Mouse/sample handling protocol:

Acclimatization:

The mice are transported in cages with bedding, food (usually apples due to high water content) and hydro gel. Upon arrival the animals are placed into the animal facility in controlled environment cabinets and their locations recorded in the “Mouse cabinet placement” sheet. Each mouse occupies an individual cage with bedding, barley and sunflower seeds, red plastic tunnel and wood stick.The food is placed in the stainless steel bar lid along with a water bottle with long nozzle. Each cage is provided with a mouse ID sticker/metal label holder (EH_ID) and ideally a second sticker showing sex, date of birth and strain information. The acclimatization should be 7-14 days long. Beddings have to be changed weekly. Mice have to be inspected and individually by placing cage on the table, removing the plastic lid and observing the animals behaviour. The animals have to be inspected every day including weekends.

Facilities:

Outline and areas:

The animal facilities comprise of 2 large rooms. The main room holds two controlled environment cabinets, a cage holding rack, shelving units, handling box, equipment shelf and a table. The side room holds two shelving units containing mouse cage parts, environment enrichment, water bottles, seeds and designated areas for “Clean” and “Dirty” equipment.
These areas have to be respected due to *Eimeria* high infective potential.
Any equipment that underwent the full cleaning procedure is stored in the “Clean” area and should be organized into the shelving units as much as possible. We are over the holding capacity so anything that doesn’t fit has to be in bags in the “Clean” area to remain clean.

Maintaining zones:

Generally speaking the main room should be regarded as an infection hazard area. When entering one has to put on disposable shoe protectors, wear gloves when handling samples, animals or touching surface areas that get exposed to infectious agents. Any material and equipment taken out of the main room should be cleaned or sealed (including polystyrene boxes) to prevent contamination outside the room. Upon leaving, gloves and shoe protectors should be discarded into the bin INSIDE the main room as this waste has a lid, gets autoclaved and te bin never leaves the room. Once a bag, bin, container or cage has been taken into the main room, it should remain there until put into an autoclave bag (or sprayed with Neopredisan) and taken through the full cleaning procedure. Micro zones between *Eimeria* strain specific equipment have to be strictly respected (e.g.: only use E88 tweezers

for E88 infected mice). Any equipment/consumable shortage has to be resolved or reported before commencing work.

Cleaning:

Any cages and equipment that were taken into the main room have to be cleaned or bagged, organized, placed in the “Dirty” area, autoclaved and taken to the Animal facility (Karin) for washing. SIMPLE AUTOCLAVING DOES NOT COUNT AS COMPLETE CLEANING (infectious fecal material may remain and cross contaminate animals). To arrange a washing call Karin in advance and request a time to drop off the equipment.

Prior to commencing work inside the main room every surface should be checked to be clean, if it doesn’t appear to be, clean with Neopredisan. The metal handling box has to be inspected for Neopredisan left overs and cleaned before handling animals inside.

While working with mice, relative cleanliness should be maintained, especially when disposing of cage contents into the bin and handling cages on the large table.

After finishing all tasks, the equipment which came into contact with animals or animal samples has to be cleaned by either ethanol and burning or Neopredisan. The large table has to be swept of any particular matter and sprayed with Neopredisan. The equipment shelf should be relatively clean, if contaminated it should be cleaned with Neopredisan (including the scales).

Once a week the floors should be swept and cleaned with bleach solution. Frequently touched areas should be cleaned with Neopredisan. If a large amount of chemicals is used and animals are kept in the room, the windows should be left open for few hours but closed overnight.

Sample collection:

In the conceptual phase of a project, a plan should be made about which samples to collect and what their storage should be. Based on that, make sure there is freezer space available and any reagents in plentiful supply (e.g.: space and towers in -80 freezers, Potassium Dichromate for fecal samples, Nitrogen for flash freezing).

Ideally all tubes should be labeled in advance and organized on lab bench for flawless workflow. The tubes have to be labeled by randomized alphabetical codes for future processing and their storage/handling explicitly stated, preferably with a legend (e.g.: C = Cytokines (Nitrogen then -80), A = Antibodies (-80), F = Feces (Potassium Dichromate)).

An organization sheet has to be made for longer or more demanding experiments to organize work with colleagues, making sure samples get collected and animals inspected.

In addition a collection of sheets (1 per day) has to be placed in the animal facility to record experiment relevant variables such as weight, weight change, feces weight, etc…

When working with multiple infectious agents, make sure to have appropriate protective equipment, collection equipment and the necessary information for handling the pathogen. Each distinct infectious agent has to have a designated set of collection tools, containers and storage areas (ideally not touching any bare surfaces).

If the location of mouse cages changes due to experimental design or reorganization, this has to be recorded on a new “Mouse cabinet placement” sheet and respected thereon after.

Animal handling:

The animals should always be handled as gently as possible to avoid stress, which could influence the experiment results. This generally consists of keeping noise to minimum, handling cages without slamming and dropping of their parts. Rodents are very sensitive to metallic sounds, keep this in mind when handling the animals. Same as the Acclimatization section, mice have to be inspected daily and beddings changed weekly.

For inspection and tracking purposes “Score sheets” have to be used for animal experiments. This has an official version from the German authorities and is used for tracking of animal behaviour, observed health status and proposed dates of experiment start and end. These can be obtained from Emanuel or GitHub repositories (../../Eimeria_Lab/data/2_designTables) or Google Drive (https://drive.google.com/file/d/1-n4Nafj-H8_bVhSnBEPFgZzU7_98N1Zh/view?usp=sharing)

When weighing the mice, make sure to use the strain designated buckets/containers. Tare between batches or after introducing a tissue on the bottom. Always let the mouse explore the bucket and take measurements only when the animal has all 4 feet touching the bottom of the container and is still, otherwise the shift in weight changes the results.

When infecting mice, the animals have to be scuffed well to prevent needle injury, held above the metal box to prevent escape and held up straight. Make sure to take care that the eyes don’t pop out too much as this can be a source of eye infection because the eyes are very sensitive

When sacrificing mice, catch the animal by the tail, lift it’s posterior half up so that back feet are in the air and front feet are holding onto the grid. This prevents the mouse from backing up and makes cervical dislocation easier and cleaner. Make sure to use finger tape when sacrificing animals on both hands as they will defend themselves. In a case of a mouse bite, do not shake your hands, simply blow air into the animal’s nose to make them release the bite. Clean the area after killing as other mice can associate the area with danger and will become more stressed.

In all cases, the least amount of handling of the animals is the best.