

Submission ID #: 69751

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21274103>

Title: Oral Gavage in Neonatal Mouse Pups and Functional Assessment of Gut Barrier Integrity Using Ussing Chambers

Authors and Affiliations:

Louis Berthet¹, Camille Tardiveau¹, Maryline Roy¹, Emilie Viennois^{1,2}

¹INSERM U1149, Center of Research on Inflammation, Université Paris Cité

²CHRU Nancy, IHU Infiny

Corresponding Authors:

Émilie VIENNOIS emilie.viennois@inserm.fr

Email Addresses for All Authors:

Louis BERTHET	louis.berthet@inserm.fr
Camille TARDIVEAU	camille.tardiveau@inserm.fr
Maryline ROY	maryline.roy@inserm.fr
Émilie VIENNOIS	emilie.viennois@inserm.fr

Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES, all done**

If **Yes**, can you record movies/images using your own microscope camera?

YES

SCOPE shots: **3.1.1 to 3.7.1 , 3.9.1 to 3.11.1**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 38 (Scope 20)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**. Answers are slightly edited according to journal guidelines.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Émilie Viennois:** We study the role of micro-RNA in host-microbiota interaction and explore their potential as new therapeutics in IBD.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Use last take**

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Émilie Viennois:** Current methods lack reliable tools to assess early-life gut barrier integrity. This protocol addresses that gap by enabling accurate investigation of developmental factors underlying chronic diseases.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Use last take**

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Louis Berthet:** This protocol offers a powerful strategy to study how specific molecules impact neonatal gut barrier function.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Use last take**

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Louis Berthet:** Alongside training, swiftness and precision are required to ensure correct handling of the animals and optimal conditions for permeability assay.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Use last take**

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Louis Berthet:** Colonic permeability changes can be validated using independent assays, such as qPCR quantification of tight junction–related transcripts to confirm barrier modulation.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Videographer's NOTE: Use last take**

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the French Ministry of Higher Education, Research and Innovation

Protocol

2. Gavage of Neonatal Mouse Pups

Demonstrator: Louis Berthet

2.1. To begin, place clean paper towels on a disinfected heating pad set to approximately 38 degrees Celsius [1].

2.1.1. Talent placing clean paper towels onto the surface of a disinfected heating pad.

2.2. Wash gloves with 70 percent ethanol prior to opening the animal cages to reduce odor transfer to the pups and limit the risk of cannibalization [1].

2.2.1. Talent applying 70 percent ethanol to gloved hands and rubbing them thoroughly.

2.3. Now, transfer the dam to a separate cage located in another room to minimize stress caused by the pups' vocalizations [1]. Rub cleaned gloves with nesting materials to prevent the transfer of external odors to the pups during gavage [2]. Attach the head of the feeding needle to a syringe using sterile technique [3].

2.3.1. Talent gently moving the dam from the original cage to a clean cage, then exiting the room.

2.3.2. Talent rubbing gloved hands against nesting material inside the cage.

2.5.1,. Talent using sterile technique to connect a feeding needle to a syringe. **Videographer's NOTE: Use take 2**

2.4. Measure the distance from the xiphoid process to the snout of a pup to mark the maximum insertion length on the feeding needle [1].

2.4.1. Talent aligning a pup beside a feeding needle and marking the measured distance between the xiphoid process and the snout on the needle.

Videographer's NOTE: Use take 2

2.5. To begin intra-esophageal gavage, ~~attach the head of the feeding needle to a syringe using sterile technique [1]~~ and draw more than the desired volume of gavage solution to avoid air bubble administration [2].

- ~~2.5.1. Talent using sterile technique to connect a feeding needle to a syringe.~~
Videographer's NOTE: Moved after 2.3.2
- 2.5.2. Talent drawing up excess gavage solution. **Videographer's NOTE: Use take 2**
- 2.6. Using the thumb and index finger of the non-dominant hand, gently pinch the skin between the scapulas to lift the pup [1].
- 2.6.1. Talent lifting the pup by pinching the skin between its scapulas.
- 2.7. After lubricating, Hold the pup in a near-horizontal position and insert the feeding needle into the oral cavity [1], advancing it perpendicularly toward the pharynx until it reaches the back of the throat [2]. Then, slide the needle to either side of the oral cavity to avoid the tongue while maintaining the same angle and depth [3].
- 2.7.1. Talent positioning the pup horizontally. **Videographer's NOTE: 2.7.1, 2.7.2, 2.7.3, 2.8.1 2.8.2, 2.9.1 and 2.9.2 are combined. Take 1-2 show side view and 3-5 show top view for all these shots**
- 2.7.2. Talent guiding the needle into the oral cavity until it reaches the throat.
- 2.7.3. Talent adjusting the needle position laterally within the pup's mouth while keeping it at the same depth.
- 2.8. Slowly tilt the pup's head backward while adjusting the needle angle [1] until the syringe, head, and back are aligned, with the head slightly inclined toward the back [2].
- 2.8.1. Talent tilting the pup's head back.
- 2.8.2. Shot of aligned syringe, head, and spine in a continuous line.
- 2.9. Then, let the syringe descend slowly under its own weight, without applying pressure [1], until the insertion mark approaches or reaches the level of the snout [2].
- 2.9.1. Talent holding the pup still as the syringe naturally lowers. **Videographer's NOTE: shows mouth in close up**
- 2.9.2. Shot of syringe near the marked insertion point at the snout.
- 2.10. Quickly dispense the desired volume of gavage solution once the insertion mark is reached or nearly reached [1]. Next, gently withdraw the needle, maintaining the same angle as during insertion [2].
- 2.10.1. Talent quickly pressing the syringe plunger to deliver the solution.

2.10.2. Talent slowly pulling the needle out while preserving the same alignment.

2.11. Place the pup on the paper towel previously laid on the heating pad and monitor for recovery, ensuring normal breathing resumes within 20 seconds [1]. Once the pup has recovered, place it back into the nest and regroup it with the dam [2].

2.11.1. Talent setting the pup down on the heated paper towel.

2.11.2. Talent gently transferring the pup back into the nest with the dam.

3. Neonatal Mouse Pup Colon Retrieval and Tissue Mounting

Demonstrator: Émilie Viennois

3.1. After euthanizing the animal, place it under a dissection binocular [1] and apply 70 percent ethanol to the abdomen of the animal [2-TXT].

3.1.1. Talent placing the animal under dissection binoculars. **TXT:**
Perform euthanasia following institutional guidelines

3.1.2. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:00-00:05 .

3.2. Using forceps, pinch the abdominal skin and peritoneum [1], then make a large transverse incision with fine scissors, oriented perpendicular to the rostral–caudal axis, without cutting into the intestine [2].

3.2.1. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:06-00:11.

3.2.2. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:12-00:20.

3.3. Now, gently insert the fine scissors into the incision and extend the cut laterally through the peritoneum on both sides [1].

3.3.1. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:25-00:31.

3.4. Using forceps and fine scissors, open the superior and inferior parts of the peritoneum by cutting from the thoracic region to the lower pelvic area [1]. Then, identify the distal colon and make an incision parallel to its axis, moving toward the rectum [1].

3.4.1. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:45-00:50.

3.4.2. SCOPE: 3.1.2---3.6.1-(take-1).mp4 00:55-01:05.

- 3.5. Now, make an incision in the terminal portion of the colon to mobilize it [1] and gently pull the colon to proceed toward the cecum, removing any connective or fat tissue encountered [2].
- 3.5.1. SCOPE: 3.1.2---3.6.1-(take-1).mp4 01:08-01:12.
- 3.5.2. SCOPE: 3.1.2---3.6.1-(take-1).mp4 01:13-01:22.
- 3.6. Free the colon from the abdomen by cutting at the ileocecal junction, just above the cecum [1]. Discard the cecum and the initial few millimeters of the proximal colon [2], then collect one or two colon samples that are larger than the aperture of the Ussing slider [3].
- 3.6.1. SCOPE: 3.1.2---3.6.1-(take-1).mp4 01:24-01:30.
- 3.6.2. SCOPE: 3.6.2---3.8.1.mp4 00:05-00:13.
- 3.6.3. SCOPE: 3.6.2---3.8.1.mp4 00:18-00:22.
- 3.7. With the scissors closed, apply light pressure and slide from the proximal to distal end to remove fecal content from the flattened biopsy [1].
- 3.7.1. SCOPE: 3.6.2---3.8.1.mp4 00:23-00:32.
- 3.8. Next, place the emptied piece of colon into ice-cold Krebs-Ringer bicarbonate buffer [1]. Lightly dry the previously cleaned colon sample using a paper towel to remove any excess moisture [2].
- 3.8.1. SCOPE: : 3.6.2---3.8.1.mp4. 00:33-00:40
- 3.8.2. SCOPE : 3.8.2.mp4.
- 3.9. Then, place the colon sample under a magnifying glass [1] and carefully insert one blade of fine scissors into the luminal part to cut it open [2].
- 3.9.1. SCOPE: 3.9.1---3.11.1.mp4 00:00-00:04.
- 3.9.2. SCOPE: 3.9.1---3.11.1.mp4 00:06-00:20.
- 3.10. Using fine forceps, mount the tissue on the white part of the slider with the luminal side facing upward toward the operator [1].
- 3.10.1. SCOPE: 3.9.1---3.11.1.mp4 00:30-00:40.

3.11. Finally, close the slider under a binocular microscope and inspect for any tissue displacement [1]. While maintaining gentle pressure to keep the slider closed, insert it into the Ussing chamber with the lumen, marked by the transparent part of the slider, facing to the left [2-TXT].

3.11.1. SCOPE: 3.9.1---3.11.1.mp4 01:00-01:14.

3.11.2. Talent pressing lightly on the slider and inserting it into the Ussing chamber with correct orientation. **TXT: Perform the colonic permeability assay**
Videographer's NOTE: MED + CU shot

Results

4. Results

- 4.1. Colonic permeability was quantified by calculating time-dependent flux of fluorescein isothiocyanate–dextran or horseradish peroxidase from Ussing chamber measurements for each experimental group [1]. The flux values significantly increased in biopsies from animals treated with micro-RNA-B [2], but not micro-RNA-A [3] when compared to controls. [4].

4.1.1. LAB MEDIA: Figure 7 *Video editor: Highlight the curve for PBS control*

4.1.2. LAB MEDIA: Figure 7 *Video editor: Highlight the curve for mirR B and the “*” above these data points*

4.1.3. LAB MEDIA: Figure 7 *Video editor: Highlight the curve for miR A*

4.1.4. LAB MEDIA: Figure 7 *Video editor: Highlight the curve for PBS control*

- 4.2. Flux evolution of individual samples was verified to identify potential anomalies, as flux values were expected to remain stable across time points [1].

4.2.1. LAB MEDIA: Figure 8. *Video editor: Serially highlight the curves 1 to 5.*

- 4.3. Addition of 10 millimolar EDTA at time zero resulted in a marked increase in flux compared with control samples while maintaining a similar overall flux evolution pattern [1].

4.3.1. LAB MEDIA: Figure 8. *Video editor: Highlight the red curve corresponding to sample number 6 (EDTA treated).*

Xiphoid

Pronunciation link: <https://www.merriam-webster.com/dictionary/xiphoid>

IPA: /'zai fɔɪd/

Phonetic Spelling: zai·foyd

Cannibalization

Pronunciation link: <https://www.merriam-webster.com/dictionary/cannibalization>

IPA: /,kæn.ə.bəl.ə'zeɪ.ʃən/

Phonetic Spelling: kan·uh·buh·luh·zay·shuhn

Gavage

Pronunciation link: <https://www.merriam-webster.com/dictionary/gavage>

IPA: /gəˈvɑːʒ/, /gəˈvæʒ/

Phonetic Spelling: guh·vaazh

Intraesophageal

Pronunciation link: <https://www.merriam-webster.com/dictionary/intraesophageal>

IPA: /ˌɪn.trəˈsɑːfəˈdʒiːəl/

Phonetic Spelling: in·truh·ih·saa·fuh·jee·uhl

Scapula

Pronunciation link: <https://www.merriam-webster.com/dictionary/scapula>

IPA: /ˈskæp.jə.lə/

Phonetic Spelling: skap·yuh·luh

Pharynx

Pronunciation link: <https://www.merriam-webster.com/dictionary/pharynx>

IPA: /ˈfer.ɪŋks/

Phonetic Spelling: fair·ingks

Euthanizing

Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>

IPA: /ˈjuː.θəˌnaɪz/

Phonetic Spelling: yoo·thuh·nyz

Peritoneum

Pronunciation link: <https://www.merriam-webster.com/dictionary/peritoneum>

IPA: /ˌper.əˈtəːniːəm/

Phonetic Spelling: peh·ruh·tuh·nee·uhm

Rostral

Pronunciation link: <https://www.merriam-webster.com/dictionary/rostral>

IPA: /ˈrɑːstrəl/

Phonetic Spelling: rah·struhl

Caudal

Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>

IPA: /ˈkɔːdəl/

Phonetic Spelling: kaw·duhl

Thoracic

Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracic>

IPA: /θəˈræs.ɪk/

Phonetic Spelling: tuh·ras·ik

Pelvic

Pronunciation link: <https://www.merriam-webster.com/dictionary/pelvic>

IPA: /'pel.vɪk/

Phonetic Spelling: পেঁল·vik

Cecum

Pronunciation link: <https://www.merriam-webster.com/dictionary/cecum>

IPA: /'si:.kəm/

Phonetic Spelling: see·kuhm

Ileocecal

Pronunciation link: <https://www.merriam-webster.com/dictionary/ileocecal>

IPA: /,ɪl.i.oo'si:.kəl/

Phonetic Spelling: il·ee·oh·see·kuhl

Biopsy

Pronunciation link: <https://www.merriam-webster.com/dictionary/biopsy>

IPA: /'baɪ.ɑ:p.si/

Phonetic Spelling: bye·op·see

Krebs-Ringer

Pronunciation link: <https://www.merriam-webster.com/dictionary/Krebs-Ringer%20solution>

IPA: /'krɛbz 'rɪŋər/

Phonetic Spelling: krebz·ring·er

Bicarbonate

Pronunciation link: <https://www.merriam-webster.com/dictionary/bicarbonate>

IPA: /baɪ'kɑ:r.bə.neɪt/

Phonetic Spelling: bye·kar·buh·nayt

Luminal

Pronunciation link: <https://www.merriam-webster.com/dictionary/luminal>

IPA: /'lu:.mə.nəl/

Phonetic Spelling: loo·muh·nuhl

Ussing

Pronunciation link: <https://www.merriam-webster.com/dictionary/Ussing>

IPA: /'ʊs.ɪŋ/

Phonetic Spelling: uhs·ing

Fluorescein

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescein>

IPA: /,flɒr.ə'si:.ɪn/

Phonetic Spelling: floor·uh·see·in

Isothiocyanate

Pronunciation link: <https://www.merriam-webster.com/dictionary/isothiocyanate>

IPA: /,aɪ.sou,θaɪ.ou'saɪ.ə.net/

Phonetic Spelling: eye·soh·thye·oh·sy·uh·nayt

Dextran

Pronunciation link: <https://www.merriam-webster.com/dictionary/dextran>

IPA: /'dɛk.stræn/

Phonetic Spelling: dek·stran

Peroxidase

Pronunciation link: <https://www.merriam-webster.com/dictionary/peroxidase>

IPA: /pə'rɑ:k.sə,deɪs/

Phonetic Spelling: puh·rok·suh·days

Millimolar

Pronunciation link: <https://www.merriam-webster.com/dictionary/millimolar>

IPA: /,mɪl.ə'moʊ.lər/

Phonetic Spelling: mil·uh·moh·ler

EDTA

Pronunciation link: <https://www.merriam-webster.com/dictionary/EDTA>

IPA: /,iː.diː.ti:'eɪ/

Phonetic Spelling: ee·dee·tee·ay