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Monitoring *Citrobacter rodentium* infection dynamics in vivo using biophotonic imaging

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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 from anaesthetised animals using biophotonic imaging.

Biophotonic imaging machines comprise a charge-coupled device (CCD) camera mounted within a light-tight specimen chamber. The shelf of the imaging chamber is heated to enhance the well-being of the anaesthetised animals. Commercially available imaging systems also have a gaseous anaesthesia manifold located inside the imaging chamber so that once sufficiently anaesthetised, animals can be transferred to the imaging chamber and anaesthesia maintained.

Typically, a photographic reference image is acquired under weak illumination and then the bioluminescent signal is captured in complete darkness, which may take from seconds to minutes depending on the strength and location of the signal. The CCD camera spatially encodes the intensity of incident photons which are then displayed as a pseudocolour image superimposed on the grey-scale photographic image. Variations in colour within the pseudocolour image represent variations in light intensity at a given location. Depending on the software, it is also possible to quantify the bioluminescence in specific regions of individual mice using the region of interest tool and from these, calculate an area under curve (AUC) value for each individual animal for the period they were infected.

Using this protocol, mice may be imaged daily if required. We routinely image immediately after oral gavage to determine whether animals have been dosed properly, and then at several time points during infection, for example, days 1, 3, 6/7, 10, and 14 post infection.

Guidelines

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

Signal impedance by melanin: In our experience, there is at least a 10-fold quenching of the bioluminescent signal from mice with black fur compared to mice with white fur. Depending on the region the bioluminescent signal comes from, it may be advisable to remove the fur from dark animals. We routinely remove the abdominal fur from dark animals using a cordless rechargeable beard trimmer. We have previously used a moisturising hair-removal cream but have found the beard trimmer to be ideal.

Anaesthesia: Most commercially available imaging machines require mice to be anaesthetised for restraint purposes. The level of bioluminescence signal emitted by the bacteria will determine the time required for the animals to remain under anaesthesia, but will usually be in the range of 5–15 min in total, with imaging times of 1–10 min. It is preferable to anaesthetise mice using inhalational agents such as isoflurane. The advantage of maintaining animals on gas is the greater control of the level of anaesthesia. Inhaled agents are mainly eliminated by the lungs, whereas injectable agents need to be metabolised by the liver and excreted by the kidneys, a process which can be prolonged. Recovery is therefore more rapid from inhaled agents, which is important in regaining normal physiology. Inhalational agents are also suitable for high-frequency imaging.

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.

Materials

Materials

In vivo optical imaging equipment. Options include the IVIS range (Perkin Elmer, USA), NightOWL (Berthold Technologies, Germany), Photon Imager (Biospace Lab, France), and the Lago, Ami, and Kino imagers (Spectral Instruments Imaging, USA).

Isoflurane

Handheld facial hair trimmer (for example, VS Beard Buddy [VSM703A])

Troubleshooting

1 Place mice into the anaesthetic induction chamber.

2 Induce anaesthesia using a flow rate of 1 L/min oxygen combined with 5% isoflurane.

3 When the animals have lost their righting reflex, remove them from the induction chamber and placed them on their backs within the imaging chamber. Ensure each animal's nose is correctly positioned within its nose cone.

4 Maintain anaesthesia at 1.5–2% isoflurane with an oxygen flow rate of 0.4–0.5 L/min.

5 Monitor the animals for a few minutes to ensure they are in the proper anaesthetic plane. If they are too lightly anaesthetised, they may regain consciousness while in the imaging chamber. If they are too deeply anaesthetised, their vital functions may be compromised.

Their 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 is too deeply anaesthetised, remove it from the imaging chamber and place it into its home cage and monitor. If its breathing fails to quickly return to normal, place it back in the anaesthesia induction chamber and administer supplemental oxygen.

6 Optional: Shave the abdominal area of each mouse using a handheld facial hair trimmer to minimise any potential signal impedance by melanin within pigmented skin and fur.

7 Image following manufacturer's instructions for the machine. We generally set the sample shelf to give a field of view of 12.5 cm. The level of luciferase expression (bacterial numbers) will determine the amount of bioluminescence to be detected. This in turn will determine the time required to detect the signal, which for *C. rodentium* ICC180 usually ranges from less than 1 minute at the peak of infection to 5-10 minutes as the infection is clearing.

8 After imaging, remove mice from the imaging equipment for recovery from anaesthetic. When using isoflurane, animals will recover within minutes of being returned to their cage.