

Bsal Hygiene Protocol for Fieldwork and Amphibian Husbandry

Materials for disinfection:

- Brushes
- Buckets
- Disinfection liquid
- Hand soap
- Spray bottles/pump sprayers
- Disposable powder-free gloves
- Plastic bags
- Trash bags

Top Tips for *Bsal* Hygiene protocols in the field

1. All organic material (soil, plants, small invertebrates, debris, biological material and secretions etc.) should be removed from equipment before any chemical disinfectants are used. Organic material will inactivate chemical disinfectants and/or render the concentration of these solutions ineffective to kill pathogens. The presence of any material organic or inorganic may impede the contact of the disinfectant with the surface which is meant to work on.
2. The following chemical disinfectants can be used in the field to disinfect fomites from *Bsal*:

***Bsal* can be killed using most of the common disinfectants (Table 1). This table was taken from a paper that has been submitted for publication (Van Rooij et al., 2017). Hydrogen peroxide shows poor activity against *Bsal*. Heat treatment is to be expected to result in fast killing of all life stages of *Bsal* but needs further study. The fungus tolerates high temperatures poorly: *Bsal* cultures are killed after incubation for 5 days at 25°C (Bloo et al., 2015). If *Bsal* responds to**

heat as its sister species *B. dendrobatidis*, exposing materials to 60°C for 5 minutes or 100°C for 1 minute should be efficient (Johnson et al., 2003). Several of the disinfectants mentioned may cause harm to humans, animals, the environment and to materials (including clothes). Please always carefully consult the disinfectant’s manual. Virkon S is widely used (relatively safe, highly efficient) but its use in the field may require derogations from existing legislation. Disposal of disinfectants in the natural environment should be avoided.

Disinfectant	Concentration	Minimal exposure time for 100% killing of <i>Bsal</i>
Ethanol (EtOH)	70%	30s
Disolol®	undiluted	30s
Hibiscrub®	0.25, 0.5, 0.75%	30s
Chloramine-T®	0.5%	5 min
	1%	2 min
Bleach	1:5 dilution	5 min
	4%	30s
Kickstart®	0.05%	5 min
	0.1%	2 min
Potassium permanganate (KMnO₄)	1%	10 min
	2%	5 min
Virkon S®	0.5%	5 min
	1%	2 min
Dettol medical®	1:20 dilution	5 min
Biocidal®	undiluted	30s
Safe4®	undiluted	30s
F10 ®	1:100 dilution	30s
	1:250 dilution	30s
	1:500 dilution	30s
	1:1000 dilution	30s
Sodium chloride (NaCl)	10%	10 min

Table 1: Minimal exposure time for 100% killing of *Bsal* spores and sporangia at room temperature

3. Please ensure that you utilize the disinfectant in the concentrations and manner stipulated by the references above and/or the manufacturer in order to achieve the intended results.

Hygiene protocol for:

1. Swabbing amphibians in one or multiple site(s)/population(s)

Non-invasive sampling of amphibians using skin swabs is the preferred method to survey for *Bsal* infections. This procedure can be done after minimal training, does not result in animal injury when properly done and produces a usable sample.

- a. Ensure that all equipment has already been disinfected prior arrival on the site.
- b. Use powder-free vinyl (not latex) disposable gloves when handling amphibians. Gloves should be changed for each individual amphibian handled then discarded.
- c. Remove cotton swab from protective case, be careful that you only touch the upper part of the swab that was meant for holding so as to avoid contamination.
- d. Place the tip of the cotton swab on amphibian skin and rub firmly on the point of contact with the skin (especially areas where there are obvious lesions), ideally 10 times the abdomen, 10 times the ventral tail and 10 times the underside of a foot. Samples should be done in duplicate.
- e. Reinsert swab into protective case.
- f. Label the swab's protective case with identifying information such as date, site, sample number and species.
- g. Place used gloves in a trash bag for disinfection and disposal later.
- h. Put on a fresh pair of gloves.
- i. Repeat steps c-g.

- j. Store all samples from one site/population together. Swabs should be stored dry and preferably frozen. Frozen samples can be stored for long periods (several months). If swabs have to be stored longer than one week and cooling is not available, they may be stored in a small volume (200 µl) of 70% ethanol.
- k. When moving to a new site or population please follow the hygiene protocol described for “Hygiene protocol used between sites or populations and Hygiene protocol used on equipment”.

2. Tissue Sampling in amphibians

Tissue sampling is not recommended for surveying amphibian populations for the presence of *Bsal*, since this is an invasive sampling method that may produce serious health issues in the animal sampled (wound infections, mechanical hindrance). Non-invasive sampling using skin swabs as described above should be preferred. In case tissue samples are being collected (e.g. for reasons of genetic research), these can be used for *Bsal* detection when properly collected. Collecting tissue samples from live amphibians requires advanced skills and should be approved by an ethical committee.

- a. Ensure that all equipment has already been disinfected or utilize disposables.
- b. Use powder-free vinyl (not latex) disposable gloves when handling amphibians. Gloves should be changed for each individual amphibian handled then discarded.
- c. Clip small portion of amphibian skin, particularly in location where there are lesions.
- d. Preserve skin sample in 70% EtOH or frozen in a cryotube.
- e. Put on a fresh pair of gloves.
- f. Store all samples from one site/population together.
- g. Repeat steps a-f. Disinfect clipper between animals.
- h. When moving to a new site or population please follow the hygiene protocol described for “Hygiene protocol used between sites or populations and Hygiene protocol used on equipment”.

3. Hygiene protocol for equipment and clothes used between sites or populations

When sampling multiple sites in one field visit, in order to minimize the potential for transmission or spread of disease it is important to establish a plan as to the order in which these sites will be visited. It is good biosecurity practice to first visit sites/populations where there have been no cases or no suspected incidence of disease. Later, where there is no information on the status of sites/populations, one may visit these next. Finally, the sites or populations with known cases of disease may be visited. All material, including footwear and clothes should be subject to proper disinfection procedures between visits to different sites.

- a. Ideally, when visiting multiple locations, travel with as many bags of clothes, shoes and other equipment necessary to organize a dedicated set of clothes, shoes, equipment for each site/population etc. or to store dedicated clothing and equipment for each site/population on each actual site. The use of disposable materials effectively prevents disease transmission. Alternatively, all materials must be thoroughly cleaned and disinfected between sites as outlined below.
- b. All mud, soil, leaves and other organic and non-organic material should be brushed off of all equipment and field gear.
- c. The equipment should then be rinsed to remove all residues of organic material or any inorganic material which impedes contact of the disinfectant with the fomites which need to be disinfected.
- d. Small equipment should then be submerged in disinfection liquid (prepared according to the manufacturer's instructions) or larger equipment should be thoroughly covered with disinfection liquid sprayed on.
- e. Rinse the disinfectant from the equipment or allow mixture to dry in case of volatile disinfectants (e.g. ethanol).
- f. It is important to get all disinfectant residues off the equipment as they can be harmful to amphibian skin. At the new site/population, water from any body of water present can be used to rinse the equipment. This should be done at least 50m from the body of water and preferably on an impermeable surface.
- g. Place all gloves and other waste material in trash bags so that they can be disinfected and disposed of properly at the office or other suitable location.
- h. Vehicles used during sampling or carrying out surveillance for amphibians may have residual soil or water that could contain *Bsal* zoospores. Therefore, the following steps may be required to ensure that no *Bsal* can be spread to other sites via the vehicles:

- clean wheels and tyres
 - disinfect wheels and tyres
- i. Hands, arms and any other body parts which have come into contact with water, soil or debris should be:
- cleansed of all organic material
 - disinfected with suitable solution. Not all disinfectants are appropriate for use on human skin.

Please carefully consult the manufacturer's instructions. Frequent use of disinfectants on skin may produce side effects.

6. Hygiene protocol for amphibian husbandry

The cornerstone of avoiding spill-over of pathogens from amphibians from captivity to the wild is certified absence of *Bsal* (and other pathogens like *Bd* and ranaviruses) from the captive animals. *Bsal* infections can be treated rather easily (by housing the infected animals at 25°C for 10 days, with subsequent assessment of absence of *Bsal*). Absence of *Bsal* can be achieved by having:

- 1) all animals present tested using a skin swab for the presence of *Bsal*
- 2) all newly arrived animals maintained under strict quarantine for at least 40 days. During this period, the animals should be sampled for *Bsal*, *Bd* and ranaviruses. If positive, the animals should be treated until total clearance of any *Bsal* infection. During this quarantine period, all materials and terrarium contents should be disinfected thoroughly before disposal or use elsewhere. Before discarding any wastewaters or terrarium contents which may have been contaminated by salamanders or newts, these should be disinfected. Heat treatment is the preferred way of treatment given its relative ease of application and absence of environmental contamination. All waste should be treated for at least 30 minutes at, at least, 60°C before disposal.
- 3) ill or dead animals examined by a competent veterinarian.

Captive salamanders and newts kept as pets should neither be released into the wild nor have direct or indirect contact with native salamanders or newts (direct contact: for example, in outside terraria, indirect contact: for example, by using dipnets, containers, buckets etc. both for terrarium animals and for wild animals). Even in the absence of obvious signs of clinical illness, some amphibians are still able to carry and shed the pathogen. If pet owners are no longer able to care for their pets then

they should contact their local herpetology society, zoo, a local veterinarian, or local animal welfare organization to care for them.

Trade in captive salamanders and newts should be restricted to certified *Bsal* free animals.