g
	NaCl in 1L dH_2O	10 g

- MacConkey agar plates: Dissolve and mix 50 g in 1 L dH_2O, heat with frequent agitation, boil for 00:01:00 to completely dissolve powder and autoclave (121 °C , 00:15:00) (Difco; REF 212123)
- MacConkey agar Petri dishes supplemented with chloramphenicol: Use chloramphenicol at final concentration of 20 µL (667 µL of 30 µL stock in 1 L MacConkey)
- Sterile 1x PBS
- Autoclaved glass beads for spreading bacteria on Petri dishes
- Orbital shaker
- 37°C incubator
- Sterile bacterial glass culture tubes
- 5ml push cap tubes (Sarstedt; 55.526.006)
- Ice bucket
- Gavage needle (22G)
- X machine in BSL2
- Glass beads
- diam. 1.0mm (sterile)
- V-bottom 2ml cryotubes (sterile)
- Analytical balance
- MagNA lyser

Tube, 5 ml, (LxØ): 75 × 12 mm, PP **Sarstedt Catalog #** 55.526.006

MacConkey Agar **Difco Catalog #**212123

Prepare Master Stock of Citrobacter rodentium (1 time)

1 On day 1, streak *Citrobacter rodentium* wild-type or chloramphenicol-resistant DBS100 strains from 1 mL frozen stocks on LB agar plates (with or without chloramphenicol) and incubate at 37 °C Overnight .

> Note
>
> C. rodentium has characteristic colony morphology of a red center and white rim (only on MacConkey, not on LB: the overnights are grown on LB, colonies are 'normal' buff color).

2 On day 2, pick one colony and inoculate 3 mL of LB (no antibiotic, regardless of strain) in a labelled culture tube and shake at 220 rpm, 37°C, 17:30:00 .

3 On day 3, mix 250 µL of 50% glycerol with 250 µL of overnight culture (final of 25% glycerol) in a labelled cryotube, vortex and freeze at -80 °C .

> Note
>
> Ensure to clearly label vials and box with pathogen, strain, antibiotic resistance, and date.

Prepare *Citrobacter* Inoculum and *In Vivo* Infection

4 On day 1, streak *Citrobacter rodentium* wild-type or chloramphenicol-resistant DBS100 strains from 1 mL frozen stocks on LB agar plates (with or without chloramphenicol) and incubate at 37 °C Overnight .

5 On day 2, pick one colony and inoculate 10 mL of LB (no antibiotic, regardless of strain) in a labelled glass culture tube (prepare in duplicate) and shake at 220 rpm, 37°C, 17:30:00 .

6
- The day of the infection, spin down the inoculum at 4000 rpm .
- Resuspend in 1 mL of plain LB media.

- Pool total 6 mL into twist cap 15ml tube.
- Place the tube of dose in a sealed bag for transport to the BCL-2 and pathogen transport container Saf-T-Pak.

7 The day of the infection, gavage mice with 100 µL of overnight culture per mouse using a 22G gavage needle.

Note

See Substance administration SOP. The average dose is 2-3x10^9 *Citrobacter rodentium* per 100 µL.

8 Perform 1 in 10 serial dilutions of dose injected into mice, and plate 10^{-6} and 10^{-7} dilutions on LB plates. Take 100 µL bacteria (vortex very well) in 900 µL saline. Plate 100 µL per plate.

9 The next day, record CFU counts, and calculate actual dose infected in mice.

Monitor *Citrobacter*-Infected Mice and Time Point Studies

1h 11m

10 Record mouse weight daily. Monitor for clinical signs and symptoms up to 30 days post-infection. Mice must be humanely euthanized when they reach clinical endpoints:

- Loss of >20% bodyweight
- Body score condition <2
- Loose stools, bleeding from anus, ruffled fur, lessened activity/lethargy, dehydration, piloerection, and sunken eyes and abdomen.

11 At specific time points post infection (day 3, 6 and 9), collect feces and/or euthanize mice.

Note

See Euthanasia and mouse necropsy SOP.

12 To collect feces:

10m

- Place individual mice in an empty cage (no bedding) for on average 00:10:00 (can take up to 2 hours) and collect 4 fecal pellets per mouse.
- Weigh a 2ml tube with beads before and after adding fecal pellets and record weight of feces.
- For highly susceptible animals (ex. C3H mice have no feces on day 9 post-infection).

12.1 To prepare 2ml V-bottom cryotubes with beads:

- add beads to individual cryotubes, tighten lid, place tubes with beads in a glass beaker, cover with aluminum foil and autoclave.

13 Add 1 mL PBS to each tube and incubate for 01:00:00 at 4 °C to soften stool. 1h

13.1 Homogenize fecal pellets using MagnaLyser at speed 6000 rpm, 00:01:00 . 1m

13.2 Plate undiluted to 10^{-6} dilutions (variable) on MacConkey agar supplemented with chloramphenicol to determine bacterial load.

13.3 Keep samples On ice in the cold room, you may need to replate some the next day.

14 The next day, count and record CFU per gram feces.