



Dec 23, 2021

Ligated intestinal loop mouse model protocol.

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[dx.doi.org/10.17504/protocols.io.bvzin74e](https://doi.org/10.17504/protocols.io.bvzin74e)

PLOS ONE Lab Protocols
Tech. support email: plosone@plos.org

Pablo Castro-Córdova

In the present protocol, we provide a step-by-step protocol with all the critical information to perform an intestinal ligated loop in a mouse model. Using this technique, we describe the infection of intestinal loop with *C. difficile* spores and immunofluorescence of whole mounted intestinal tissue to study the interaction of *C. difficile* spores with the intestinal mucosa. Also, this work provides information of the processing and mounting of the tissues to acquire high-resolution confocal images, and that may be used to quantify the spore adherence and internalization to the intestinal mucosa.

DOI

[dx.doi.org/10.17504/protocols.io.bvzin74e](https://doi.org/10.17504/protocols.io.bvzin74e)

<https://journals.plos.org/plosone/article/citation?id=10.1371/journal.pone.0261081>

Pablo Castro-Córdova, Maria José Mendoza-León, Daniel Paredes-Sabja 2021.
Ligated intestinal loop mouse model protocol.. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bvzin74e>



protocol

Castro-Córdova P, Mendoza-León MJ, Paredes-Sabja D (2021) Using a ligate intestinal loop mouse model to investigate *Clostridioides difficile* adherence to the intestinal mucosa in aged mice. PLOS ONE 16(12): e0261081. <https://doi.org/10.1371/journal.pone.0261081>

intestinal ligated loop, ileal loop, colonic loop, *C. difficile* spore

protocol ,

Jun 22, 2021

Jun 23, 2021

50954

Biosafety and personal protection elements

During the surgery, the use of personal protective equipment is required, such as a disposable laboratory coat, goggles, gloves, cap, and mask when you are manipulating the fixing solution. The surgery is not performed under the biological safety cabinet. However, processes that produce aerosol, such as preparing the syringe with *C. difficile* spore inoculum, opening and cleaning the tissues infected with *C. difficile* spores, were performed under the biosafety cabinet. Finally, all the used surfaces were disinfected with a solution of 0.5% bleach and 1:200 Virkon-S.

Solutions

- 10× Phosphate buffered saline (PBS):** Stock solution; Calculate the reagents required to prepare **1 L** of **1.37 Molarity (M)** NaCl, **27 Milimolar (mM)** KCl, **100 Milimolar (mM)** Na₂HPO₄, **18 Milimolar (mM)** KH₂PO₄, and dissolve it in **800 mL** of Milli-Q water. Then the pH was adjusted to **pH 7.5** with HCl and fill with Milli-Q water to **1 L**. Sterilize by autoclaving for **00:20:00** at **121 °C** with **1 Bar**, and store at **Room temperature**

 Sodium

Chloride Sigma Catalog #S9888

 Potassium chloride Millipore







Sigma Catalog #P3911








 Sodium phosphate dibasic Sigma

Aldrich Catalog #7558-79-4

 Potassium phosphate monobasic Merck



















Millipore Catalog #P5655

- 2 **1× (PBS):** Dilute 10× PBS stock solution 1:9 ( 100 mL of 10× PBS into  900 mL of Milli-Q water) and then sterilize by autoclaving for  00:20:00 at  121 °C with  1 Bar . Store at  Room temperature .

- 3 **Saline solution:**  0.9 % (w/v) NaCl. Dissolve  9 g of NaCl in  1 L of Milli-Q water. Pass through 0.45-µm filters and sterilize by autoclaving for  00:20:00 at  121 °C with  1 Bar . Store at  Room temperature .

 Sodium



Chloride Sigma Catalog #S9888

- 4 **Fixing solution:**  30 % (w/v) sucrose in PBS–  4 % (w/v) paraformaldehyde. In the first place, a solution of PBS–  4 % (w/v) paraformaldehyde was prepared as follows: for  1 L , add  40 g of paraformaldehyde powder to  800 mL of 1× PBS. Heat to  60 °C in a fume hood (no dot boil). If it does not dissolve, raise the pH adding  5 N NaOH drop by drop until a clear solution is formed. Cool the solution, adjust the pH to  pH 8.0 , and adjust the volume to  1 L with 1× PBS. Pass through 0.45-µm filter to remove particles. Aliquot in small volume and store at  4 °C for use in 1 to 2 weeks or store at -  20 °C for up to 1 year. To prepare  100 mL of  30 % (w/v) sucrose in PBS–  4 % (w/v) paraformaldehyde,  30 g of sucrose were dissolved in PBS–  4 % (w/v) paraformaldehyde with a final volume of  100 mL .

 Paraformaldehyde Sigma

Aldrich Catalog #P6148

 Sucrose Winkler Catalog #013900

- 5 **Permeabilizing solution:** a PBS–  0.2 % (v/v) Triton X-100 solution was prepared as follows: a stock solution of  10 % (v/v) Triton X-100 was prepared in PBS. To prepare  10 mL of the stock solution, dilute  1 Molarity (m) of Triton X-100 in  9 mL of PBS

with gentle shaking. PBS– [M]0.2 % (v/v) Triton X-100 solution was made by dilution 1:49 of the stock with sterilized 1× PBS. The stock solution was stored at 4 °C .

 Triton X-100 Merck

Millipore Catalog #X100

- 6 **Blocking solution:** PBS– [M]3 % (w/v) BSA solution. To prepare 10 mL of the solution, 0.3 g of BSA was dissolved in 8 mL of 1× PBS, then add PBS to 10 mL and sterilize by filtering with 0.2-µm syringe filter. The solution was stored at 4 °C .

 Bovine Serum Albumin Sigma

Aldrich Catalog #A9647

Surgery

7 Day 1

All surgical procedures were performed under clean but non-sterile conditions. The surgical scissors and forceps were autoclaved before usage except the Forceps Dumont N°5, that are not autoclavable, so they were washed with soap, [M]0.5 % (v/v) bleach, and [M]70 % (v/v) ethanol.

All the required materials such as isoflurane, povidone-iodine, [M]70 % (v/v) ethanol, ophthalmic ointment, heat-pad, a stainless-steel surgical tray attached to an isoflurane mask, syringe with saline solution, syringes with *C. difficile* spores, silk braided silicon suture, autoclaved towel paper, masking tape, and the surgical material as forceps, scissors, were organized in a manner that they are easily accessible to the hand (Fig 1).

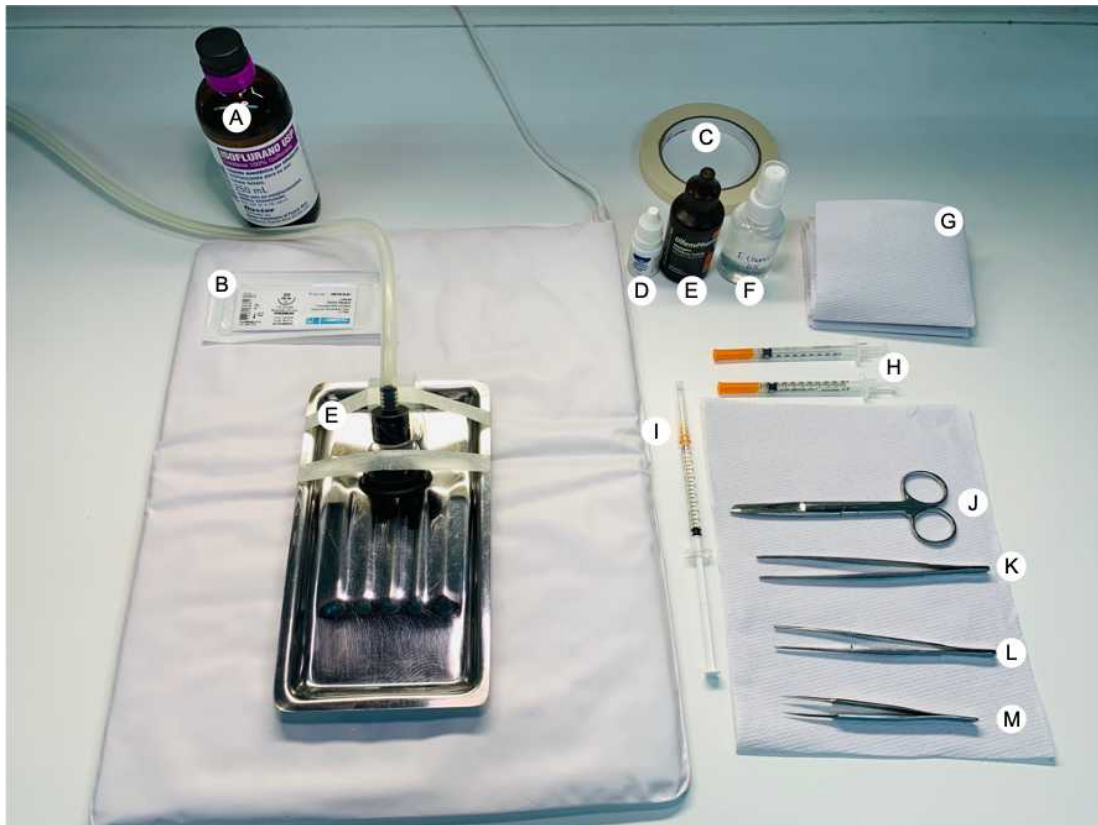


Fig 1. Surgical preparation for intestinal ligated loop procedure. In a clean, disinfected, but non sterile condition, the surgical instruments were prepared as follow: (A) isoflurane-USD, (B) silk braided silicon-coated suture 3-0 HR20, (C) masking tape, (D) ophthalmic ointment, (E) 10% povidone-iodine solution, (F) 70% ethanol solution, (G) sterilized paper towels, (H) 29G insulin syringe with 100µL of 0.9% NaCl containing 5×10^8 *C. difficile* spores, (I) 29G insulin syringe containing sterile 0.9% NaCl to hydrate the tissues), (J) surgical scissors Sharp/blunt, stainless steel, (K) anatomical forceps, stainless steel, (L) anatomical forceps fine, stainless steel, (M) forceps Dumont N°5, super fine tips.

- 8 Male or female, 18–25g mice C57BL/6 of 8–12 weeks were fasted overnight (🕒 15:00:00) before the surgery with free access to water.
- 9 Depth anesthesia was induced in ~ 🕒 00:03:00 with [M] 4 % (v/v) Isoflurane Baxter and a flow of 0.6L/min of with an isoflurane induction chamber.

Small animal Anesthesia Machine

RWD RWD-R510IP

- 10 Take out the mouse from the isoflurane induction chamber, put the mouse prone into the surgery tray, and put the snout (nose and mouth) into the isoflurane mask. The surgery bed is over a heating pad to avoid hypothermia during the procedure. Reduce the isoflurane to **2 % (v/v)**.

 Heating Pad **MardelSur**

- 11 Add ophthalmic drops in the eyes to avoid corneal drying.

 Ophthalmic ointment **Pharmatech**

- 12 Turn the mouse to supine position.

- 13 Check anesthetic depth by non-response to hind limb toe pinch.

- 14 Using small pieces of masking tape, fix the limbs of the mice to the surgery bed.

- 15 Dampen the hair of the abdominal area with **70 % (v/v)** ethanol either by spraying or by dipping. Clean with a paper towel. Let it dry.

 95% Ethanol **Winkler**

- 16 With a disposable razor, shave the abdominal zone.

- 17 Clean the shaved abdominal zone with povidone iodine, and clean it with towel paper (**Fig 2A**).

 Povidone iodine DifemPharma

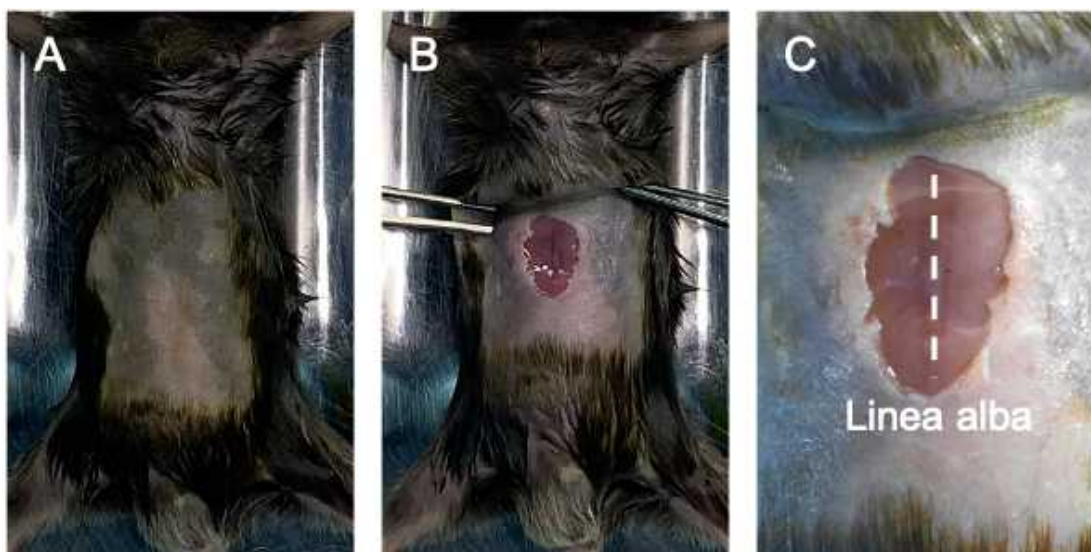


Fig 2. Schematic identification of linea alba. (A) The abdominal skin of the anesthetized mouse was disinfected with 70% ethanol, then was shaved, and the skin was cleaned with povidone-iodine. (B) The incision in the skin was performed parallel to the linea alba. (C) Identification of the linea alba as a semitransparent white line in the peritoneum.

- 18 For steps 8–17, see **S1 Video** in the next link:

https://www.dropbox.com/s/bnnu71l2rewa220/S1_Video.mp4?dl=0

S1 Video. Mouse preparation for surgery (steps 8–17). This video shows how to anesthetize the mouse, apply ophthalmic solution, disinfect, and shave the abdomen.

- 19 Using forceps and a surgical scissor sharp/blunt, perform an incision of ~ **2 cm** in the midline of the abdominal skin (**Fig 2B**).
- 20 The skin is separated from the peritoneum using anatomical forceps.
- 21 Identify the linea alba, a semitransparent longitudinally white line in the peritoneum (**Fig 2C**).

Open the abdominal cavity, incise the abdominal musculature on the linea alba. For this, gently

22 grab the musculature with anatomical forceps and retracting up. The abdominal organs are not adjacent to the muscles. Using a scissor or a scalpel makes a small opening into the peritoneal cavity in the linea alba. Extend the incision in the midline until it reaches the size of the skin cut.

23 For steps 19–22, see **S2 Video** in the next link:

https://www.dropbox.com/s/2ynycw9f8vwz1ot/S2_Video.mp4?dl=0

S2 Video. Midline laparotomy(steps 19–22). This video shown how to open the abdomen skin, identify the linea alba and open the peritoneal cavity.

24 Using forceps, gently move the intestines to identify the cecum, a large J-shaped blind sac curved. Extract the cecum through the incision and identify the ileum and colon (**Fig 3A**). The ileum is the distal last part of the small intestine that is attached to the cavity by mesentery tissue that derives blood supply from the mesenteric artery. The proximal colon is the first part of the colon that begins in the cecum.

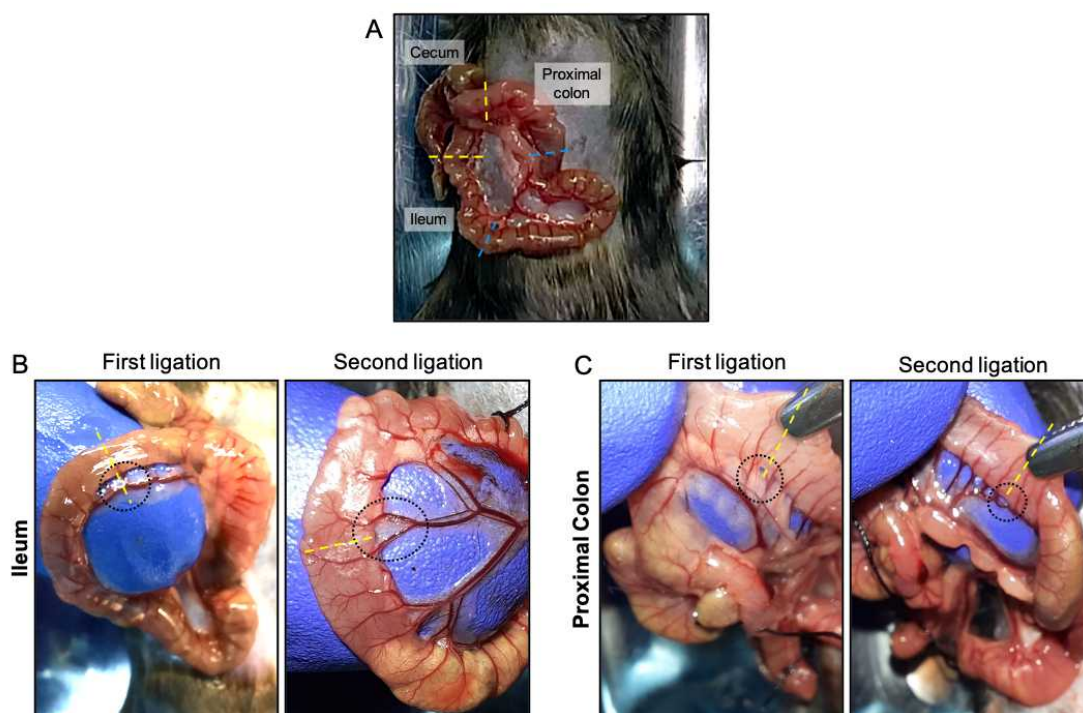



Fig 3. Identification of regions of interest to perform ligations between blood vessels.(A) Identification of ileum and colon using as reference the cecum. The regions of interest to be ligated are indicated with dotted lines. The yellow line and blue line denote the first and second ligation, respectively. Ligations are spaced ~1.5 cm. The ligatures with surgical silk sutures were performed between the intestine and the blood vessels. The identification of regions of interest are shown in (B) ileum and (C) proximal colon. As a reference, the first ligation was performed close to the cecum.

25 When the ileum or colon has fecal material, it can be removed, pressing gently with a blunt tip of the forceps against your fingers and move it in the direction of the flow of the fecal material

or to the cecum.

- 26 In the ileum, identify regions to be ligated where blood vessels are finely separated from the ileum's external wall (**Fig 3B**). Once identified, pass the fine tip of the Dumont N°5 Forceps between the outer wall of the ileum and the blood vessels having care of not damage or puncture the blood vessels. With the tip of the Dumont N°5 Forceps, on the other side of the hole formed between the external wall of the ileum and the blood vessels, grasp the thread of the surgical suture, and gently pass to the other side of the hole.
 - 27 Perform a firm but gentle double "simple knot," performing a blind knot so as not to cut the tissue and having special care of not ligate neither interfering with the blood flow. Note: When necessary, hydrate the intestines with drops of sterile saline solution.
 - 28 A second ligation is then performed at ~ **1.5 cm** of distance from the first ligation using the same strategy described above (**Fig 3B**). However, in this case, perform a simple knot without closing.
 - 29 At **0.5 cm – 1 cm** out of the second ligation, insert the needle of a tuberculin syringes with 5×10^8 *C. difficile* spores in **100 µL** of saline in the direction of the ligation and cross the ligation by inside the intestine the and close the knot with the syringe needle inside. *C. difficile* spores strain R20291 (CM210) were purified as was described previously (Calderón-Romero et. al. 2018)
-  **1 ml tuberculin syringes BD**

Biosciences Catalog #REF 309659
- Calderón-Romero P, Castro-Córdova P, Reyes-Ramírez R, Milano-Céspedes M, Guerrero-Araya E, Pizarro-Guajardo M, Olguín-Araneda V, Gil F, Paredes-Sabja D (2018). Clostridium difficile exosporium cysteine-rich proteins are essential for the morphogenesis of the exosporium layer, spore resistance, and affect C. difficile pathogenesis.. PLoS pathogens. <https://doi.org/10.1371/journal.ppat.1007199>
- 30 Release the *C. difficile* spores inside the loop, keeping the pressure in the knot. This is performed to avoid inoculum loss and splashing that occurs when the ligated loop is injected directly.

31 Remove the syringe and close the ligation with a simple double knot.

32 For steps 24–31, see **S3 Video** in the next link:

https://www.dropbox.com/s/8ckq11o8enpc8s4/S3_Video.mp4?dl=0

S3 Video. Procedure to ligate loops(steps 24–31). This video shows how to identify the ileum and the proximal colon, remove fecal material from the section to be ligated, identify the sites to be ligated. Also, shown how to perform the ligations without interruption of the blood vessels and injection of *C. difficile* spores on ileum and colon.

33 To perform the colonic loop, identify the regions to be ligated (**Fig 3C**) and repeat the points 24–31 in the proximal colon.

34 Carefully using anatomical forceps return intestines to the abdominal cavity.

35 For steps 33 and 34, see **S3 Video**.

36 Close the peritoneum of the abdominal wall by a continuous or interrupted suture using silk suture.

37 Close the skin of the abdominal wall by continuous or interrupted suture using silk suture.

38 Remove mice from the isoflurane mask and from the heating pad and allow the mouse to recover from the anesthesia under a heat lamp. The awareness recovery usually takes
🕒 00:01:00 – 🕒 00:02:00 .

39 For steps 37–38, see **S4 Video** in the next link:

https://www.dropbox.com/s/5kaqmdjw1tu2rlq/S4_Video.mp4?dl=0

S4 Video. Midline laparotomy closure with suture (steps 37–38). This video shows how to suture the abdominal wall and the abdominal skin with silk suture by continuous suture technique to close the incision and let mice recover from the procedure.

- 40 Apply postoperative analgesia when required according to the animal care protocol at your institution.
- 41 Usually, the complete procedure for one mouse and one loop takes ~ 🕒00:15:00 : and with 2 loops ~ 🕒00:10:00 . Animals were kept in the cage for 5h with free access to water and close to a heat lamp. Animals were monitored every 🕒00:30:00 .


Necropsy and tissue collecting

- 42 Depth anesthesia is induced by isoflurane inhalation, as is indicated above in step 9.
- 43 Check anesthetic depth by non-response to hind limb toe pinch.
- 44 Perform cervical dislocation by separating the vertebrae in the cervical area with a firm pinch to the neck using a rigid metallic tool and firmly pull the mouse from the tail. The separation of the skull and brain from the spinal cord is caused by anterior pressure applied in the skull base.
- 45 Using forceps, gently grab the skin and retract it up, so the abdominal organs and the ligated loops are not adjacent to the muscles.
- 46 Using scissors, open the abdominal cavity, cutting the skin and peritoneum. Extend the incision to visualize the intestines and the loops.
- 47 Remove the ligated ileum and colonic loop by cutting at ~ ✂0.5 mm from the outside of ligatures and put the intestinal loops in a petri dish.
- 48 For steps 46–47, see **S5 Video** in the next link:

https://www.dropbox.com/s/1wl5v3u3qadfwiv/S5_Video.mp4?dl=0

S5 Video. Extraction of the ligated loop (steps 33–34). This video shows how to extract the ligated loop in a euthanized mouse.

Fixing the tissues

- 49 In a biosafety cabinet, put drops of **1 mL** of PBS over a petri dish.
- 50 To fix the tissues, prepare a "fixation chamber": we used a petri dish, but you can use any other tupperware with lid that you have available. Put inside a filter paper (extra thick blot paper). If the filter paper is larger than the used container, cut the filter paper with scissors o fit it inside the container.
- 51 Imbibe filter paper with the solution of **30 % (w/v)** sucrose in PBS– **4 % (w/v)** paraformaldehyde enough to wet the entire filter paper without adding an excess of solution. Remove the excess of fixing solution from one edge using a **100 µL – 1000 µL** micropipette.
- 52 To remove the ligatures, cut the ligated loop as close as possible from the ligation. A liquid with gelatinous consistency comes out of it.
- 

Be careful with the handling because that liquid has a high concentration of *C. difficile* spores.
- 53 Put the scissor tip inside the lumen of the intestine and perform a longitudinal cut in the tissue to extend it.
- 54 Grasp the tissue from one end with the forceps and wash the tissue by immersion in the PBS drops for ~20 immersions and repeat in 2–3 different drops of fresh PBS as is necessary for each tissue.
- 55 With anatomical forceps, grasp the opened intestinal tissues from a corner with the muscular layer downwards and the luminal side upwards. This can be identified because when the longitudinally cut tissue is grasped with the forceps from one end, it tends to recover its uncut shape, where the luminal side is inwards and the muscular side is outside. Using 2 pairs of forceps, stretch the tissues on the filter paper with the fixing solution.
- 56 Using a **100 µL – 1000 µL** micropipette, add fixing solution directly over the tissues.

Repeat each ~ 🕒 00:05:00 .

57 Let samples fixing for at least 🕒 00:15:00 .

58 Using forceps, transfers each tissue to one independent 🧴 1.5 mL microcentrifuge tube containing 🧴 1 mL of [M]30 % (w/v) sucrose in PBS– [M]4 % (w/v) paraformaldehyde solution, having care that the tissue is completely submerged in the fixing solution and there are no air bubbles in the tissue. Is common that some tissues should be folded in half so that it remains immersed in the solution. If the intestine has adipose tissue attached, it will tend to float. Therefore, it is recommended to remove the adipose tissue using scissors and forceps. Incubate the intestines in fixing solution 🕒 Overnight at 🌡 4 °C .

59 For steps 50–58, see **S6 Video** in the next link:

https://www.dropbox.com/s/w1xacz5hqxc0m4z/S6_Video.mp4?dl=0

S6 Video. Washing and fixing of extracted tissues(steps 50–58). This video shows how to open and wash the infected ligated loops and the procedure of fixing with 30% sucrose in PBS–4% paraformaldehyde.

Immunofluorescence

60 Day 2

Using forceps, transfer the tissues to a new 🧴 1.5 mL microcentrifuge tube containing 🧴 1 mL of PBS. Perform this step carefully to avoid paraformaldehyde splashing. Incubate for ~ 🕒 00:05:00 a 🌡 Room temperature .

61 Wash the tissues. Using a 🧴 100 µL – 🧴 1000 µL micropipette, remove the PBS and discard it to an autoclavable glass bottle, from now on, waste bottle. Add 🧴 1.0 mL of PBS to the edges of the tube. And repeat one more time.

62 Using forceps put the tissues over a clean and sterile open petri dish, and using surgical scissors, cut a section of the tissues of ~ 📏 5 mm × 📏 5 mm .

63 Using forceps, transfer the tissues to a 🧴 0.5 mL microcentrifuge tube containing 🧴 150 µL

permeabilizing solution; PBS– **0.2 % (w/v)** Triton X-100 and incubated for **02:00:00** at **Room temperature**

- 64 Using a **20 µL – 200 µL** micropipette, remove the permeabilizing solution as much as possible from the walls of the tube and discard it in the waste bottle. Add **200 µL** of PBS to wash the samples and incubate for ~ **00:03:00** at **Room temperature** in an orbital shaker at **60 rpm** . Repeat 2 more times.
- 65 In the same tubes, incubate the tissues with **150 µL** of blocking solution; PBS– **3 % (w/v)** BSA for **03:00:00** at **Room temperature** in an orbital shaker at **60 rpm** .
- 66 Using a **20 µL – 200 µL** micropipette, remove the blocking solution and discard it in the waste bottle.
- 67 Add **70 µL – 90 µL** of 1:1,000 chicken primary antibody anti-*C. difficile* spore IgY batch 7246 (AvesLab, USA) and 1:150 phalloidin Alexa-Fluor 568 (A12380 Invitrogen, USA); in PBS– 3% BSA overnight at **4 °C** . This antibody does not immunoreacted with epitopes of vegetative cells neither with murine microbiota [see references]. After adding the antibody solution, check that there are no bubbles in the tube and that the tissue is completely submerged.

Castro-Córdova P, Mora-Urbe P, Reyes-Ramírez R, Cofré-Araneda G, Orozco-Aguilar J, Brito-Silva C, Mendoza-León MJ, Kuehne SA, Minton NP, Pizarro-Guajardo M, Paredes-Sabja D (2021). Entry of spores into intestinal epithelial cells contributes to recurrence of *Clostridioides difficile* infection.. Nature communications. <https://doi.org/10.1038/s41467-021-21355-5>







Pizarro-Guajardo M, Díaz-González F, Álvarez-Lobos M, Paredes-Sabja D (2017). Characterization of Chicken IgY Specific to Clostridium difficile R20291 Spores and the Effect of Oral Administration in Mouse Models of Initiation and Recurrent Disease.. Frontiers in cellular and infection microbiology.







<https://doi.org/10.3389/fcimb.2017.00365>

 **Alexa Fluor™ 568 Phalloidin Thermo**

Fisher Catalog #A12380

68 Day 3

Wash the tissues. Using a  **20 µL** –  **200 µL** micropipette, remove the primary antibody solution as much as possible from the walls of the tube and discard it in the waste bottle. Add  **200 µL** of PBS to wash the samples and incubate for ~  **00:03:00** at  **Room temperature** in an orbital shaker at  **60 rpm** . Repeat 2 more times.

69 In the same tube, incubate the tissue with  **70 µL** –  **90 µL** of 1:350 secondary antibodies goat anti-chicken IgY Alexa Fluor-647 (ab150175, Abcam, USA) and  **4.5 µg/µL** of Hoechst 33342 and incubate for  **03:00:00** at  **Room temperature** in an orbital shaker at  **60 rpm** .

 **Hoechst 33342 Invitrogen - Thermo**

Fisher Catalog #H1399

 **Goat Anti-Chicken IgY Alexa Fluor-**

647 Abcam Catalog #ab150175

70 Wash the tissues 3 times as was described in step 68.

Tissue Mounting

71 At this point is difficult to identify the luminal side and the muscular side of the tissues at the naked eye. However, sample mounting is essential to identify the tissue orientation. For this:

- 72 Using forceps, place the samples in a clean glass slide.
- 73 First, using a light -upright or -inverted microscope with 20× or 40× magnification coupled to epifluorescence with a blue filter to visualize Hoechst 33342 staining, orientate the tissues to put the luminal side up as follow.
- 74 In the case of the ileum, villi can be visualized, and in the case of the colon, crypts are easy to identify. In both cases, on the other side, the muscular layer is seen.

Note 1: if you are using upright microscopy, when you see the crypts or villi, keep the orientation of the tissue to the glass slide. If you use an inverted microscope, when you see crypts or villi, flip the tissue to the other side and put them in the glass slides. (**Fig 4A and B**).

Note 2: During this process, don't let the tissues dry because it causes autofluorescence. If samples begin to dry, add PBS with a micropipette.

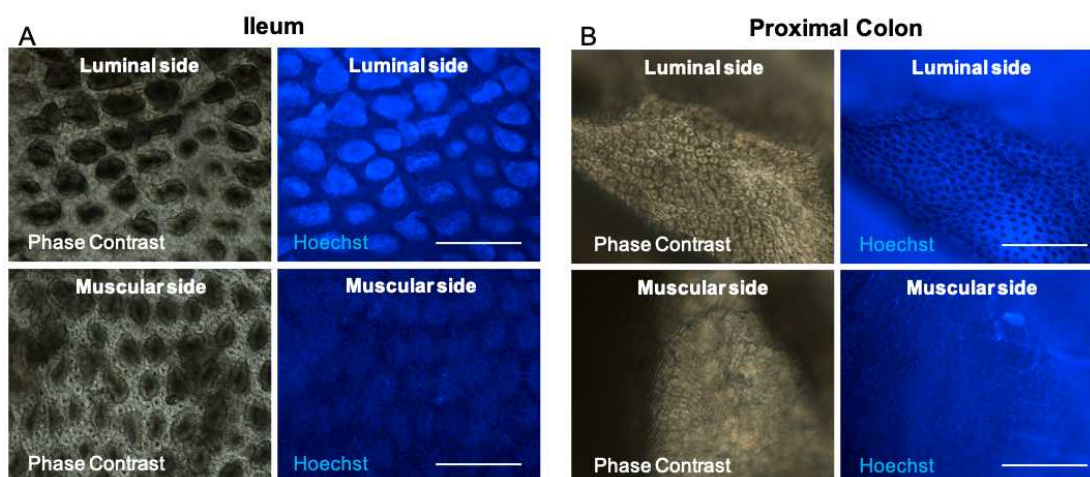


Fig 4. Tissue orientation under microscopy for mounting. Immunostained tissues were visualized under an upright light/epifluorescence microscopy to identify the tissue orientation to mount them with the luminal side up. Representative phase-contrast and Hoechst staining micrograph of (A) ileum and (B) proximal colon with the luminal or muscular side up. Scale bar 400µm.

- 75 Clean the coverslips and slides with **70 % (v/v)** ethanol and towel paper.

- 76 Using towel paper removes the excess PBS from the edges of the tissues.
- 77 In a new clean glass slide, using a **2 µL – 20 µL** micropipette, put a drop of **5 µL** of fluorescent mounting medium for each tissue to be mounted.
- [Fluorescence Mounting](#)
[Medium](#) **Dako Catalog #S3023**
- 78 Using forceps put the tissues over the drops of the mounting medium of the clean slide.
- 79 Using a **2 µL – 20 µL** micropipette, put **15 µL** of fluorescent mounting medium over the tissues having care of not to damage the tissue with the tip of the micropipette.
- 80 Put Scotch transparent tape (3M, USA) on the upper and lower edges of the coverslip, leaving half of the Scotch transparent tape on the coverslips and the other half free.
- 81 Put a coverslip over the samples, not allowing air bubbles to remain in the tissue.
- 82 With your fingers, fold the piece of Scotch transparent tape under the slide firmly.
- 83 Seal the remaining edges with Scotch transparent tape.
- 84 Store the samples at **4 °C** **Overnight**, and then the samples are ready to visualize under confocal microscopy.

Note: After mounting, we usually observe the samples as soon as possible and with no more than 1 week because sometimes samples begin to dry, making impossible the confocal visualization. If you need to store the samples for a longer time, you can store them in a wet chamber: in a tupperware with lid, put a layer of towel paper on the bottom and wet it with water (not in excess), then put a layer of parafilm or plastic wrap, and over it, you can put the samples and then close the cage.

85 For steps 74–84, see **S7 Video** in the next link:

https://www.dropbox.com/s/to07xfy6fyi4xtd/S7_Video.mp4?dl=0

S7 Video. Mounting of immunostained tissues for confocal microscopy (steps 74–84). This video shows how to orientate the tissues to put the luminal side up of the ileum and the colon, the mounting using mounting medium, and sealing it with Scotch transparent tape.