

University Animal Care Committee Standard Operating Procedure		
Document No: 7.32	Subject: Humane Endpoints in Mice with Cecal Ligation and Puncture-Induced Sepsis	
Date Issued: April 19 th , 2024	Revision: Original	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe humane endpoints in mice with cecal ligation and puncture-induced sepsis.

Introduction and Definitions: Mice are commonly used for sepsis-related research and cecal ligation and puncture (CLP) is induced by a surgical procedure. Sepsis research has prompted animal welfare concerns about the use of death as an experimental endpoint. Obtaining health parameters after surgical sepsis induction is important to establish humane endpoints for the mice. Cage side monitoring is essential to obtain these parameters and should only be performed by appropriately trained personnel.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**

Scoring System:

Using the chart below you will have the choice of two different scoring methods:

1. **Two-Parameter Scoring System:** This includes the ASR and eye scores. A number of 5 or higher will determine endpoint with the mouse.
2. **Temperature:** If the animal has a subcutaneous microchip and the temperature falls below 32 degrees Celsius, it has reached endpoint.

Either score must be completed daily after the puncture induced surgical procedure is performed and the scores must be completed and signed by the personnel completing the scoring.

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Scoring Sheet:

Date							
ASR							
Eyes							
Temperature							
TOTAL							

Activity, Response to Stimulus (ASR):

- Begin with an initial cage side evaluation. What is the animals activity level?
- If the animal has had a spinal cord injury, obtain an updated weight, and do not administer the stimulus.
- The stimulus used to determine the animal's response is a tail pull. The tail pull is very brief and very gentle.
- If the mouse reacts by moving backwards a second tail pull is elicited to observe a forward motion.

Eyes:

- Eyes are assessed for degrees of openness or orbital tightening.
- This is done after the respiratory status and the ASR test, the eyes of the CLP mice are observed to resume their pre-stimulus orbital tightness after the gentle pull of the tail was completed.

Temperature:

- Subcutaneous temperature microchips are the least invasive method of evaluating the body temperature in mice. Studies have shown temperatures less than 32 degrees celcius indicates sepsis.
- The temperature is highly recommended to be monitored but not essential.
- Rectal temperatures can cause undue stress on the mice.

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PARAMETER	SCORE	PARAMETER DESCRIPTION
Activity, Response to Stimulus (ASR)		
	0	Mouse is performing any prior to stimulus: Climbing, running, fighting. Normal spontaneous walking. Mouse displays normal reaction in response to stimulus.
	1	Slightly suppressed activity prior to stimulus. Spontaneous walking but slower than normal. Mouse moves forward in response to stimulus but slower than normal.
	2	Moderately suppressed activity. No spontaneous walking observed prior to stimulus. Mouse moves at least two steps forward in response to stimulus but much slower than normal.
	3	No activity. No spontaneous walking observed prior to stimulus. Mouse moves less than 2 steps forward in response to touch. Mouse may experience tremors in response to touch.
Eyes		
	0	Open
	1	Partially closed
	2	Half-closed
	3	Mostly or completely closed
Temperature		
If temperature falls below 32 degrees Celsius, endpoint the animal.		



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References:

Ferguson, LT, Rashied AA, Liang Z, Yumoto T, Anyalebechi JC, Swift DA, Hernandes MS, Krafty RT, Coopersmith CM, Lee VK. 2023 A Novel Scoring System for Humane Endpoints in Mice with Cecal Ligation and Puncture-Induced Sepsis. [A Novel Scoring System for Humane Endpoints in Mice with Cecal Ligation and Puncture-Induced Sepsis - PubMed \(nih.gov\)](#)

SOP Revision History:

[illegible]