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Fecal Carmine Red Protocol

Forked from Fecal Carmine Red Protocol

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Adam Hamilton[1], Ian Krout[1], Tim Sampson[1]

[1]Emory University

Anastasiya Moiseyenko
California Institute of Technology

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We use this protocol and it's working

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Abstract

This assay is used to determine whole gut transit time. Mice are given an oral gavage containing a known volume of bright red carmine dye. Mice are placed into empty cages and are then observed in 15-minute intervals until they produce a bright red fecal pellet. The time between gavage and pellet expulsion is the time the dye takes to travel down the length of the GI tract, which is influenced by gastric emptying, and small and large intestinal transit.

Materials

Carmine Red Solution
6% w/v carmine red (Sigma cat #C1022),
0.5% w/v methylcellulose (Sigma cat #M7027) in water.
Sterile Cages
Gavage needles

Pre-protocol

1 Prepare Carmine Red Solution (see materials).

2 Autoclave Carmine Red solution, if sterility is required. (i.e. microbiome analysis)

3 Shake well or stir on low heat to homogenize

4 Shake/vortex each right before gavage.

Day of Assay

3h

5 Orally gavage mice with 100 µL carmine red solution. Generally, soft-tipped, disposable (Instec FTP-20-30, or similar) feeding needles, attached to a 1 mL slip-tip syringe.

Equipment

Polypropylene Feeding Tubes for Rodents	NAME
Gavage Needle	TYPE
Instech Labs	BRAND
FTP-20-30	SKU
https://www.instechlabs.com/products/feeding-tubes/polypropylene	LINK

6 Record time of gavage for each mouse.

7 Following gavage, place animals back into home cage with cage mates, food, and water.

8 Allow animals to rest with food, water, in home cage for 03:00:00 3h

9 Separate individual mice into single-housed, clean (sterile, if needed) cages, with no bedding, which will interfere with the observation of a red fecal pellet. Cover with cage top.

10 Assay can be set up with or without food as long as all comparable runs are done the same way. If
adding food, place 1-2 food pellets into a portion cup with water to create moistened chow (sterile if needed). Moistened chow reduces the risk of mice spilling water in the cage. Glass dishes can be used as cups to prevent flipping of the cups.

11 Every 00:30:00 , check cages for a bright red pellet. Can observe through sides 30m
(with pen light if needed) or by opening cage top. Placing the cages on a white sheet of paper, rather than a
black benchtop, will also make visualization easier. If unclear, a pellet can be taken out of the cage and smeared on a napkin to determine if dye is present. If food was provided, check in the portion cups for red pellets as well. ***Ensure that whichever method of observation is used, all animals are disrupted similarly (ie. all animals have their cages opened).***

12 Record time at which the first red pellet is observed for each mouse. For a normal, healthy adult mouse, this will be ~3-4hrs following gavage, but can range from 2-8+ hrs. Assay maximum time is set to 5 hours, but can be altered as needed, but can be altered as needed up to 10hrs.

12.1 To prevent stress associated with single-housing of mice, limit the single-housed portion of the assay to 6-8hrs, putting the maximum recordable transit time at 8-10hrs post-gavage.

13 Once a red pellet is observed, return that mouse to its home cage.

14 If the maximum allotted time is reached without production of a red pellet, return the mouse to their
home cage, and record their transit time as the maximum.