

SOP No: AHP 88

SUBJECT: Intestinal Permeability Assay in mice

POLICY: Only experienced operators are to perform this procedure.
Careful monitoring of individual mice is required when performing this procedure.

PRECAUTIONS: Gloves, mask, long-sleeve gown, closed in shoes

EQUIPMENT: FITC-Dextran (40mg/ml in sterile PBS)
Heparin
Eppendorf tubes

PROCEDURE:

1. Weigh mice.
2. Gavage mice with 10µl/g body weight of FITC-Dextran (according to [AHT 42](#)). Allow 5min between each mouse (this gives adequate time to take blood samples at step 3).
3. Bleed the mice 4hr later (according to [AHT 29](#)) – collect ~150-200µl of blood in an eppendorf tube containing heparin. Bleed the mice in the order they were gavaged – keep to the 5min time limit for each mouse.
4. Mix the blood and heparin. Store the tube on ice in the dark until ready to collect the plasma.
5. Mice are gavaged and bled by the above procedure once weekly for not more than 2 weeks (total of 2 occasions). Mice are sacrificed immediately after the final blood sample has been taken.
6. Sacrifice mice (either [AHT 35](#) or [AHT 36](#)).

RECOMMENDATIONS:

FITC-Dextran is non-toxic to mice. Care needs to be taken when doing the oral gavage and bleeding procedures according to the referenced SOPs.

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REVISED:

REFERENCES

1. Wang, Q., C.H. Fang, and P.O. Hasselgren, *Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice*. *Am J Physiol Regul Integr Comp Physiol*, 2001. 281(3): p. R1013-23.
2. An, G., et al., *Increased susceptibility to colitis and colorectal tumors in mice lacking core 3-derived O-glycans*. *J Exp Med*, 2007. 204(6): p. 1417-29.

SCORE SHEET FOR ASSESSMENT OF MICE WITH INTESTINAL PATHOLOGY/DEVELOPING COLITIS/BMT



DAILY OBSERVATIONS

Experiment number: _____

Mouse number: _____

Date and time of challenge: _____

Drug (if any): _____ Dose: _____

Pre-challenge weight (g): _____

<i>DATE</i>												
Time post-challenge												
Days post-DSS/or alternative challenge	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Observations from a distance												
Inactive												
Hunched posture												
Ruffled fur												
Rate of breathing												
Crusty Eyes												
Shivering												
Diarrhoea												
Rectal bleeding												
Rectal prolapse												
<i>On handling</i>												
Not inquisitive or alert												
Bodyweight (% change from start/score)*												
Any other abnormal behaviour or signs noted												
ACTION TAKEN^												
NOTES												
TOTAL SCORE												

Scoring Details:

0 = normal/equivocal symptoms, 1 = mild symptoms, 3 = severe symptoms. (see next page for descriptions) Total of 3 or over = mouse culled.

0 = <5% weight loss, 1 = 5-15% weight loss, 2 = 15-20%, 3 = >25% weight loss

Symptom	Mild Symptoms	Severe Symptoms
Inactivity	fully mobile, but more inactive than usual	mouse moving only occasionally and largely unresponsive to prompting
Hunched posture	small change in normal posture	mouse noticeably hunched over without regaining normal posture
Ruffled fur	some restricted ruffling of fur	obvious ruffling of most fur
Rate of breathing	slightly above or below normal	excessive panting or markedly diminished rate of breathing
Crusty eyes	small build up of excretion in eyes	significant excretion in both eyes, vision likely to be impaired
Shivering	occasional shivering	constant prominent shivering
Diarrhoea	loose stools	stools have completely liquid consistency
Rectal bleeding	some blood in stool on close inspection	clear bleeding from rectum
Rectal prolapse	rectum threatening to prolapse on defecation	permanent prolapse
Not inquisitive/alert	some reduced response to stimuli	mouse largely unresponsive to stimuli