



## Hygiene protocol to contain the spread of Chytridiomycosis during fieldwork

Amphibians are suffering population declines due to Chytridiomycosis, a disease caused by a fungus called *Batrachochytrium dendrobatidis* (abbreviated “Bd”).

Catastrophic declines have been observed in Australian, North American, Central American, South American and Caribbean species. In Europe, mass mortality and declines associated with Bd have been observed in Spain and France but our knowledge of Bd prevalence in Europe remains poor.

There is no unambiguous explanation for how Bd is vectored among locations. Nevertheless, the general consensus is that Bd fits the model of the Novel Pathogen Hypothesis, where Bd has, and continues to be, introduced into areas where it once did not occur. It has been hypothesized the fungus can be transferred from place to place through movement of materials that have come into contact with Bd, water containing infectious zoospores or amphibians infected with Bd. Experiments have shown that infections can be transmitted among amphibians. The economic trade in amphibians and consequent introduction of nonnative amphibian species and translocations of native species must therefore be treated as risks. Overall, human activities are assumed to be a major cause for the spread of the fungus and should be considered a risk for amphibian populations. Specifically, activities in or close to aquatic sites (from nature watching to scientific investigations) may increase the spread of the disease.

Individual amphibians can be effectively treated with fungicide, but currently infection with Bd has not been successfully mitigated in wild populations. However, simple disinfection procedures are effective at decontaminating equipment, clothing and people, and application of these should reduce the risk of the fungus being passively transferred among locations. The aim of this document is to provide a set of simple, preventative measures suitable for anyone involved in amphibian and, more generally, freshwater aquatic research in the field. Whilst focusing on chytridiomycosis, these precautions may be useful in controlling the spread of other diseases, invasive plants and animals. Some of these procedures may be applicable to a laboratory or zoos, but we do insist that those who use amphibians in research or in zoo settings must confer with their local veterinary representative before applying these procedures. Biosecurity requirements for captive populations are likely to differ substantially from those for field work.

## General rules

There are several disinfectants available that are effective at killing Bd and other infectious agents (e.g. 70% alcohol, saturated salt solution and bleach). Virkon® is a broad spectrum disinfectant with known capability at killing bacteria, fungi and viruses, including Bd. We also recommend the use of this disinfectant because of its lower environmental impact. Release to water bodies should however be avoided and disposal through sewer systems is recommended by the manufacturer. Read and follow the product label instructions and information including the precautionary statements before use (<http://www2.dupont.com/>).

Make sure that ALL your equipment has been properly disinfected before you embark on field work. If uncertainty remains, disinfect.

If several aquatic sites are visited during the same field trip, apply the disinfection protocol when you move among sites. When visiting one large aquatic site (marsh, river, lake, etc.) regularly disinfect the equipment.

When handling amphibian, use disposable, powder-free gloves. Whenever possible, captured amphibians should be housed individually (e.g. using zipper storage bags or other sealable and plastic bags). Preventing cohousing amphibians during collection limits contacts and disease transmission among animals.

When conducting fieldwork in an area where Bd presence is suspected (mortality, the presence of nonnative species, etc.) or validated, the hygiene protocol must be rigorously applied.

## Standard disinfection protocol

- 1) Prepare a **1% Virkon® solution (10g/l)**. The Virkon® solution should only be used as long as the color is at least a medium pink (we recommend to prepare fresh solution each day). The solution can be also prepared in the field, using brook or pond water and a hand-held plant spray to hold the disinfectant.



- 2) When leaving the water, **clean off equipment** (boot, waders, net...) with a brush to remove mud and plant and other fragments.

- 3) Squirt Virkon® solution on all equipment in contact with water, and wait **5 minutes before reusing**, and preferably until the equipment has dried.

Small material in contact with amphibians (e.g. balances, scissors, etc.) can be disinfected by immersion in Virkon® solution and/or alcohol wipes.

*If you want to rinse your equipment with clean water, then do this when you return to your home/laboratory.*



- 4) Squirt 1% Virkon® onto the base of your boots/wellingtons before walking away from the fieldsite.



- 5) Store disinfected equipment in disposable plastic bags, then in a plastic tank in the vehicle.

- 6) Disinfect your hands with alcohol handwipes (or diluted 70% alcohol solution)

- 7) On return to your home or lab, field clothes can be disinfected by washing at 60°C. Store all disposable items (gloves, bags, etc.) in a plastic bag, and disinfect them with Virkon® before throwing them away in the garbage.



### **Consumable list**

- Brush
- Hand sprayer
- Virkon® (pellets) (add here where ordering in your country, e.g. in UK: [www.VWR.co.uk](http://www.VWR.co.uk), veterinary offices in France, etc.) if you think it is necessary and if the address of sellers will not change.
- Alcohol handwipes or 70% alcohol solution
- Disposable powder-free gloves
- Disposable plastic bags of different sizes
- Store containers (staying in the vehicle and regularly disinfected)

*(if Virkon® is not available during the field trip, and Virkon® is not available locally you can use alcohol solution ( $\geq 70^\circ$ ). However, Virkon® should be used whenever possible).*