{% extends 'assays/detail_assay2.html' %} {% block measured_values %}

Small Intestine

Large Intestine

{% endblock %} {% block min_models %}

3M + 3F

{% endblock %} {% block assay_content %}

Data

{% for assay in measures %} {% endfor %}

Mouse ID	Mouse Genotype	Timepoint ({{object.timesteps_in}})	Measurement Date	In sm
{{ assay.mid }}	{{ assay.mid.genotype| default_if_none:"" }}	{{ assay.timepoint| default_if_none:"" }}	{{ assay.measurement_date| default_if_none:"" }}	{{ as de

{% endblock %} {% block assaytype_content %}

A **Histological** phenotypic assay was performed on **{{total}} mice**.

The charts show the results of measuring **Alanine aminotransferase** in **{{females}} female**, **{{males}} male** mutants compared to **362 female**, **316 male** controls.

The mutants are for the **Tnfrsf1a$^{tm2b(EUCOMM)Wtsi}$** allele.

{% endblock %} {% block equipment_content %}

Protocol

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HPIBD-02

Enteropathy Histopathology

Date published 7/5/2019
Version **2**
Team Histology Unit
Purpose Experimental Design Equipment Supplies Procedure Notes References

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Purpose

Histological evaluation of enteropathy caused by a-CD3 administration.

Experimental Design

- **Minimum number of animals :** 3M + 3F
  3 male and 3 female mice per genotype for anti-CD3 administration and 3 male and 3 female mice per genotype for anti-IgG administration to be used as controls.
- **Age at test:** 6-8 weeks

Equipment

- Tissue embedder (Meditel TBS88)
- Histokinette (Leica TP 1020)
- Microtome (SLEE/Microm HM 200 Ergostar/Leica RM2125)
- Waterbath (Meditel TFB35/Leica HI1210)
- Hot plate (Leica HI1220)
- Slide stainer (Leica AutostainerXL)
- Glass coverslipper (Leica CV5030)
- Microscopes (Primo Star Zeiss, Nikon Eclipse E200)
- MilliQ H2O system (for type 1 ultrapure water; Merck, Synergy Water Purification System)
- Hot plate with magnetic stirrer (DragonLab, MS-H-S)
- Scales (MRC, Precision Balance, BPS-750-C2-V2)
- SigmaPlot Software (Systat Software Inc.)
- Image J Software (NIH)

Suplies

- Phosphate Buffer Saline (PBS) (homemade)
- Sodium chloride (Fisher Scientific), BP358-1
- Potassium chloride (Riedel-de Haën), 31248
- Sodium phosphate dibasic (Sigma), S7907
- Potassium phosphate (VWR), 26936.260
- Formaldehyde 4% (VWR Chemicals BDH), 9713.500
- Papanicolaou’s solution, 1a Harris Hematoxylin Solution (Merck), 1.09253.2500
- Eosin Y (Merck), 1.15935.0100
- 50%, 70%, 80% Ethanol (homemade)
- 96% Ethanol (Fisher Chemical), M/4000/PC25
- Ethanol absolute (VWR Chemicals BDH), 20821.330
- Xylene (Fisher Chemical), X/0250/17
- Magnesium sulphate 7-hydrate (Analar BDH), 101514Y
- Potassium bicarbonate (Sigma), P9144
- Acetic acid glacial (Fisher Scientific), A/0360/PB17
- Schiff’s reagent (Merck), 1.09033.0500
- Periodic acid (Merck), 1.00524.0025
- DPX (Sigma), 06522
- Paraffin (VWR Chemicals), 361334C

- Microscope slides (Knittel Glass), KA-01001004
- Cover slips (Knittel Glass), KA-01101008
- Microtome blade S-35 (Feather), 207500000
- Embedding cassettes (Simport), QTY/PK
- Biopsy pads (Bio - optica), 07-7290
- Forceps (Stainless Germany)
- Scissors (at least one medium size and one small) (Stainless Germany)
- 10 ml syringes with needles 21G (BD M

Procedure

3.1 Calculate and record the volume of anaesthetic solution required for intraperitoneal (IP) injection.

3.2 Anesthetize the mice.

3.3 Monitor the animal carefully until unconsciousness by ensuring that the mouse is adequately sedated.

3.4 Weigh the mouse and record the value.

3.5 Measure the length of the mouse as follows and record the value (accuracy ±0.1cm)

3.5.1 Place the unconscious mouse on a disinfected ruler so that its nose is at zero

(figure 1).

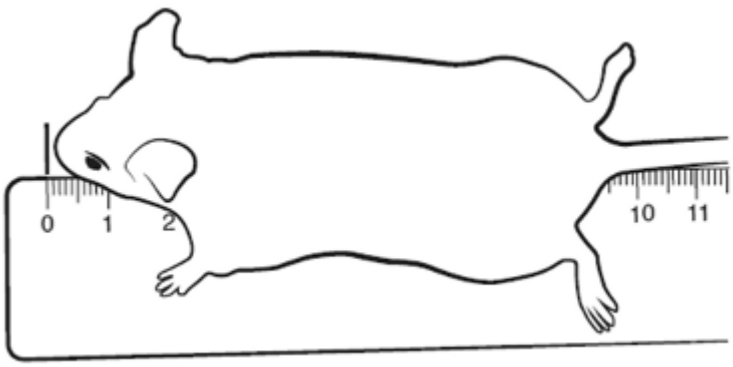

Figure 1

3.5.2 To measure the entire length of the head press gently against the ruler

(figure 2) and gently pull the tail to ensure that the spine returns to its full

length (figure 3).

Figure 2

Figure 3

3.5.3 Measure the length starting from the nose (0cm) to the beginning of the tail

(figure 4). Record the measurement the accuracy is within 0.1cm. For

example in figure 4 the length of the mouse is 9.5cm.

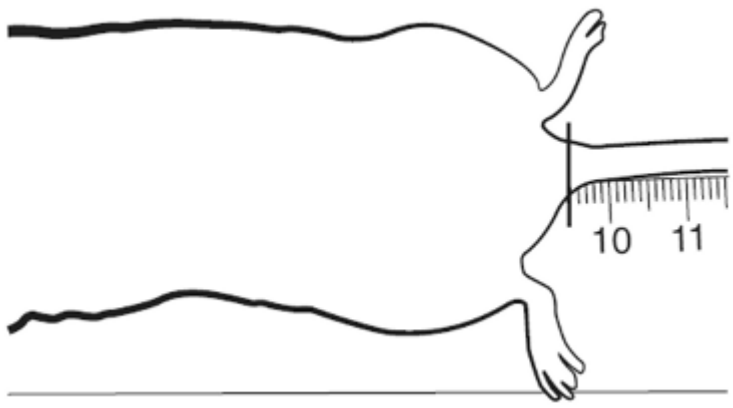

Figure 4

3.5.4 Disinfect the ruler and contact area after the measurement has been taken.

3.6 Place the unconscious mouse into the DEXA analyser.

3.7 Perform a scout-scan.

3.8 Optimise the area of interest and perform a measure-scan.

3.9 Note that the exposure dose per mouse is 300?Sv.

3.10 For the analysis of the data, regions of interest must be defined. The standard analysis comprises of a whole body analysis excluding the head area.

Continue with X-ray analysis or

3.11 Remove the mouse once the image is captured. Place the mouse on a heated mat, set at 37°C, in a cage and monitor closely until consciousness is regained.

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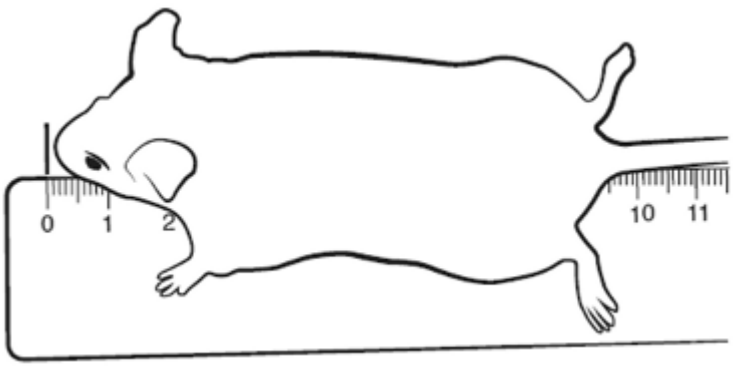

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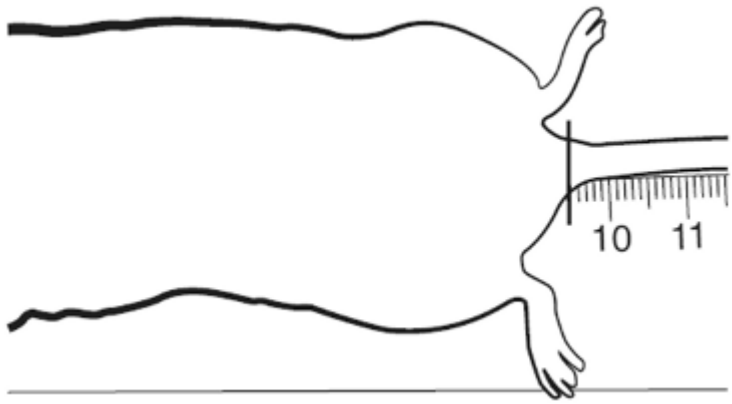

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Notes

1. For every large intestine segment (3 seg) score 3 fields (40x) with maximum phenotype. Total number of apoptotic bodies /9 = Apoptotic bodies /field.

2. Evaluate the Pas stained slide in conjunction to control.

3. Evaluate enlarged and distended lymph and blood vessels.

References

1. M. Merger, J. L. Viney, R. Borojevic, D. Steele-Norwood, P. Zhou, D. A. Clark, R. Riddell, D. Maric, E. R. Podack, K. Croitoru (2002). Defining the roles of perforin, Fas/FasL, and tumour necrosis factor a in T cell induced mucosal damage in the mouse intestine. Gut, 51:155–163.

2. U. Erben, C. Loddenkemper, K. Doerfel3, S. Spieckermann, D. Haller, M. M. Heimesaat, M. Zeitz1, B. Siegmund, A. A Kühl (2014). A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol, 7:4557-4576.

{% endblock %} {% block info_content %}

Histological Scoring for a-CD3 mediated enteropathy

Small intestine

Score	**0**	**1**	**2**	**3**	**4**
Epithelium flattening		Minimal (<10%)	Mild (10-25%)	Moderate (26-50%)	Marked (>51%)
Apoptotic bodies/field	0	1-3	4-5	8-13	>13
Goblet cell depletion (relative to baseline by Pas stain)		Minimal (<10%)	Mild (10-25%)	Moderate (26-50%)	Marked (>51%)
Lumen exfoliation		Minimal	Mild	Moderate	Marked
Villus shortening			Mild (< 25%)	Moderate (26-50%)	Marked (>51%)
Intestinal gland damage			Mild	Moderate	Marked
Paneth activation (relative to baseline by Pas stain)			Mild		Marked
Peyer patch	1-2		3-5		>6
Score (min 0, max 32)					

Large intestine

Score	**0**	**1**	**2**	**3**	**4**
Apoptotic bodies/field	0		1-3	4-8	>9
Goblet cell depletion (relative to baseline by Pas stain)		Minimal (<10%)	Mild (10-25%)	Moderate (26-50%)	Marked (>51%)
Lumen exfoliation		Minimal	Mild	Moderate	Marked
Crypt damage			Mild	Moderate	Marked
Endothelial cell activation			Mild		Marked
Score (min 0, max 32)					

{% endblock%} {% block chart_content %}

Line Chart

Area Chart

Bar Chart

Donut Chart

Line Chart

Line Chart

{% endblock %}