Whole Intestinal & Colonic transit time (*in vivo*)

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Ref: Koster ST et al. STAR Protocols 2021

Summary:

This protocol is to collect gut transit times (total and regional (upper and lower GI tracts) in mice. This test is used to evaluate gut motility in response to treatments, drugs , etc.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Carmine powder	Millipore-Sigma	C1022
Methylcellulose	Millipore-Sigma	M0512
Glass beads (sterile) 1.5 to 3 mm	Fisher	
Double distilled H20		
Hot plate w. magnetic stirrer, stir bars		
50 ml conical tubes, glass beakers		
Dissection scissors/forceps		
Feeding syringes (1 ml)		
Gavage needles		
Lab coats/gloves/PPE		
CO2 gas tank		
Isoflurane USP bottles		
Isoflurane vaporizer/ delivery systems	Kent Scientific	
Charcoal cannisters		
Anesthesia induction chamber		
70% ethanol in squirt bottle		
Disinfectant Novalsan 10%		

Protocol:

1. SET-UP

a. Prepare the 0.5% methylcellulose solution: Warm up ~80% of total water volume on a hot plate with agitation (add stir bar). Add 0.25 grams of methylcellulose powder, let dissolve. Complete to final volume with the remaining water. Let cool down and stored at 4°C in a 50 ml conical tube.

b. Prepare the 6% carmine red dye solution: dissolve 1.2 grams of carmine red dye powder into 20 ml 0.5% methylcellulose solution on a hot plate with agitation until completely in solution. Let the solution cool down and transfer to a 50 ml conical tube and store at 4°C.

NOTE: Methylcellulose and carmine are stable at 4°C for 1 year and protected from light; we recommend preparing fresh 6% carmine solution for each experiment; calculate ~ 200ul of solution per mouse. Methylcellulose and carmine powder can be autoclaved if necessary.

2. PROCEDURE

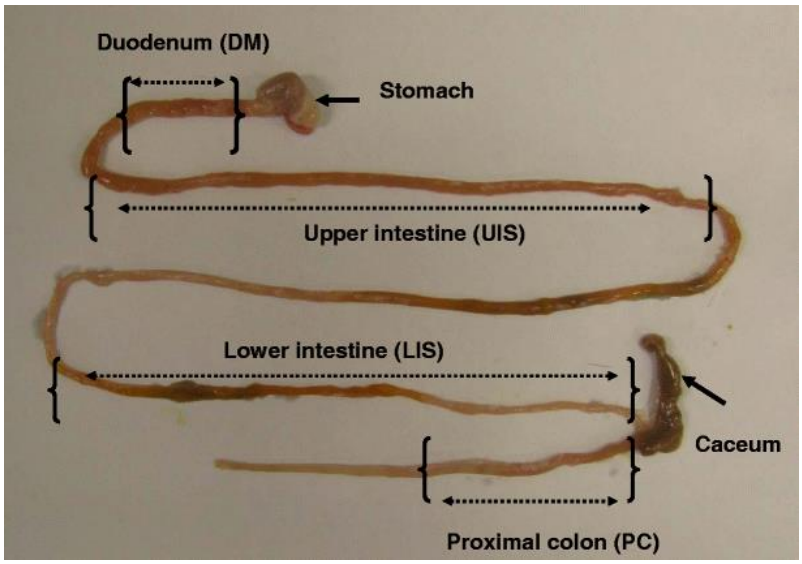
Whole Intestinal transit time:

- **a.** Gavage mouse with 150 µl of the 6% carmine solution (in 0.5% methylcellulose) and record time of administration.
- **b.** Place mouse in a single clean cage with no bedding to collect fecal pellets. The whole intestinal transit time will typically be greater than 2 hours. After a ~2h pause, check each cage (mouse) periodically (at least every 5-10 min) for the presence of fecal pellets. Record time all times when checks are made even if no pellets are produced. Continue periodical checking until the red dye is clearly seen in the fecal pellets. **Note the time**.
- **c.** Once the mouse has defecated carmine fecal pellets, remove mouse from the collection cage and return to its home cage.
- **d.** The total GI transit is determined as the time between the gavage and the time of the expulsion of the first red pellet. A fecal pellet that is partially red may constitute a red pellet if the red color is carefully examined and convincingly felt to represent carmine dye.
- **e.** Time to clearance is defined as the amount of time for the carmine red to be passed in its entirety (ie, until there are no more red fecal pellets). This data are not typically collected.

- **IMPORTANT NOTES:** When in doubt, pellets can be crushed on a paper towel to visualize the presence of the red dye.
- DO NOT apply pressure on the mouse abdomen/anus to stimulate transit time.
- Water and food can be provided during the transit time assay, but it is important to standardize the conditions (light/dark, water/food, etc) between all cohorts of a given study.

Proximal (upper GI) transit time:

- **a.** To determine upper GI transit time, mice are euthanized ($C0_2$ asphyxiation) **15 minutes** after oral administration of the 6% carmine dye solution.
- **b.** Wet abdomen with 70% ethanol and cut a v-shape incision through the ventral abdominal wall from the pubis to the base of the ribcage.
- **c.** Remove the entire length of the small intestines and place – stretched on a paper tower.
- **d.** Measure the distance traveled by the red dye.
- **e.** Proximal transit time is expressed as the **percentage of distance of the red dye** over the entire length of the small intestines (measured from duodenum to the caecum).



Distal (colon) transit time:

a. Mice are fasted for 16h prior the procedure.

b. Place mouse in induction chamber and anesthetize with 2-5% isoflurane. When mouse is ready, switch isoflurane flow to nose cone and set at 2%.

c. Apply petroleum jelly to the anus of mouse with a sterile Q-tip and slowly insert a sterile ~3mm glass bead into the rectum using Q-tip. Insert at a distance of 2 to3 cm from the anus opening.

d. Place mouse in a clean, plastic empty cage and measure the time when bead is expelled. The time between when the mouse awakes and when the bead is expelled is measured.

NOTE: the mouse must be awake before expelling the bead.