Procedure Type: Compound Administration/Drug Administration
Procedure Title: Acute Peritonitis
Species: Mouse
Pain/Distress Category: D

Background Information:
Please make sure that your purpose for performing this procedure is fully described below in the section: "How does this procedure fit into or address your overall research goals?"

General compound administration guidelines: Doses will comply with ACUC Guidelines for "Dosing Techniques and Limits." If doses will not comply with ACUC Guidelines, insert variation with justification below in the section: "How does this procedure fit into or address your overall research goals?"

Provide a list of all compounds that will be administered, dosage, route(s) of administration, and any known toxicities in the Other Agents Utilized tab.

Procedure Description Tab:
Procedure Description: (select all that apply):

☐	Thioglycolate or Zymosan A induced acute peritonitis	Procedural Steps: 1. Following the steps outlined below, inject mice intraperitoneally with ≤ 3ml of sterile thioglycollate medium (3% w/v of an autoclaved stock prepared from dehydrated thioglycollate medium and sterile saline water). Dose volume for mice ≥2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months. 2. Following the steps outlined below, inject mice intraperitoneally with ≤ 3ml of sterile water suspension containing < 1.5 mg of Zymosan A. Zymosan A is not soluble in water so the stock suspension is vortexed immediately prior to injection. Dose volume for mice ≥2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months. *IP Procedural Steps:* a. Animals will be restrained manually or placed in a plastic decapicone bag to facilitate restraint. Restraint will be < 5 minutes. b. The injection is made using a 22-27-gauge needle in the left or right lower quadrant of the abdominal cavity, in between the midline and medial side of the hind leg to

		avoid the liver and the bladder. The needle should be angled 20-30 degrees relative to the animal to avoid penetration of the abdominal organs. c. Prior to injecting the compound, pull back gently on the plunger to confirm placement. If fluid (e.g., blood, urine or intestinal contents) is aspirated, discard needle, syringe, and solution due to contamination, and repeat with fresh supplies.

		Once proper placement has been confirmed, slowly inject solution. d. If bleeding occurs at the injection site, apply pressure until hemostasis is achieved. e. Return mouse to its cage. *Note: To reduce discomfort, use a new needle for each animal, bring solutions for injection to 37°C, and avoid injecting material with a high or low pH.* 2. Mice will be euthanized for recovery of peritoneal inflammatory cells and exudates at various time points following injection. Leukocytes will accumulate rapidly in normal mice in response to the injection (e.g., large numbers of cells can be recovered after 24-96 hours). Some strains may require up to 4 days to produce adequate numbers of cells. Distinct cell types are recruited to the peritoneal cavity during the inflammation and resolution time course of acute peritonitis; so the study can last 1-9 days post injection, after which animals will be euthanized as outlined in the Animal Disposition section. **Potential Adverse Events:** Lethargy, weight loss, perforated bowel or bladder, peritonitis, damage to internal organs.

How does this procedure fit into or address your overall research goals? (Insert protocol-specific rationale here.)

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.
Please see "Potential Adverse Events" listed under the Procedure Description.

Describe post procedure monitoring that will be performed.
Mice will be examined immediately following compound administration and daily thereafter. Mice will be assessed for general appearance and activity level, as well as the potential adverse events listed above.

Treated mice will be monitored on a daily basis for signs of discomfort, including hunched posture, ruffled fur and lack of movement around the cage. If any abnormal signs are noted, an OLAC veterinarian will be contacted.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?
Injected mice will be euthanized if they show any signs of hunched posture and inactivity

Anesthetic Regimen Tab:

Not applicable

Peri procedure Care/Analgesics Tab:

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Recovery Location Building Name: (Insert protocol-specific information here.) **Room Number:** (Insert protocol-specific information here.)
Responsible Personnel: (Insert protocol-specific information here.)

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)
Treated mice will be monitored on a daily basis for signs of discomfort, including hunched posture, ruffled fur and lack of movement around the cage.

Monitoring Duration
Mice will be examined immediately following injection(s) and then daily. Mice will be monitored for general appearance and activity level as well as the potential adverse events listed under the Procedure Description.

Monitoring Frequency

Mice will be observed with sufficient frequency to ensure that they are euthanized according to established endpoints.

Describe what actions will be taken if parameters monitored fall outside normal ranges:
The mouse will be euthanized.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):
The mouse will be euthanized.

Describe record keeping/documentation methods for post-procedure monitoring: A record of the compound administered, the date, and the animal's ID will be kept in the laboratory notebook.

Other Agents Utilized Tab:

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Thioglycollate	3% w/v stock prepared from dehydrated thioglycollate medium and sterile saline water Dose volume for mice ≥2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months.	Intraperitoneal (IP)	Administered once
Zymosan A	0.5-1.5 mg of Zymosan A powder is added to sterile saline (<3 ml) under aseptic conditions to generate a working stock suspension. Dose volume for	Intraperitoneal (IP)	Administered once

	mice ≥2 months of age is 2-3 ml and is 1-1.5 ml for mice that are < 2 months.		

Literature Search for Alternatives:

Suggested Keywords
Thioglycollate, Zymosan A, yeast antigen, leukocyte collection, acute peritonitis, inflammatory exudate, mouse, alternatives, refinement

Updated/ACUC approved:
Dec. 2021