BIOSAFETY PLAN ZEBRAFISH CORE FACILITY

REGISTRATION NUMBERS OF THE ZEBRAFISH CORE FACILITY

Registration number “Anläggningsbeslut för försöksdjur”: JBV: 5.2.18-05581/2020

Registration number ”Innesluten använding av genetiskt modifierade organismer”: HoV: 3779-2019

Registration number ”Vattenbruksanläggning”: 1142

Risk level of the facility “Riskklass vattenbruksanläggning”: “Låg”

IMPORTS TO THE ZEBRAFISH CORE FACILITY

The zebrafish core facility receives animals from other facilities on a regular basis. Since Sweden is classified as VHS-free country and zebrafish is a classified as a susceptible species, the European Zebrafish Center (EZRC) is the only permitted source for animals outside of Sweden (Import permit 6.6.18-13170/2023).

The following rules apply for importing of animals to the zebrafish core facility:

1) Any import must be coordinated with the veterinary team and the imported animals are classified as “high-risk” or “low-risk” together with the veterinarian. The classification depends on:
    a. The availability and quality of a health report for the animals as well as documented husbandry routines of the sending facility
    b. The presence and nature of the pathogens detected in the sending facility – both historically and currently
    c. If no health monitoring is performed by the sending facility, the classification of the import is automatically set to “high-risk”
    d. If no husbandry routines can be shared, the classification of the import needs to be based on a single-case scenario despite potentially acceptable test results shared.
    e. If the embryos are be bleached by the staff of the core facility
    f. Import of adult animals for cryopreservation is always classified as “high-risk”
2) Adult animals can only be imported for cryopreservation of sperm. Those animals are housed in the flow-through unit of the quarantine and sacrificed directly after cryopreservation
3) All imported animals are directed to the quarantine unit
4) All imported animals classified as “high-risk” are housed in the flow-through system of the quarantine unit
5) Imported animals classified as “low-risk” are housed in system 1 of the quarantine unit.
6) Animals imported into the flow-through unit must undergo a health screen by a accredited health-care provider before they can be moved to system 1 of the quarantine unit.

EXPORTS FROM THE ZEBRAFISH CORE FACILITY

- A health certificate as well as a self-declaration in accordance with Article 218 of Regulation (EU) 2016/429 is provided for all animals exported.
- Animals are only exported to research facilities
- Exported animals are removed from the animal database and information concerning the receiving facility are registered

BIOSAFETY MANAGEMENT AT THE ZEBRAFISH CORE FACILITY

The biosafety strategy of the zebrafish core facility is based on three pillars: (i) A barrier system with controlled movement of staff, animals and equipment, (ii) dedicated personal protective equipment, (iii) a cleaning and disinfection strategy for the facility.

TRANSPORT OF ANIMALS BETWEEN KMB AND THE ZEBRAFISH CORE FACILITY

1) Adult animals can only be imported for cryopreservation. Those must be housed in the flow-through unit of the quarantine.
2) Zebrafish embryos can be imported in the quarantine where they are raised. Offspring of these embryos must be surface disinfected before introducing them into the main fish room
3) Embryos for experiments may be imported into the zebrafish core facility based on a case-to-case basis. The routine should however always be that those embryos are surface-disinfected in the quarantine unit by zebrafish core facility staff followed by transferring the embryos into new multi-well plates or dishes which are then securely sealed with air-permeable seals or similar.

GENERAL INFORMATION CONCERNING THE PERSONAL PROTECTIVE EQUIPMENT

- Any personnel must wear working clothes, in some instances a lab-coat over personal clothes is allowed (e.g. for emergency services or when not coming into contact with animals).
- Working clothes must be changed at least once a day and washed at minimum 60 °C.
- Every barrier has dedicated lab shoes, those must remain in the barrier
- Gloves must be exchanged regularly and always when going from a dirty to a clean barrier.
- Gloves must never be "disinfected" with ethanol or similar as this penetrates the nitril membrane and compromises the protection against pathogenic agents.
- Visitors or service personnel must wear lab-coats or working clothes, gloves and dedicated shoes in any of the barriers of the core facility.
- Lab coats are washed at least once a week
- Gloves must be worn in all rooms of the core facility except:
    - The office
    - The entry rooms to the injection lab

OVERVIEW OVER BIOSAFETY RULES AND REGULATIONS

- Adult zebrafish can be moved freely from clean to dirty barriers
- Embryos can be moved freely from clean to dirty barriers
- Embryos can be moved from dirty barriers to clean barriers after surface disinfection only.
- Adult zebrafish can never be moved from dirty to clean barriers
- Whenever possible, staff must work in the clean barrier first before commencing work in the dirty barriers
- The rotifer culture ("barrier B+") must always be handled before entering any room with barrier B or lower
- Researchers have restricted access to the main fish room: they are only allowed to visit the room when staff is present or fin-clip their own zebrafish at the fin-clipping bench when staff is present. The fin-clipping bench is prepared and cleaned by the staff. Researchers are not allowed to handle any housing tanks connected to the CLS system.
- No equipment that has been to the BSL-2 room or the open-access unit must be taken into the injection laboratory without proper disinfection (e.g. with ytdesinfection or gassing)
- After visiting the BSL-2 unit or open-access fish room, researchers and visitors must wait at minimum of 12 hours before entering rooms of barrier "B". Alternatively, visitors/researchers can shower and change all outer clothes.
- "one net – one stock" policy which means that a net must not be used for more than one stock (genotype) without disinfection in between.
- Researchers or visitors must place their cellphones in a plastic bag before entering barrier B
- Researchers or visitors must place cell phones in a plastic bag before entering barrier C
- Staff must always remove gloves before handling cell phones.
- No Styrofoam iceboxes are allowed in any of the barriers. Insulators are available. Green insulators are to be used in barrier B, red insulators are to be used in barrier C.

THE BARRIERS OF THE ZEBRAFISH CORE FACILITY

The zebrafish core facility is divided into different barriers named “A”, “B”, “C”. Barrier “A” is the cleanest zebrafish unit, barrier “C” is the quarantine zebrafish unit housing potentially infected fish.

- **Barrier A:**
  - SPF unit (currently not established)
- **Barrier B+**
  - Rotifer culturing room: room for the continuous rotifer culture
- **Barrier B:**
  - Main fish room
  - Tank washing room
  - CLS room for the main fish room
  - Injection laboratory
  - Dry laboratory
- **Barrier B-:**
  - Procedure room
- **Barrier C:**
  - Quarantine unit
    - Separate EU: Flow-through unit (System 2)
  - Open-access unit
- **Barrier D:**
  - BSL-2 unit
- **Water treatment unit:**
  - See specific rules within the document below

BARRIER B+

ENTERING BARRIER B+

Entering routines for staff handling the rotifer culture:

- Shower and hair wash, at home or on site, if another barrier or fish facility has been visited in the last 12 hours

PPE for staff handling the rotifer culture:

- Fresh working clothes
- Gloves
- Dedicated shoes

Entering routines for service personnel or for resupplying consumables in barrier B+ only:

- Shower and hair wash, at home or on site, if another fish facility or barrier C has been visited the last 12 hours

PPE for service personnel or for resupplying consumables only:

- Lab coat
- Gloves
- Dedicated shoes

EXITING BARRIER B+:

- Take of shoes, gloves can be kept on if no door handles are touched.
- All other barriers can be visited without restriction

MOVEMENT OF CONSUMABLES AND EQUIPMENT IN AND OUT OF BARRIER B+

- Equipment can be brought into barrier B+. If the equipment has been placed in barrier C before, it needs to be gassed.
- Consumables can be brought into barrier B+ from the storage.

BARRIER B

ENTERING BARRIER B

Entering routines for staff handling animals or equipment coming into contact with animals

- Shower and hair wash, at home or on site, if barrier C has been visited the last 12 hours

PPE for staff handling animals or equipment coming into contact with animals

- Working clothes (either fresh or worn in barrier A)
- Gloves
- Dedicated shoes

Entering routines for service personnel, visitors, or for resupplying consumables only:

- Shower and hair wash, at home or on site, if another fish facility or barrier C has been visited the last 12 hours

PPE for service personnel, visitors or for resupplying consumables only:

- Working clothes or lab coat
- Gloves
- Dedicated shoes

EXITING BARRIER B:

- Take of shoes and gloves
- Barrier B, B-, C and D can be visited without restriction

MOVEMENT OF ANIMALS, CONSUMABLES AND EQUIPMENT IN AND OUT OF BARRIER B

- Only surface disinfected embryos can be transferred from barrier C into barrier B
- Animals can be exported from barrier B without any restriction
- Equipment can be brought into barrier B. If the equipment has been placed in barrier C before, it needs to be gassed or, if gassing is not possible, wiped of with Ytdes.
- Consumables can be brought into barrier B from the storage. If the consumables have been placed in barrier C before, they need to be gassed or, if gassing is not possible, wiped of with Ytdes on the surface.

BARRIER B-

ENTERING BARRIER B-

Entering routines for staff handling animals or equipment coming into contact with animals

- Shower and hair wash, at home or on site, if barrier C has been visited the last 12 hours

PPE for staff handling animals or equipment coming into contact with animals including water in which the animals are housed in

- Working clothes with long sleeves or lab coat above working clothes (either fresh or worn in barrier A or B)
- Gloves
- Dedicated shoes

*PPE for staff **not** handling animals or equipment coming into contact with animals (e.g. feeding)*

- Lab coat above working clothes (either fresh or worn in barrier A or B)
- Gloves
- Dedicated shoes

Entering routines for service personnel, visitors, or for resupplying consumables only:

- Shower and hair wash, at home or on site, if another fish facility or barrier C has been visited the last 12 hours

PPE for service personnel, visitors or for resupplying consumables only:

- Working clothes or lab coat
- Gloves
- Dedicated shoes

EXITING BARRIER B-:

- Staff or other personnel that have not come into contact with animals or water in which the animals have been housed in (e.g. feeding or resupplying):
    - Take of lab-coat, shoes and gloves
    - Free to enter barrier B or C
- Staff or other personnel that has come into contact with animals or water in which the animals are housed in
    - Take of shoes and gloves
    - Exchange working clothes to fresh ones
- Staff that have been exposed to water splashes on working clothes or skin
    - Shower and hair wash and exchange of all outer clothes to fresh ones

MOVEMENT OF ANIMALS, CONSUMABLES AND EQUIPMENT IN AND OUT OF BARRIER B-

- Animals can be transferred from barrier B into barrier B-
- Surface disinfected embryos can be transferred from barrier C into barrier B-
- Animals can be transferred from barrier B- to barrier C
- Surface disinfected embryos can be transferred from barrier B- to barrier B
- Equipment can be brought into barrier b-. If the equipment has been placed in barrier C before, it needs to be gassed or, if gassing is not possible, wiped of with Ytdes on the surface.

- Consumables can be brought into barrier B- from the storage. If the consumables have been placed in barrier C before, they need to be gassed or, if gassing is not possible, wiped of with Ytdes on the surface.

BARRIER C

ENTERING BARRIER C

Entering routines for staff handling animals or equipment coming into contact with animals

- Barrier C can be entered after visiting or working in Barrier A, B+, B, B-
- Shower and hair wash, at home or on site, if infectious agents have been handled in barrier D.

PPE for staff handling animals or equipment coming into contact with animals

- Working clothes (either fresh or worn in barrier A or B, B-)
- Gloves
- Dedicated shoes

*PPE for staff **not** handling animals or equipment coming into contact with animals (e.g. feeding)*

- Lab coat above working clothes (either fresh or worn in barrier A or B, B-)
- Gloves
- Dedicated shoes

Entering routines for service personnel or for resupplying consumables only:

- Barrier C can be entered after visiting or working in Barrier A, B+, B, B-
- Shower and hair wash, at home or on site, if infectious agents have been handled in barrier D.

PPE for service personnel or for resupplying consumables only:

- Working clothes or lab coat
- Gloves
- Dedicated shoes

EXITING BARRIER C:

- Staff or other personnel that has not come into contact with animals (e.g. feeding or resupplying):
    - Take of lab-coat, shoes and gloves
    - Free to enter barrier B or B-
- Staff or other personnel that has come into contact with animals (e.g. setting up matings):
    - Take of shoes and gloves
    - A shower and hair wash as well as fresh working clothes are required before entering barrier B+, B or B- within the next 12 hours

MOVEMENT OF ANIMALS, CONSUMABLES AND EQUIPMENT IN AND OUT OF BARRIER C

- Animals can be transferred from barrier B or B- into barrier C
- Surface disinfected embryos can be transferred from barrier C into barrier B or B-
- Equipment can be brought into barrier C.
- Consumables can be brought into barrier C from the storage.

TWO DISTINCT EPIDEMIOLOGICAL UNITS IN THE QUARANTINE

The quarantine houses “high-risk” and “low-risk” animals. High-risk animals are housed in the flow-through system, therefore the flow-through system represents a separate epidemiological unit and specific measurements must be taken to keep this unit separate from the other systems:

- Gloves must be changed after handling animals from the flow-through system or after coming into contact with water from the flow-through system.
- Matings must be set up in a dedicated area, the area must be disinfected with Ytdes afterwards
- Any surface on which tanks or other equipment from the flow-through system is placed must be disinfected with Ytdes directly afterwards.

BARRIER D

The rules below give just an overview. Specific and detailed rules are available in the SOP “working in the BSL-2 unit”.

ENTERING BARRIER D

Entering routines for staff handling animals or equipment coming into contact with animals

- No specific entering routines, barrier D can be entered after visiting or working in any other barrier

PPE for staff or researchers handling animals or equipment coming into contact with animals

- Working clothes (either fresh or worn in barrier A, B, B- or C)
- Lab-coat over working clothes
- Gloves
- Dedicated shoes

Entering routines for service personnel or for resupplying consumables only:

- No specific entering routines, barrier D can be entered after visiting or working in any other barrier.

PPE for service personnel or for resupplying consumables only:

- Lab-coat
- Gloves
- Dedicated shoes

EXITING BARRIER D:

- Follow the “working in the BSL-2 unit” for details
    - Take off lab-coat, put in laundry basket after handling infectious agents
    - Take of shoes and gloves
    - Shower and wash hair after handling infectious agents, contaminated water or animals that have been housed in the BSL-2 unit.
    - Change working clothes before entering barrier B or B-

MOVEMENT OF ANIMALS, CONSUMABLES AND EQUIPMENT IN AND OUT OF BARRIER C

- Animals can be transferred from all barriers to barrier D
- Equipment can be brought into barrier D.
- Consumables can be brought into barrier D from the storage.

WATER TREATMENT UNIT

The room of the water treatment unit is divided into two sections. Section 1 is considered the region outside the coffin; section 2 is considered the region inside the coffin.

After visiting section 1, hands must be disinfected and after that barrier B, B-, C and D can be visited.

Section 2 must only be entered after having changed to shoes/rubber boots which are standing in section 2. Gloves must be worn at any time in section 2. Other PPE might be necessary, see the respective SOP and/or risk assessment. A shower and hair was is necessary after having been to section 2 and before visiting any other barrier of the core facility.

CLEANING AND DESINFECTION STRATEGY

- Colony tanks of barrier B as well as lids, baffles, and siphons are cleaned and disinfected in the calypso dishwasher using appropriate detergents
- Colony tanks of barrier B- and C are washed in a laboratory dishwasher at > 80° C after every use.
- In all barriers, mating tanks are washed in a laboratory dishwasher at > 80° C after every use.
- In all barriers, nets, plastic plants etc. are disinfected in 70 % ethanol for at least 10 min. The ethanol bath is changed once a week.
- All surfaces, working benches etc are disinfected once a week with Ytdes (effective against Mycobacteria)
- All small items that come into contact with animals (e.g. surgical instruments, forceps, probes etc.) are disinfected in 70 % ethanol for at least 10 min. The ethanol bath is exchange once a week.

LOCATION OF DISINFECTION SOLUTIONS

1) Ytdes for disinfection of surfaces can be found in every barrier.
2) The 70 % ethanol bath used to disinfect small items such as nets, dividers and similar can be found at the sink next to the polyculture rack. See separate SOP and risk assessment for disinfection of small items with ethanol.

ZEBRAFISH STRAIN MANAGEMENT

- The zebrafish core facility has a specific strain management plan that ensures healthy and genetically strong strains.
- For all strains older than 1 year, a compulsory regeneration program is started and adult fish older than 18 months are sacrificed. In specific cases (*e.g*. important carrier fish), the strain can be kept until 24 months of age in the quarantine unit.

DAILY HEALTH MONITORING

- All colony tanks are inspected daily. Fish with early signs of human endpoints and clinical signs consistent with human endpoints must be removed from the system directly.
- Animals found dead or sacrificed animals are disposed of as biological material or kept fixated or frozen for analysis.
- In case of severely sick fish or fish with previously unseen symptoms, the manager and the veterinarian must be informed. Those fish will be sent for pathological analysis.
    - Fish from core-facility owned stocks with a health score of > 0.3 are sacrificed directly. The number of fish is adjusted in the animal database tick@lab.
    - Fish from researcher-owned stocks with a health score of > 0.4 are sacrificed directly. The number of fish is adjusted in tick@lab.
    - Fish from researcher-owned stocks with a health score between 0.3 and 0.4 are transferred into a labelled 1 l tank and the manager is informed.

QUATERLY HEALTH SAMPLING

Samples are sent for health control four times a year. The samples include:

1) Sentinel animals
2) Randomly sampled animals
3) Sludge/sump
4) Live feed

For details on the health monitoring program, please see the respective SOP.

HANDLING OF DEAD ANIMALS

- Sick or dead animals are removed from the colony directly and disposed of as biological waste.