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Infection of nalidixic-acid treated mice with bioluminescent derivatives of Citrobacter rodentium by oral gavage

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ABSTRACT

Citrobacter rodentium is a Gram-negative bacterium which infects laboratory mice in a similar way to how enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) infect humans. We routinely use a bioluminescent derivative of *C. rodentium* called ICC180 which contains the lux operon from *Photorhabdus luminescens.* This allows us to monitor infection dynamics non-invasively using biophotonic imaging.

We have previously investigated the in vivo evolution of ICC180 through 10 independent transmission chains of 20 mice each. The transmission chains were split into 2 groups, one fed on water and the other fed on water containing nalidixic acid. This protocol describes the oral infection of mice treated with nalidixic acid to assess the infection dynamics of the evolved ICC180 derivatives.

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KEYWORDS

Citrobacter rodentium, oral gavage, mouse, mouse infection model, enteropathogen, in vivo

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GUIDELINES

Experiments involving animals and pathogenic bacteria require ethical and biological safety approval. When planning experiments involving animals, consult the PREPARE and ARRIVE guidelines.

MATERIALS TEXT

Microorganism: In vivo evolved derivatives of *Citrobacter rodentium* ICC180

Growth media and chemicals:

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Item	Catalogue number	Supplier
LB (Lennox) Agar	240110	Fort Richard Laboratories
LB (Lennox) Broth	240230	Fort Richard Laboratories
Kanamycin	K4000	Sigma-Aldrich
Nalidixic acid	N4382	Sigma-Aldrich
Phosphate Buffered Saline tablets	P4417	Sigma-Aldrich
Isoflurane		MedSource NZ Ltd.

Plasticware and equipment:

Item	Catalogue Number	Supplier
Petridishes - 90mm x 14mm	S9001	medi'Ray NZ
Falcon 50mL Conical Centrifuge Tube	BDAA352070	In vitro technologies
straight 4 cm Instech stainless steel feeding needle		Harvard Apparatus
Pipette tips		
Pipettes - various sizes		
37 degree incubators - shaking and static		
Centrifuge		

Animals and husbandry:

Female 6–7 week old C57BL/6Elite mice from specific-pathogen free (SPF) stocks.

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Item	Supplier
Tecniplast Blue line 1284L cages	Tecniplast Australia Ltd
Grit-ology 1/8” corn cob	Corn-cob-ology, Mt Kuring gai NSW, Australia
EnviroDri	Biological Associates, Gladesville NSW, Australia
mouse house	Tecniplast Australia Ltd
Teklad global 18% protein	Biological Associates, Gladesville NSW, Australia

We house up to 6 animals in individually HEPA-filtered Tecniplast Blue line 1284L cages with sterile bedding materials (Grit-ology 1/8” corn cob and EnviroDri), a mouse house, and autoclaved cardboard tube for enrichment. We provided the animals with free access to sterile food (Teklad global 18% protein) and water. Conditions in the animal unit are controlled at 20-24°C, 45-65% relative humidity, and a 12-hour dark-light cycle. Lights turn on at 6:30 am and off at 6:30 pm with a 30 min dawn/dusk period starting at 6 am and 6 pm, respectively.

BEFORE STARTING

Prior to oral gavage, ensure animals have been weighed and marked in some way so that you can identify individual animals. We weigh animals by placing them in a 1ml pipette tip box placed on a set of scales. We use a marker pen to mark each animal's tail. For example, if there are 5 animals in a cage, we give each animal 1-5 marks. In our experience, a black marker pen stays visible the longest.

Preparation of bioluminescent *Citrobacter rodentium* derivatives 2d 0h 5m

1 1d

At least two days before needed, revive bacteria from frozen stocks stored at –80°C. Plate onto LB-Lennox media. At this stage, you can grow them with or without kanamycin [M] **50 ug/mL** . Incubate **Overnight** at **37 °C**

2 1d

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The day before needed, inoculate **10 mL** LB-Lennox (LB) media supplemented with kanamycin in a **50 mL** tube. We use several colonies to inoculate to provide a more heterogeneous culture for infection. Incubate **Overnight** at **37 °C** with shaking at **200 rpm** .

3 

5m

On the day of infection, centrifuge the culture at **4500 rpm** for **00:05:00** and resuspend in **1 mL** PBS to give a 10x concentrated inoculum.

4 

To retrospectively calculate the number of bacteria in the inoculum, prepare a 10-fold dilution series of the inoculum in PBS and incubate 3 **25 µL** drops of each dilution onto LB plates (with or without kanamycin). Incubate overnight at **37 °C** and count the colonies.

Addition of nalidixic acid to drinking water

5 One day prior to gavage, add nalidixic acid to drinking water to give a final concentration of **10 ug/mL** . To do this, prepare a 1000 times concentrated stock of nalidixic acid and add at 1 uL/mL. Change the water every 2-3 days, adding fresh nalidixic acid from the concentrated stock each time.

Oral gavage of mice

6 

[Optional] Animals can be lightly anaesthetised using gaseous isoflurane to aid gavage. To do this, place mice into the anaesthetic induction chamber and induce anaesthesia using a flow rate of 1 L/min oxygen combined with 5% isoflurane. Animals are sufficiently anaesthetised once the animals have lost their righting reflex. It is important that animals are not too deeply anaesthetised as their vital functions can be compromised. The respiratory rate of a normal undisturbed mouse is approximately 180 breaths per minute. A slow rate drop of 50% is acceptable during anaesthesia. Breathing should be steady. If the animals' breathing becomes ''jerky'', too much anaesthetic is being applied and this will be fatal if maintained for long periods of time. If an animal appears too deeply anaesthetised, immediately turn off the anaesthetic and administer supplemental oxygen.

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7 Prepare the inoculum in a **1 mL** syringe and attach a feeding needle.

8 ⚠

Using the feeding tube, orally gavage each animal with **200 µL** of concentrated inoculum.

This video is a good resource for people who are new to the technique: https://researchanimaltraining.com/articles/oral-gavage-in-the-mouse/.

To minimise the risk of oesophageal trauma and incorrect dosing, it is crucial that the operator is skilled both in the technique and the restraint method used. Inadvertent dosing into the lung may occur, and this usually results in the animal showing immediate signs of respiratory distress. If this is observed, then the animal should be humanely killed using an approved method.

9 After dosing, return animals to their cage and observe. If done correctly, the animals should resume normal activity within minutes.

10 Animals should be routinely monitored by measuring their weight, behaviour, and condition. The GRIMACE scale is ideal. The original study that developed the scale is online here and an explanatory poster and other resources are available here.

Depending on the size of the dose, some animals may not eat for a short period and so may experience some weight loss in the first 24 hours after gavage. If they are active and alert and their fur remains smooth and glossy, this is usually no cause for concern.

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