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Date: 27.07.2022	Scoring of Mice after EAE Induction EAE Scoring	LTK-RES-1-EN Version: I
This SOP replaces: Version: H		
Reason for Change: Action on weight loss was added		
Related SOPs:	Induction of EAE (SOP-LTK-RES-26-EN) Isoflurane anesthesia (SOP-LTK-TRT-13-EN) Adoptive Transfer EAE (SOP-LTK-RES-58-EN) CO2 Euthanasia (SOP-LTK-TRT-14-EN) Stereotactic Injection for EAE mice (SOP-LTK-RES-86-EN)	
Indication of Use:	Scoring of mice after active Experimental Autoimmune Encephalomyelitis (EAE) has been induced (e.g. by immunization with myelin antigen or by adoptive transfer of immune cells); scoring of mice which develop EAE spontaneously (e.g. 2D2/IgHMOG mice); scoring of mice in which myelin components are ablated by chemical or genetic means	
Aim of SOP:	Mice are scored to obtain a defined measures of disease severity as experimental data point. Further this protocol serves as criterion for the application of measure for animal welfare. This protocol also determines the termination points when an animal needs to be euthanized.	
Distribution:	1. Server	
Attachments:		
Generated at: 27.07.2022	Checked and approved at: 27.07.2022	
by: Vasileia Kalaitzaki	by: Thorsten Buch	

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Responsible Persons: Researcher with Module 1 after registration on animal license including EAE protocols		

Method: Physical test and observation by the researcher, weight

Principle of Method: observation, weighing

Material to be used: Cage top, grid

Machine: Scale, laminar flow/changing station

Safety:
Follow the rules for the respective animal room

Method Description:

1. Place the home cage under a sterile hood/changing station.
2. Place a second, empty, cage next to the home cage. Lift the mouse by cup handling (use the tail only if the cup handling does not work) and place it on the grid.
3. Record the EAE clinical score, in accordance with attachment A.
4. Place the mouse on the scale and record its weight.
5. Place the mouse back in their home cage and record the next one.

Score and weight are documented on paper within the animal facility through the duration of the experiment and copied to the server, as xlsx, after termination of the experiment.

Note:
In case mice present with other symptoms, record them according with attachment B & C.
Take action, when necessary, in accordance with the different attachments.

Factors influencing outcome:
The scoring should be performed at around the same time of the day

Analysis:
Describe how the data is calculated, how the outcome is interpreted

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Documentation:


The scoring needs to be documented by the individual investigator, stored as Excel file in the PROJECT folder of the LTK server and must be available in case of inspection.

Problem management:

If anything unusual is observed regarding the animals, the lab head or a vet have to be informed.

Literature:

1. Locatelli et al. 2012
2. Standard Operating Procedure Scoring of EAE of the Canton Zurich from 9. Juli 2004
3. Palle et al. 2015

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Attachment A

Clinical scoring and observation

Score	Clinics	Assessment	Action
0	No detectable signs of EAE		
0.5	Distal limp tail		
1.0	Complete limp tail		
1.5	Limp tail and hind limb weakness	Occasional grid test positive	From now on provide chow and gel packs in the cage or access to water on ground level
2.0	Unilateral partial hind limb paralysis	One leg consistently falls through the grid (cage test)	
2.5	bilateral partial hind limb paralysis	Cage grid test bilaterally positive	
3.0	Complete bilateral hind limb paralysis		euthanize if persistent >7 days
3.5	Complete bilateral hind limb and partial forelimb paralysis		euthanize if persistent >3 days
4.0	Moribund (mouse completely paralyzed)		euthanize immediately
5.0	dead		

Take further action when one of the following parameters are met:

A) Euthanize when:

- Weight loss exceeds 25% of weight at onset.
- Weight loss is 20% - 25% for more than 3 days (these 3 days, solid food and hydrogel is provided in the cage immediately)

B) Record in accordance with attachment B and/or C (Reaction to stimulus; Grimace scale; Injection site/Intervention site; Fur/body)

- If the mouse shows other type of symptoms

Start scoring when:

1) EAE and adoptive transfer EAE

Score the mice at least every other day, starting at day 5.

At clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

2) oDTR/Diphtheria toxin/cuprizone

Until day 25 score every 4-5 days

From day 25 score every 2 days


With first symptoms score daily

3) spontaneous EAE models

From three weeks of age **score every 3-4 days**


With first symptoms score **every second day**

At clinical score of ≥ 3 the mice are scored **daily** until remission below score 3.

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Experiment Code: _____
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License ID: _____ **Page 1/** _____

Cage Nr	Animal ID	75% Weight at DPI 0	DPI _		DPI _		DPI _		DPI _		DPI _	
			Weight	Score	Weight	Score	Weight	Score	Weight	Score	Weight	Score
Signature												

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Cage Nr	Animal ID	75% Weight at DPI 0	DPI _		DPI _		DPI _		DPI _		DPI _	
			Date:	Weight	Score	Date:	Weight	Score	Date:	Weight	Score	Date:
Signature												

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Cage Nr	Animal ID	75% Weight at DPI 0	DPI _		DPI _		DPI _		DPI _		DPI _	
			Date:	Weight	Score	Date:	Weight	Score	Date:	Weight	Score	Date:
Signature												

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Cage Nr	Animal ID	75% Weight at DPI 0	DPI _		DPI _		DPI _		DPI _		DPI _	
			Date:	Weight	Score	Date:	Weight	Score	Date:	Weight	Score	Date:
Signature												


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Cage Nr	Animal ID	75% Weight at DPI 0	DPI _		DPI _		DPI _		DPI _		DPI _	
			Date:	Weight	Score	Weight	Score	Weight	Score	Weight	Score	Weight
Signature												

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Attachment B

This attachment refers to:

- a. animals deviating from symptoms described in attachment A (without previous stereotactic application)**

Inform the lab head!


- b. pain management of EAE animals after stereotactic injections**

For pain management measures please refer to respective SOPs and indicative flow charts (*SOP-LTK-RES-86-A-EN Stereotactic Injection on EAE animals*, *SOP-LTK-TRT-51-EN Buprenorphine analgesia on EAE animals*, *SOP-LTK-TRT-50-EN Carprofen analgesia on EAE animals*)

Score these animals daily!

1. Parameters

- Reaction to stimulus: reaction to hand in the cage
- Mouse Grimace Scale (MCS): see attachment C
- Injection site/intervention site: check for inflammation/ulceration/necrosis
- Fur/body: check coat appearance (belly fur might provide information for urinary problems) and posture

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2. Observation and Action

Score	Stimulus reaction	Grimace Scale	Injection site	Fur/body	Action
0	immediate movement	0	normal	normal	none
1	normal but after many stimuli	1	Light inflammation	dull, unkempt coat	If signs (except pain) are persistent for 5 days: euthanasia For pain signs (grimace scale), apply analgesia according to Flow Chart (see below), relevant SOPs: SOP-LTK-TRT-50-EN Carprofen analgesia on EAE animals and SOP-LTK-TRT-51-EN Buprenorphine analgesia on EAE animals. Euthanize according to flow chart
2	slow movement after many stimuli	2	Heavy inflammation	pilo erection	For pain signs (grimace scale), apply analgesia according to Flow Chart (see below), relevant SOPs: SOP-LTK-TRT-50-EN Carprofen analgesia on EAE animals and SOP-LTK-TRT-51-EN Buprenorphine analgesia on EAE animals. Euthanize according to flow chart
3	no reaction after stimuli	-	Necrosis/ulcerization	hunched posture, sunken flanks, loss of skin turgor	euthanize



Pain management for EAE animals

EAE animals (no stereotactic inj.)

In case of pain symptoms (grimace scale (MGS) - Attachment B)

h0 Carprofen analgesia

h4

Buprenorphine analgesia

h5

Check for pain symptoms *if yes*

Buprenorphine rescue analgesia

h8

Check for pain symptoms *if yes*

↓ 1h post buprenorphine
Check for pain symptoms *if yes*

h12

Carprofen analgesia *if yes*

↓ 4h post buprenorphine
Check for pain symptoms

Check for pain symptoms

continue *if no* | *if yes* → Euthanasia

h24

Carprofen analgesia *if yes*

Check for pain symptoms *MGS1*

h36

Euthanasia *if yes*

Carprofen analgesia

Check for pain symptoms

regular EAE scoring daily


EAE animals (with stereotactic inj.)

Anesthesia

↓
Stereotactic injection


↓
Carprofen analgesia

↓
Anesthesia antidote










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Date	Cage Nr.	Mouse ID	Stimulus Reaction	Grimace Scale	Injection site	Fur/body	Weight (g/% start)	Intervention (type, date, time)	Signature

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Attachment C
Minimal Mouse grimace scale (MGS)

Not present 0	Moderate 1	Severe 2
		
Orbital tightening		
		
Nose bulge		
		
Cheek bulge		