# Rapid Response Template for *Batrachochytrium salamandrivorans*

Written and prepared by: The North American *Bsal* Task Force

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**PLEASE NOTE:** Within this document are explanatory notes and questions to stimulate discussion to help clarify the intent of the information provided for end users and to facilitate their customization of the template. These notes are placed throughout the text in [bracketed blue font]to distinguishthem from other guidance provided for the purpose responding to a detection or outbreak of *Bsal*.

**Purpose:** This document is a **template to be customized** by any agency or institution with management jurisdiction over wild or captive salamanders, respectively, when actions in response to a disease may be warranted. [This purpose statement may be further customized as needed for individual entities.]

Herein are provided an outline and guidance for **local, rapid response** actions that could be triggered upon initial or subsequent detections of *Bsal*, in either wild or captive populations. [The scenarios are based on what an entity using this plan might do after receiving information regarding *Bsal* detection status from a diagnostic laboratory with expertise in *Bsal* diagnostics. In other words, all recommended actions occur after the laboratory has made its determinations based on the case definition of *Bsal* (White et al. 2016).]Also provided are considerations for in situ containment (i.e., in the existing location of the population) as well as establishment of ex situ populations (i.e., outside of the natural location, such as in a captive assurance colony). Rapid containment and response measures may prevent broad impacts of *Bsal*. [The USGS Amphibian Research and Monitoring Initiative (ARMI) is also working to assist entities in making decisions regarding wildlife disease management, including the customization of this template. Contact the ARMI Decision Science Lead, Dr. Evan Grant (ehgrant@usgs.gov), for assistance.]

This template was produced by the *Bsal* Response Working Group as part of their work with the *Bsal* Task Force’s Technical Advisory Committee(TAC) (see [www.salamanderfungus.org](http://www.salamanderfungus.org) for additional information) and is considered a living document that will be updated as more information becomes available. At the time of this version of the template, *Bsal* is not known to occur in North America, and suggested responses are consistent with the high-alert condition of *Bsal* being yet undetected or rare in North America.

This document is intended to be incorporated into a National *Bsal* Strategic Framework, where larger surveillance and monitoring strategies, research needs, policy needs, and related prevention strategies, along with public outreach and communication, are addressed.

***Batrachochytrium salamandrivorans* (*Bsal*)**

**Rapid Response Template**

**Preliminary definitions and resources:**

This section sets forth how terms are defined within the rest of the document. [Entities customizing this template should add other definitions as they deem appropriate.]

**Definitions:**

1. ***Bsal*-susceptible host species**– We use this phrase broadly to indicate both species for which *Bsal* can be fatal and species that can be infected by *Bsal* but not develop signs of disease, thereby serving as carriers and reservoirs of *Bsal*. At the time of this writing, experimental evidence suggests that anurans may carry *Bsal* (Stegen et al. 2017). Thus, we assume that all amphibian species *may* be susceptible to *Bsal* or be carriers of *Bsal* unless it is demonstrated that a species cannot be infected. [*Bsal* has been termed the “salamander fungus” because it was described from infected Fire Salamanders (*Salamandra salamandra*) in Europe and has been shown to either infect or to be carried by several other salamander species (Martel et al. 2014). New evidence suggests that some anurans can also be infected and carry the pathogen, potentially without developing clinical signs of infection (Stegen et al. 2017, Yuan et al. 2018). This template and this definition will be updated when new evidence of species-specific susceptibility becomes available.]
2. **Wild host population**– Free-ranging population of *Bsal*-susceptible species.
	1. Naïve (no prior *Bsal* detections known at a given site)
	2. Exposed (prior *Bsal* detections documented at a given site)
	3. Unknown (no or insufficient *Bsal* surveillance has been performed)
3. **Captive host population** *–* Any population that is not free-ranging, including outdoor enclosed spaces or fenced runs where contact with wild amphibians or disease vectors may be possible (e.g., zoo, aquarium, research facility, university).
	1. Naïve (no prior *Bsal* detections known from the captive location)
	2. Exposed (prior *Bsal* detections documented from the captive location)
	3. Unknown (no or insufficient *Bsal* surveillance has been performed)
4. **Mortality event, wild** – Death of one or more free-ranging amphibians in the environment, whether or not the *Bsal* pathogen has been detected.
5. **Mortality event, captive** – Death of one or more amphibians in a captive environment, whether or not the *Bsal* pathogen has been detected.
6. **Eradication** – The assumed elimination of *Bsal* from individual amphibians *in captivity* based on four consecutive negative PCR tests, each one week apart, per individual, as described in Blooi et al. (2015a).
7. **Participating Laboratory** – The particular laboratory that has been engaged during a testing or response effort; see also the Resources section below regarding the Diagnostic Laboratory Network.
8. **Reporting Individual(s)** – The individual(s) who submitted the sample(s) (e.g., swabs, carcasses, live animals) to a laboratory for diagnostics. This is the person(s) the laboratory is to contact to provide results. In some cases, this person may be a scientific researcher. [At the time of this version, *the Bsal* Task Force is developing a statement and working with key scientific journal editors to ensure that sharing of scientific findings with management agencies to facilitate early detection and rapid response actions will not diminish the value or integrity of the scientific findings or the person(s) involved.]
9. **Core Response Team (CRT)** – The group of authorized professionals, and other parties involved in the initial discovery, that evaluates the situation and makes recommendations for next steps. The CRT may include other trusted parties, as appropriate, where information can be securely shared and will not compromise scientific integrity (see suggested CRT composition in Resources)**.** [We reference the use of such a team as part of the recommended actions in the response scenarios described in this template. We suggest that certain members of this team be identified in advance to facilitate a rapid response. Below, we offer additional suggestions regarding the team’s role and composition. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.]

**Resources**

1. **Diagnostic Laboratory Network.** A consortium of participating laboratories equipped to handle *Bsal* testing requests and to employ specific protocols (as recommended by the *Bsal* Task Force’s Diagnostics Working Group) for quality assurance and quality control (QA/QC). The network assists with coordination of sample handling. The list of known laboratories capable of *Bsal* testing is provided on the *Bsal* Task Force website ([www.salamanderfungus.org/resources/labs](http://www.salamanderfungus.org/resources/labs)). [Entities customizing this template may benefit from contacting their nearest laboratory(-ies) to understand their sample submission protocols, fees for services (as applicable), and any other requirements relevant for sample submission in the event of a disease outbreak (whetherBsal or another pathogen).]
2. **Core Response Team (CRT).** [As noted above, we reference the use of such a team as part of the recommended actions in the response scenarios described in this template. Here, we offer suggestions on the charge and composition of the team. However, the use or role of the team is ultimately at the discretion of the entity customizing this template.]
	1. **Purpose:** The CRT is an advisory group that discusses the specific response scenario and helps to make initial decisions regarding response actions and related communications. Any member of the CRT is expected to keep the shared information **confidential** until the **management agency or entity with jurisdiction** (i.e., the authority to make decisions about the species or the lands affected)indicates how, where, and when information may be shared.

**Composition**: [The composition of this team may change depending on the specific circumstances. Below is a brief explanation of the suggested composition. Note that the ***Bsal* Technical Advisory Committee** includes appropriate expertise in the event of a *Bsal* outbreakand is at your disposal for confidential advisory assistance.]

* + 1. Reporting Individual(s) – [The individual who discovered the mortality event or was involved in research that led to a *Bsal*-positive detection may have ability to assist in response-related actions or follow-up work at the site.]
		2. Agency or entity with jurisdiction over the affected species or lands – [The agency with management jurisdiction or the land manager will be able to confirm actions that can or cannot be taken.]
		3. Land or facility manager(s)/owner(s) where samples were collected, if different from the entity in (2)(b)(ii)
		4. State agency personnel in charge of amphibians – [The state or provincial/territorial fish and wildlife agency is the primary management authority for amphibians and can assist with appropriate species management actions on non-federal lands.]
			1. NOTE: The Association of Fish and Wildlife Agencies’ Amphibian & Reptile Program Manager can assist in determining the appropriate state contacts.
		5. Key amphibian expert scientists who can provide recommendations, **in a confidential consulting capacity**, for short and long-term responses based on best available science – [Amphibian experts can advise on most current science.]
			1. NOTE: Please consider contacting the *Bsal* Technical Advisory Committee leadership (response@salamanderfungus.org); one or more members will be available to assist in a confidential advisory capacity.

1. **Points of contact (PoCs).** [Entities customizing this template should populate it with the appropriate PoCs.]
	1. Compile a list of key contacts in a given state, federal agency, or management unit (e.g., unit director or manager, staff veterinarian, lead herpetologist, or wildlife biologist) to inform and coordinate response actions to a positive diagnostic test result.
	2. Include permit coordination contacts (state, federal, provincial, local, etc.)
2. **Wildlife Health Expert Networks.** Qualified wildlife experts to assist in treatment of captive or privately-owned animals and in issuing health certifications or other documentation to verify animal health, emergency responses, etc. may be found via:
	1. The Diagnostic Laboratory Network established via the *Bsal* Task Force’s Diagnostics Working Group (see [www.salamanderfungus.org](https://www.salamanderfungus.org/)).
	2. Veterinary experts:
		1. [Association of Reptile and Amphibian Veterinarians](https://arav.org/) (ARAV)
		2. [American College of Zoological Medicine](https://www.aczm.org/content.aspx?page_id=22&club_id=366916&module_id=48992) (ACZM) list of board-certified zoological medicine veterinarians
		3. The [American Association of Wildlife Veterinarians](http://www.aawv.net/) (AAWV)
		4. The [Canadian Wildlife Health Cooperative](http://www.cwhc-rcsf.ca/) (CWHC)
	3. Wildlife epidemiologists or wildlife disease ecologists
3. **Facilities.** A list of available captive housing or breeding facilities (e.g., [Amphibian Ark](https://www.amphibianark.org/) [AArk], [Canada’s Accredited Zoos and Aquariums](https://caza.ca/) (CAZA), [Association of Zoos and Aquariums](https://www.aza.org/?locale=en) [AZA]-accredited zoos, or other local facilities), with contacts.
	1. Treatment. [Entities customizing this template should identify secure emergency facilities in their network to temporarily house moribund (dying, unable to right themselves) or sick but potentially treatable animals.]
	2. Rescue colonies. [Entities customizing this template should identify facilities to house rescued animals or those collected for the purpose of captive breeding and reintroduction.]
	3. Museums or other storage facilities. [Entities customizing this template should identify facilities for vouchered animals or archived tissue samples, swabs, or extracted DNA.]

**Questions**

What AArk, CAZA, or AZA facilities are local? Are you familiar with the appropriate contacts there? What local museums are able to accession animals? Can they also accession tissues, swabs, DNA?

1. **Protocols.** [Along with those below, consider other protocols that may be useful, e.g., data submission or management protocols.] Recommended guidance can be found at the *Bsal* Task Force website ([www.salamanderfungus.org](http://www.salamanderfungus.org)) via the Diagnostics or Research pages but see also Pessier and Mendelson (2017), including:
2. Biosecurity protocols for field, lab, use of live cultures, etc.
3. Swabbing and storage (and transportation) protocols.
4. **See also Appendix I**, where pertinent portions of the guidance manual have been included and adapted for quick reference.

**SCENARIO-SPECIFIC MANAGEMENT RESPONSES**

**NOTE:** The scenarios below pertain to mortality or PCR detection events (and subsequent confirmation of causative agent); however, any suspicious-appearing amphibians should be investigated. Examples of suspicious-appearing amphibians that should be reported include sick or lethargic individuals, those with black circular or oblong lesions, or those unable to right themselves. The Partners in Amphibian and Reptile Conservation (PARC) national Disease Task Team has established the “Herpetofaunal Disease Alert System” (HDAS) to help connect people to the appropriate experts and authorities quickly; please see the [PARC Disease Task Team website](http://parcplace.org/resources/parc-disease-task-team/) for information and for how to send a report to herp\_disease\_alert@parcplace.org.

**Scenario 1: Mortality event, cause unknown; Wild**

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These actions should be implemented at the discretion of the jurisdictional management unit depending on the level of response they are able to take to help minimize potential impacts. Contact [your local amphibian expert or member of a Veterinary Expert Network] to assist. [Entities customizing this document should identify appropriate amphibian experts local to their jurisdiction.]

**When uncertain how to proceed or whom to contact**, the PARC Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to herp\_disease\_alert@parcplace.org.

**Mandatory action:**

**Notification to agency with management jurisdiction.** To facilitate early *Bsal* detection and rapid response, contact the management agency with jurisdiction where the mortality event occurred (which may be your own agency) to ensure they are aware of the testing event and impending results. [**Important –** Given the heightened state of alert for *Bsal* and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the management agency with jurisdiction where the mortality event occurred, even if this may be your own agency, to be sure they are aware that a mortality event has occurred and testing is underway, while results are pending.]

**Questions**

Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state? (If not, the PARC Disease Task Team may be able to assist; send a message to herp\_disease\_alert@parcplace.org requesting information on the appropriate contacts.) **For management agencies:** Are there other partners that you need to engage, and if so, should it be at this stage or after results are received?

**Recommended actions (in no priority order and as feasible):**

1. **Tissue collection for diagnostics.**
	1. Collect any live but apparently moribund (dying, unable to right themselves) or lethargic animals, using humane euthanasia procedures, as applicable (see Appendix I, Section A), for submission to Participating Laboratory. Swabs alone are insufficient to confirm a *Bsal* diagnosis.
	2. Collect carcasses, fresh-dead (see Appendix I, Section A), for diagnostic necropsy and submission to Participating Laboratory.
	3. Sample other live amphibians (e.g., swabbing skin for use in a PCR assay), if area is high risk and if feasible (Appendix I, Section B).
2. **Biosecurity protocols**, as established (Appendix I, Section A(3)), implemented for all field gear, especially as part of implementing action #2 above, and also upon leaving the die-off site.

**Question**

Have you considered establishing an approved set of biosecurity protocols for sampling or surveillance in a disease-affected site?

1. **Heightened alert considerations**.
	1. Increased surveillance
	2. Local personnel notification. [It may be helpful to form and consult the CRT (see Resources above) or to assess notifications at this stage; notifications could be handled on a “need to know” basis.]
2. **Containment considerations**. The following are options that might help prevent spread of pathogens.
	1. Restricted public access to the die-off site.
	2. Signage at or around the exposed area(s).
	3. Local personnel notification and access restrictions to the exposed area(s). [Again, it may be helpful to consult the CRT or to assess notifications at this stage, and notifications could be handled on a “need to know” basis.]
3. **See “Definitive detection, Wild” scenario below for additional responses.**

**Scenario 2: Mortality event, cause unknown; Captive**

Mortality events may be due to any number of causative agents. The actions below include collection of samples to confirm a diagnosis and activities to be considered while results are pending. These actions should be implemented at the discretion of the captive management facility depending on how conservative or comprehensive of a response they are able to take to help minimize impacts. Contact [your local amphibian expert or member of a Veterinary Expert Network] to assist.

**When uncertain how to proceed or whom to contact**, the Partners in Amphibian and Reptile Conservation (PARC) Disease Task Team has established an alert system to help connect people to the appropriate experts and authorities; send a report to herp\_disease\_alert@parcplace.org.

**Mandatory action:**

**Notification to state or provincial/territorial fish and wildlife agency.** To maintain transparency and open communications regarding *Bsal* and to facilitate early detection and rapid response, we recommend contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred to ensure they are aware of the testing event and impending results. [**Important –** Given the heightened state of alert for *Bsal* and the critical nature of early detection and rapid response, when customizing this template, please consider including this recommended action of contacting the state or provincial/territorial fish & wildlife agency where the mortality event occurred, to be sure they are aware of the mortality event and that testing is underway, while results are pending. This notification allows them to consider additional surveillance or management actions to further protect wild populations.]

**Questions**

Do you know the appropriate contacts for disease response in the agencies with management jurisdiction in your state or province/territory? (If not, the PARC Disease Task Team may be able to assist; send a message to herp\_disease\_alert@parcplace.org requesting information on the appropriate contacts.) **For management agencies or industries:** Are there other partners that you need to engage, and if so, should it be at this stage or after results are received?

**Recommended actions (in no priority order and as feasible):**

1. **Tissue collection for diagnostics.**
	1. Collect tissue and/or moribund (dying, unable to right themselves), abnormally behaving, or co-located live animals, as feasible and using humane euthanasia procedures, as applicable (Appendix I, Section A), for submission to the facility’s pathologist, where applicable, or, after confirming the closest lab able to handle the specific case, to a Participating Laboratory (see also the Diagnostic pages of [www.salamanderfungus.org](http://www.salamanderfungus.org)).
	2. Collect carcasses, fresh-dead animals, for diagnostic necropsy; submission to Participating Laboratory (Appendix I, Section A).
	3. Consider collecting swabs from living animals without signs of disease contained in the same enclosures or nearby.
2. **Biosecurity protocols**, as established in Pessier and Mendelson (2017), implemented for:
	1. Disinfection of captive caging/housing facilities and materials prior to reuse for treated or new animals.
	2. Treatment and disinfection of water prior to disposal.
	3. Treatment of plant or soil substrate materials prior to disposal.

**Question**

Have you established/considered establishing an approved set of biosecurity protocols for disease-affected population/housing materials in captivity?

1. **Containment considerations**. For exposed captive animals that remain living, we suggest the following:
	1. Individual quarantine for all potentially exposed animals until causative agent is determined.
		1. Consult with your local amphibian or veterinary expert and consider prophylactic treatments, and post-treatment testing and monitoring, as per guidance in Blooi et al. (2015a,b).
	2. Halt transport/commerce of exposed, co-located, co-shipped, or all amphibians until health conditions and pathogen eradication can be verified.
	3. Retrieve chain-of-contact/custody information (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
		1. Inform all personnel at potential points of transmission and recommend they follow quarantine, testing, and treatment recommendations.
	4. Ensure biosecurity standards have been met (see action #3) prior to resumption of any transport or commerce of animals or caging materials, in accordance with existing federal, state or provincial/territorial, or local laws.
2. **See “Definitive detection, Captive” scenario below for additional responses.**

**Scenario 3: Detection of *Bsal* Presence by Polymerase Chain Reaction (PCR) (Wild or Captive)**

**This scenario is defined as:** Detection of *B. salamandrivorans* DNA, as determined by a Participating Laboratory, based on PCR testing of swab or tissue samples of individual amphibians or samples from the environment (e.g., environmental DNA [eDNA] sampling). [Ideally, the Participating Laboratory will have also verified the result by a second Participating Laboratory.] This scenario indicates potential presence of *Bsal*, but it is NOT considered a “definitive detection” of *Bsal* until additional evidence of *Bsal* has also been determined. The guidance below is to facilitate early detection, rapid response efforts while confirmation of *Bsal* presence is pending.

[A detection of *Bsal* presence via PCR could occur a) in an instance where no clinical sign or histopathologic evidence, nor evidence of a current mortality event, exists that is indicative of an active *Bsal* outbreak, b) as an outcome of Scenarios 1 or 2 above, or c) independently via surveillance or research of wild or captive populations.]

**Actions recommended (one or more, as feasible):**

1. Initial diagnostic results communicated by Participating Laboratory to:
	1. Reporting Individual(s), who in turn informs:
		1. Detection site landowner/manager
		2. Wildlife agency or entity with management authority
2. Agency or entity with management authority forms and convenes the CRT. [Some entities customizing this template may consider developing an [incident command system](https://en.wikipedia.org/wiki/Incident_Command_System) to help coordinate across other agencies or stakeholders.]
	1. Consider also engaging the *Bsal* Task Force Technical Advisory Committee (TAC) leadership (response@salamanderfungus.org), who are available to assist by advising on resources and responses, and will keep the information confidential. [Through the Task Force’s working groups, additional assistance can be provided on next steps following a PCR detection.]
	2. Consider developing a communications plan that facilitates internal agency and CRT communications to external stakeholders and the public (including signage for affected sites and intended visitor behavior modifications). [These are potential, suggested components of a communications plan; customized actions may differ.]

**Questions**

Is there any cultural or archaeological significance of the site? Is it a popular visitor site that may require a visitor management plan or additional staffing to advise the public and help avoid disturbance or public contact with affected areas?

1. **Further investigation**. Additional diagnostic testing should be conducted as feasible (e.g., sequencing and phylogenetic analyses, isolation by fungal culture, necropsy, and histopathologic examination of associated dead animals or tissues where applicable) by a Participating Laboratory for a definitive diagnosis (White et al. 2016).
2. **Management actions**, **wild populations**
	1. Biosecurity protocols, as established (Appendix I, Section A(3)), implemented for all field gear used at the *Bsal*-positive site.
	2. Increased surveillance at the *Bsal*-positive site.
		1. If available, test any archived amphibian tissues from the site of detection for *Bsal*.
		2. Evaluate known amphibian species composition at the site, with special consideration for the presence of federally-listed, state-listed, and at-risk salamander species.
			1. If listed and/or at-risk species are present, evaluate the need and opportunity available for taking healthy individuals from the wild and placing them in captivity for establishment of a breeding (captive assurance) colony.
		3. Conduct additional sampling of amphibians and water at the site of detection.
		4. Evaluate movements of other animals in or out of the site
	3. Heightened awareness by managers at the *Bsal*-positive site.
		1. Collect any morbid or dead amphibians at that site and submit them to Participating Laboratory for testing.
		2. Review any existing data from the vicinity of the site for evidence of population or mortality trends.
		3. Initiate population monitoring of affected amphibian species to determine if the population is stable or declining.
	4. Containment considerations. Consider options that might help prevent the spread of *Bsal*:
		1. Restricted public access to the exposed area(s).
		2. Signage at or around the exposed area(s).
		3. Local personnel notification and access restrictions to the exposed area(s).

Direct actions should consider risk from multiple perspectives, and assessment should extend beyond the immediate area of concern. Consider working with decision scientists to take effective actions in the context of multiple management objectives and varying risk profiles.

**Questions**

**[add as needed]**

Is drying or treating the site an option? Is the harm of taking an extreme action greater than doing nothing?

1. **Management actions**, **captive populations**
	1. Containment:
		1. Ensure no shared water sources or water flowing out of the affected animals’ caging/housing.
		2. Individual quarantine. Isolate affected animals and any others that were housed with affected individuals.
			1. Perform additional diagnostics on co-located individuals.
			2. Eradicate *Bsal* sources.
				1. For live, captive animals whose samples return a positive *Bsal* result, eradication may be attempted:

For fail-safe eradication, we recommend humane culling or euthanasia. See Section 8.6 in Pessier and Mendelson (2017) or humane methods in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals ([AVMA 2020](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)) and either:

Preservation of infected individuals for further histological analysis (consult with your CRT and your Participating Laboratory to confirm necessity).

Disposal of infected individuals using strict biosecurity protocols.

If there are reasons to maintain the animals, eradication of *Bsal* may be possible and has been demonstrated in published literature (Blooi et al. 2015a,b). [There may be reasons to maintain and treat animals, e.g., with threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.]

Treat per guidance in Blooi et al. (2015a,b). [As new treatments and research are being investigated, we will update this template. **Please note:** The methods tested to date have only been confirmed in Fire Salamanders (*Salamandra salamandra*); keep in mind that species differences may come into play with respect to treatment validity and effectiveness, which is why multiple swabs for PCR testing over time are necessary to confirm eradication.]

Swab treated animals post-treatment (see Appendix I, Section B) and submit repeat samples to a Participating Laboratory to confirm *Bsal* eradication.

Repeat treatment regime(s) and post-treatment swabbing until confirmation of *Bsal* eradication.

* 1. Disinfection, per Pessier and Mendelson (2017), of:
		1. All caging/housing materials and equipment prior to reuse.
		2. All water prior to disposal.
		3. All plants, soils, or other organic materials prior to disposal.
	2. Captive population monitoring. Evaluate the exposure of other co-located amphibians, including:
		1. Determine other places *Bsal* could be in the facility and disinfect those areas.
		2. Assess other potential sources of spread or origin of the pathogen, including through shared water sources, and quarantine or disinfect these sources.
		3. Assess the entire population to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
	3. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).

At minimum, swab amphibians for PCR analysis throughout the chain of custody.

Consider additional monitoring, as in 5(c) above.

1. **Document *Bsal* treatment**. Prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying *Bsal* treatment and eradication for *each individual animal* that tested positive for *Bsal* and was treated and for which *Bsal* was shown to be eradicated. [Entities customizing this template should keep in mind that each state may or may not have specific laws regarding “official” health certifications or alternative options; it is important to consult your state fish and wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.]
2. **Additional management guidance via CRT**
3. Messaging considerations.
	* 1. CRT will advise on and assist in development of preliminary detection messaging for the Reporting Individual(s) or the agency/entity with management jurisdiction over the site of detection to disseminate information.
4. Movement restrictions, voluntary or mandatory, implemented by landowner/manager, captive population owner, or agency with jurisdiction over the captive animals, to reduce further transmission (e.g., prohibitions on collecting salamanders from the wild site; temporary moratorium on movement or sale of salamanders from the captive facility until further information is known). [Entities customizing this template may consider including additional guidance for tracking animals that were documented to be infected and then treated, including reporting or other requirements upon relocation to new jurisdictions.]
5. **Subsequent communications**:
	1. If the *Bsal* Technical Advisory Committee has not been engaged in prior steps, consider contacting them regarding the findings and actions (response@salamanderfungus.org).
	2. Internal communications as required by the Reporting Individual’s agency/organization.
	3. Internal communications within the agency or entity with management jurisdiction of the detection site as management decisions are made, on a need-to-know basis.
	4. Local stakeholder and chain-of-contact/custody outreach.
	5. No further communications until detection status is definitive. [Limiting communications to a “need to know” group of people until confirmations of *Bsal* (or other pathogen) detection is received, may help to avoid unnecessary attention or public reaction.]

**Scenario 4: Definitive detection, Wild**

**This scenario is defined as:** Evidence of *both* 1) the **presence of *Bsal*,** as determined by the Participating Laboratory through either PCR-testing or isolation of a *Bsal* fungal culture as identified with genetic sequencing *and* 2) **histopathologic lesions** that confirm *Bsal* infection as the cause of disease or mortality.

**IMPORTANT**

Evidence of Bsal presence without confirmation with a second diagnostic test or demonstration of histopathologic lesions is not enough to determine definitive detection of Bsal chytridiomycosis (see Iwanowicz et al. 2017). Interpretation of laboratory results should follow the case definition for Bsal chytridiomycosis (White et al. 2016) accepted by the Diagnostics Working Group of the Bsal Task Force.

**Actions recommended (one or more, as feasible):**

1. Results communicated by Participating Laboratory to:
	1. Reporting Individual(s), who in turn informs:
		1. Detection site landowner/manager
		2. Wildlife management agency with jurisdiction over species and/or land
2. Agency or entity with management authority forms and convenes the CRT.
	1. Consider also engaging the *Bsal* Task Force Technical Advisory Committee leadership (response@salamanderfungus.org), who are available to assist by advising on resources and responses and will keep the information confidential.
3. Subsequent communications (in order of priority):
4. Internal communication as required by the Reporting Individual’s agency/organization.
5. Notification to the *Bsal* Task Force Technical Advisory Committee leadership (response@salamanderfungus.org), if they have not yet been informed.
6. Formal stakeholder notifications (e.g., partner institutions or agencies).
7. Public announcement/press release as appropriate.
8. Local stakeholder outreach (e.g., public groups who use the affected sites and could be asked to disinfect gear and report observations of dead amphibians).
9. Statement in scientific publication outlet.
10. Entry into *Bsal* reporting database.
11. Emergency meeting convened among parties identified in 2a and possibly 3a–b above to discuss:
	1. Risk/threat assessment. [Some areas to assess for potential risk include species movements, people’s activities, water movements, etc. and risk level to co-occurring species.]
	2. Management actions and considerations:
		1. Containment of mortality/detection site:
			1. Landowner/manager restrictions on public access to site, except for approved personnel.
			2. Strict use of approved biosecurity protocols (Appendix I, Section A(3)) for all personnel, their gear, vehicles, etc. when exiting site.
				1. Establish dedicated equipment/gear, including nets, footwear, etc., for the site.
			3. Deployment of fencing or other containment measures to reduce or prevent spread by other wildlife.
			4. Demarcation of the affected area(s) to minimize or prevent trespass by personnel or public.
		2. Establishment of ex situ colony(-ies):
			1. Engage additional partners (Amphibian Ark, CAZA, AZA, American Association of Zoo Veterinarians, etc.) to assist.
			2. Initiate rescue/captive assurance populations:
				1. Based on conservation status (e.g., federally or state/provincially-listed).
				2. Based on proportion of local population affected and proportion of total population represented locally.
				3. As an attempt to salvage/save affected but treatable individuals.
		3. Priority surveillance:
			1. Detection site:
				1. Sampling of other amphibian species at the detection site, particularly any within those families shown to be susceptible in Martel et al. 2014 and Stegen et al. 2017 (or more recent publications, if available).
				2. Additional sampling of exposed amphibian species or substrates.
			2. Non-independent sites (e.g., potential transmission pathways of water bodies connected to the detection site by permanent or ephemeral water flow or watershed considerations as well as adjacent terrestrial areas).
			3. Adjacent waters or lands within natural movement distances of the affected species.
			4. Nearby sites that may serve as refugia for translocating uninfected salamanders.
		4. Movement restrictions and prohibitions on collections of wild salamanders from the affected site.
		5. Other interventions as feasible, e.g., antifungal treatments for surviving animals, as described by Blooi et al. (2015b), or possibly habitat treatments or disinfection. [As new information becomes available on pending research and mitigation strategies, we will update this template. Preliminary data show some habitat treatments may be effective in eradicating the related pathogen, *Batrachochytrium dendrobatidis* (*Bd*; Bosch et al. 2015). In the early stages of *Bsal detection* and rapid responses, the following treatments may be the best options to attempt containment and local eradication at site-level habitat:
12. Culling/euthanasia
13. Bleaching site
14. Draining
15. Site closures (including physical barriers)
16. Signage or additional staffing to address desired visitor behavior modifications]

**Questions**

Whom might you contact for each of the above possible actions? Is there an “expert team” you could develop and have on call for the different actions above? The *Bsal* Task Force can assist in identifying a few national contacts, and perhaps also some local contacts, as a start.

What local, state or provincial/territorial, or federal resources are there to accomplish the actions above (e.g., laboratories, chemical application, or water draining equipment)?

What local, state or provincial/territorial, and federal laws may apply for environmental compliance? Do agency or local law enforcement contacts need to be informed or engaged?

**Scenario 5: Definitive detection, Captive**

**This scenario is defined as**: Evidence of *both* 1) the **presence of *Bsal*,** as determined by the Participating Laboratory through either PCR-testing or isolation of a *Bsal* fungal culture as identified with genetic sequencing; *and* 2) **histopathologic lesions** that confirm *Bsal* infection as the cause of disease or mortality. [Evidence of *Bsal* presence without confirmation with a second diagnostic test or demonstration of histopathologic lesions is not enough to determine definitive detection of *Bsal* chytridiomycosis (see Iwanowicz et al. 2017). Interpretation of laboratory results should follow the case definition for *Bsal* chytridiomycosis (White et al. 2016) accepted by the Diagnostics Working Group of the *Bsal* Task Force.]

**Actions recommended (one or more, as feasible):**

1. Results communicated by Participating Laboratory to:
	1. Reporting Individual(s), who in turn informs:
		1. Captive animal owner/captive facility manager or veterinarian
		2. State or provincial/territorial agency(-ies) with jurisdiction over captive animal health and movement (e.g., wildlife management agency or state/provincial/territorial department of agriculture)
2. Agency or entity with management authority forms and convenes the CRT.
3. Consider also engaging the *Bsal* Task Force Technical Advisory Committee leadership (response@salamanderfungus.org), who are available to assist by advising on resources and responses, and will keep the information confidential.
4. Subsequent communications (in order of priority):
	1. Internal reports within agency/organization (if applicable).
	2. Notifications to pet store, or importer, or zoological institution where animals were acquired.
	3. Notifications to chain-of-contact/custody stakeholders (i.e., individuals or entities throughout the history of possession of the affected amphibians and other associated individuals or entities).
	4. Formal stakeholder notifications (per CRT guidance).
		1. State veterinary health official.
		2. AZA Taxonomic Advisory Group or Species Survival Plan contacts.
	5. Statement via scientific publication outlet.
	6. Entry into *Bsal* reporting database.
	7. Public announcement/press release as appropriate (and in collaboration with captive animal/facility owner).
5. Emergency meeting convened among parties identified in 2 and possibly 3(a–c) above to discuss:
	1. Risk/threat assessment.
	2. Management actions.
		1. Containment.
			1. Ensure no running water out of the animals’ housing area.
			2. Eradicate *Bsal* sources.
				1. For live, captive animals whose samples return a positive *Bsal* result, eradication may be attempted:

For fail-safe eradication, we recommend humane culling or euthanasia and disposal of infected individuals using strict biosecurity protocols. See Section 8.6 in Pessier and Mendelson (2017) or AVMA Guidelines for the Euthanasia of Animals ([AVMA 2020](https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf)).

If there are reasons to maintain the animals, eradication of *Bsal* may be possible and has been demonstrated in published literature (Blooi et al. 2015a,b). [There may be reasons to maintain and treat animals, e.g., threatened or endangered species. However, there may also be reasons to maintain infected animals, e.g., for additional diagnostics or research. Consult with the CRT and your Participating Laboratory to determine options.] In such instances, we suggest the following:

Treat per guidance in Blooi et al. (2015a,b). As new treatments and research are being investigated, we will update this template. **Please note:** The methods tested to date only are confirmed in Fire Salamanders (*Salamandra salamandra*); keep in mind that species differences may come into play with respect to treatment validity and effectiveness, which is why multiple swabs over time are necessary to confirm eradication.

Swab treated animals post-treatment (see Appendix I, Section B), and submit samples to a Participating Laboratory to confirm *Bsal* eradication.

Repeat treatment regime(s) and post-treatment swabbing until confirmation of *Bsal* eradication.

* + 1. Quarantine. Isolate any potentially affected individual animals, including any that were housed nearby or co-located with affected individuals.
			1. Perform additional diagnostics on quarantined, co-located individuals.
			2. Employ strict use of biosecurity protocols (see Pessier and Mendelson 2017) for all people/personnel handling the affected species, particularly prior to exiting the quarantine area.
		2. Disinfection, per Pessier and Mendelson (2017):
1. All caging/housing materials and equipment prior to reuse.
2. All water prior to disposal.
3. All plants, soils, or other organic materials prior to disposal.
	* 1. Captive population monitoring. Evaluate the exposure to other co-located amphibians, including:
			1. Determine other places *Bsal* could be in the facility, and disinfect these areas.
			2. Assess other potential sources of spread or origin of the pathogen, including through shared water sources, and quarantine or disinfect these sources.
			3. Assessment across the captive population to determine whether it is clinically stable or if there is a trend of increasing morbidity and mortality.
			4. Evaluate other sources of infection, including new acquisitions.
		2. Reporting, and additional testing, throughout the chain of custody (i.e., individuals or entities throughout the history of possession of the animals or lot in which the affected amphibians originated).
			1. At minimum, swab amphibians for PCR analysis in either direction throughout the chain of custody.
			2. Consider additional monitoring, as in 4(b)(iv) above.
		3. Voluntary surveillance of affected populations.
			1. Additional sampling of affected species and captive environment (plants and other substrates).
			2. Sampling of all other amphibian species in the facility.
			3. Sampling of stock of original importer or zoological collection
				1. Exposed animals
				2. Other co-located animals
			4. Sampling throughout the chain-of-contact/custody of exposed individual animals.
		4. Voluntary movement restrictions/prohibitions of movement or sale of affected species.
			1. Place a temporary moratorium on the sale or movement of all salamanders from the same zoological collection, captive breeder, pet supplier, or importer. [The entity customizing this document can determine whether to qualify this action as “mandatory” or “required” or use another descriptor. When there is a definitive detection of Bsal, we suggest the strongest possible measures to reduce risk of spread and facilitate containment.]
			2. Document *Bsal* treatment. If animals are treated prior to resumption of transport or sale (in accordance with existing federal, state, or local laws), consider obtaining a health certificate or other documentation from a member of one of the Veterinary Expert Networks verifying *Bsal* treatment and eradication for *each individual animal* that tested positive for *Bsal* and was treated and for which *Bsal* was shown to be eradicated. [Entities customizing this template should keep in mind that each state may or may not have specific laws regarding “official” health certifications or alternative options; it is important to consult your state fish and wildlife agency and state department of agriculture regarding either the recommendation being an official or unofficial form of documentation.]

**Appendix 4.1**

**Protocols and procedures for sampling from mortality events,**

**and for sampling from living animals, for diagnostic testing**

Text adapted, with permission, from:

Pessier, A.P., and J.R. Mendelson III (Eds.). 2017. A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs, version 2.0. IUCN/Species Survival Commisstion (SSC) Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA. (Available from: <https://www.cpsg.org/disease-manual-amphibians-update-2017>)

**[NOTE: This appendix will be updated to reflect any new information as it becomes available.]**

**Tissue collection during mortality events.** Mortality events where multiple animals are found dying or dead are observed in amphibian survival assurance colonies as well as wild amphibian populations. Although well‐known infectious diseases of amphibians (e.g., chytridiomycosis or *Ranavirus* infection) may be strongly suspected, it is important to keep an open mind and always consider other potential causes. Many different disease conditions can initially look very similar and require laboratory investigation to achieve a definitive diagnosis.

The initial goal of investigating mortality events is to collect and preserve representative samples that can be used for the different types of laboratory techniques that may be needed. It is always advisable to contact the lab where you intend to send samples and discuss with them their preference on how to prepare and ship the animals. [If possible, well in advance of a mortality event, consider contacting your nearest diagnostic laboratory to find out their preferences for preparing and shipping animals or samples in various scenarios of a mortality event.] Complex protocols can be designed for sample collection during mortality events—especially if veterinary guidance is available. However, a simple and basic approach is also sufficient for most situations.

* If wildlife health expert guidance is not available or if animals are small:
	+ Perform the carcass-fixation necropsy method (see Chapter 9 in Pessier and Mendelson 2017) on one‐half to two‐thirds of the dead animals.
	+ For the remaining animals, freeze the carcasses whole as soon as possible and label with the species name, individual identification number, and date.
		- For freezing of entire carcasses or individual tissue samples, ultracold temperatures (–70°C or below) or liquid nitrogen are preferable. However, regular household freezer temperatures (–20°C) are sufficient for short‐term storage.
		- As a last resort, if a freezer or liquid nitrogen is unavailable, fixation of carcasses or tissue samples in 70% ethanol (instead of formalin) may still allow application of some molecular diagnostic techniques.
* If wildlife health expert guidance or an individual experienced with amphibian anatomy is available, perform the dissection necropsy method (see Pessier and Mendelson 2017) on the dead animals.
* In addition to saving samples from all major organs in fixative solution for histopathology, freeze additional samples of individual organs.
	+ Suggested samples for freezing include skin, liver, kidney, lung, intestine, brain, and any tissue thought to be abnormal during dissection (e.g., enlarged or discolored organs or organ nodules). In addition, stomach contents, coelomic fat bodies, and skeletal muscle can also be saved, especially if exposure to a toxic substance is a possibility.
	+ Organ samples are saved in sterile Whirl‐Pak® style bags (Nasco, USA, [www.enasco.com](file:///C%3A%5CUsers%5Cmjfg-aire%5CDownloads%5Cwww.enasco.com)) or cryovials such as Nunc CryoTubesTM or Vangard CryosTM (Sumitomo Bakelite Co., Ltd., Japan, [www.sumibe.co.jp/english/](http://www.sumibe.co.jp/english/)).
	+ Containers should be labeled with the species name, individual animal ID number, specimen type, date, and county and state where collected.
* If moribund (dying) animals are found, consideration should be given to humanely euthanizing some of these individuals for necropsy and sample collection (see Section 8.6 in Pessier and Mendelson 2017). This approach provides very fresh samples that are ideal for most laboratory methods used for disease investigation.
	+ - 1. **Basic tissue sample collection protocol for amphibian mortality events** (wildlife health expert not available or in a field situation with limited equipment).
* For half of the dead animals, make an incision into the coelomic cavity and expose the internal organs.
	+ For very small animals or if a knife is not available, just fix the carcasses intact.
	+ Place the opened carcass into a fixative solution, such as 10% neutral buffered formalin (preferred) or 70% ethanol. The ideal ratio is one part animal carcass to nine parts fixative solution.
* For the other half of the dead animals, freeze the carcasses whole or keep them cool (such as in a portable ice‐chest) until they can be transported to a location where freezing is possible.
	+ It is always better to save both fixed (formalin or ethanol) and frozen samples. If this is not possible, preference should be given to saving tissues fixed in formalin or ethanol.
	+ Saving only frozen samples should be a last resort (but is better than no samples at all).
		- If freezing of samples is not possible, fixation in ethanol may allow for both histopathology as well as some molecular diagnostic tests (e.g., PCR)
			1. **Shipment of samples** (shipment of tissues that have been preserved in a fixative solution). Once carcasses or tissues have been in formalin or another fixative solution for a minimum of 48 hours, remove from fixative, wrap in paper towels or gauze moistened with fixative, pack into sealed plastic bags, and ship to a pathologist. This approach minimizes the potential for leakage during shipment and reduces package weight (and shipment costs).
* Materials should be shipped in a manner that follows International Air Transport Association (IATA) regulations for Dangerous/Hazardous Materials (see also <https://www.gpo.gov/fdsys/pkg/FR-2011-07-20/pdf/2011-17687.pdf>). Some general guidelines include:
	+ Samples should be enclosed in a primary receptacle that is leak‐proof.
	+ The primary receptacle is then placed within a leak‐proof secondary receptacle.
	+ An absorbent material (e.g., paper towels) should be placed between the primary and secondary receptacles. The volume of material should be sufficient to absorb all of the fluid within the primary receptacle.
* Major shipping companies have guidelines available to help with proper shipping of biological samples. More information available here: <http://images.fedex.com/downloads/shared/packagingtips/pointers>
	+ - 1. **Disinfection and biosecurity in the field.** Concerns about the possibility of moving amphibian pathogens to new locations as the result of field research conducted on wild amphibians have led to a number of protocols for reduction of this risk (e.g., <http://northeastparc.org/disinfection-protocol/>). There are variations and sometimes contradictions between the different protocols; however, the basic principles of biosecurity for biologists working on wild amphibian populations are similar. Peer‐reviewed publications including the addition of risk calculators to assist the biologist in making good biosecurity decisions have recently become available (St‐Hilaire et al. 2009; Phillott et al. 2010). A summary of recommended field practices includes:
* **Definition of the field site.** The first precaution against the possible spread of disease among amphibian populations is careful definition of the field site or sites. Researchers should use natural and man‐made boundaries to help define the sites. Whenever possible, plans should be made ahead of time to work in only one site per outing or have different groups working at each individual site to avoid cross‐contamination (and transmission of disease) between sites.
* **On‐site hygiene and biosecurity of equipment.** The use of disposable equipment discarded after use at a single site or on a single individual amphibian reduces the risk of spreading disease. All reusable equipment, including footwear, should be disinfected between sites or dedicated to a single site (e.g., a single pair of rubber boots is purchased for each field site and used ONLY at that site). Consult the table in Section 5.10 of Pessier and Mendelson (2017) for details on the use of specific disinfectants, including recommended concentrations and contact times.
	+ Footwear and other reusable equipment should be made of materials that are easy to clean and disinfect (e.g., rubber boots are better than leather hiking boots).
	+ Thorough cleaning of equipment is essential for removal of dirt and organic material prior to disinfection in the field. As noted in other sections, organic material inactivates many disinfectants. Scrub brushes and other implements to remove dirt should be part of the field equipment. If disinfectant solutions become contaminated with organic material or dirt, they should be changed.
	+ The quaternary ammonium compounds (see Section 5.2 in Pessier and Mendelson 2017) have been recommended for field situations because they are concentrated and easy to transport into field situations (Johnson et al. 2003, Webb et al. 2007).
	+ If disinfection is undertaken in the field, consideration should be given to the toxicity of chemicals to the environment. The quaternary ammonium compounds and Virkon® (see Section 5.2 of Pessier and Mendelson 2017) are more environmentally friendly options compared to chlorine bleach (Johnson et al. 2003, Webb et al. 2007, von Rütte et al. 2009). If ranaviruses are a special concern, Virkon® may have some advantages over the quaternary ammonium compounds (Bryan et al. 2009). Powdered bleach is another easily portable suggestion.
	+ Vehicles are less likely to be a vector for the transmission of disease than are footwear and field equipment but still should be disinfected, especially if used to cross or enter a known contaminated site. The wheels and tires should be cleaned of all dirt and organic material and disinfected prior to leaving the site by using the same disinfectant that was used on footwear. Always remember to disinfect footwear before getting into a vehicle to prevent pathogens from transferring to the floor or pedals.
* **Handling and collection of samples from amphibians.** When handling amphibians in the field, even within the same site, precautions should be taken to minimize the risk of transmitting pathogens between individual animals.
	+ Non‐powdered disposable gloves are the best choice when handling amphibians. Powdered gloves should be rinsed free of powder. A new pair of gloves should be used for each animal. If gloves are unavailable, it is slightly preferable to use bare hands, and wash hands between handling different animals (Mendez et al. 2008).
	+ The greatest risk for spreading disease when handling amphibians occurs when animals are placed together in the same container or when containers are reused without being disinfected. Do not reuse collecting bags—utilize a new one for each animal.
	+ Always handle animals as little as possible. Procedures that are quick, even if potentially painful, may cause less stress than longer procedures.
	+ Animals should only be released at the site of capture, and any sick or dead amphibians found should be preserved in 10% buffered formalin solution and submitted for disease diagnosis (see Chapter 9, Necropsy, in Pessier and Mendelson 2017).
	+ Instruments used for sample collection should be disinfected between use on different animals. For surgical instruments (e.g., scissors) and weighing equipment, 70% ethanol is rapidly acting against the amphibian chytrid fungus (Johnson et al. 2003).
	+ Although mentioned in some amphibian handling protocols, the use of iodine-based compounds for sanitizing the animal’s skin prior to procedures such as toe‐clipping or microchip implantation is not recommended because of toxicity concerns. Potential substitutes include 0.75% chlorhexidine or 2 mg/L benzalkonium chloride (Wright 2001).

**Sample collection for *Bsal* PCR.** [As of this version, some of the sample collection options for *Bsal* have not yet been documented; this information is provided based on techniques used for *Batrachochytium dendrobatidis* (*Bd*) and will be updated as new information becomes available.] Based on what is known for *Bd*, the PCR procedure can be performed using a variety of different sampling methods including skin swabs, water bath, and tissue samples (e.g., toe clip; Hyatt et al. 2007).

* Skin swabs. The skin swab procedure is simple, minimally invasive, and it samples multiple areas of the skin that may be infected with *Bsal* (increasing the likelihood that infected areas will be sampled). Skin swabs generally are the preferred sampling method for *Bsal* PCR.
* Water bath. Samples using the water bath procedure require immediate centrifugation or micropore filtration and are not practical in many settings.
* Tissue samples. Toe clipping is an invasive procedure with associated ethical concerns and has the disadvantage of sampling only a small portion of potentially infected skin.
	+ - 1. **Materials needed for skin swabbing.** The materials listed below are general guidelines needed to perform the skin swab procedure for *Bsal* PCR using realtime or quantitative PCR (qPCR) methods. There may be differences depending on the preferences of the laboratory processing the samples and the environmental conditions under which the swabs are obtained.
* Powder‐free latex or nitrile disposable gloves.
* Sterile applicators (“swabs”); see “Swab Selection” in Pessier and Mendelson (2017).
* 1.5 ml microcentrifuge tubes/cryovials.

Storage of dry swabs at controlled room temperature/refrigeration or freezing is preferred, but 70% ethanol is an alternative, especially if samples will be exposed to variable climate conditions, such as heat. Individual laboratories may have preferences about sample storage conditions; be sure to check in advance with the Participating Laboratory to which samples will be sent. For additional information, see the section on “Storage of Skin Swab Samples” below.

* + - 1. **Swabbing procedure 101.** Several videos demonstrating swabbing and associated biosecurity and prevention of contamination have been developed: <https://amphibiaweb.org/chytrid/swab_protocol.html>
			2. **Avoiding cross‐contamination of samples.** The PCR assays are very sensitive tests and can detect very small amounts of *Bsal* DNA. This sensitivity is good for detecting animals that have very low‐level infections with *Bsal*, but it increases the risk that samples from a non‐*Bsal* infected animal can have false-positive results if they become contaminated with even small amounts of *Bsal* DNA from an infected animal. Therefore, it is very important to take precautions to avoid sample cross‐contamination. These precautions include:
* Use a new pair of disposable latex or nitrile gloves for each animal handled for testing (Mendez et al. 2008).
* Avoid contact of swabs (especially swab tips) with surfaces or substrates other than the skin of the animal to be tested.
* If instruments are used to cut the tip of the swab into cryovials, use a freshly disinfected instrument for each sample.
	+ To disinfect instruments for this purpose, dip in 70% ethanol followed by flaming under an alcohol lamp.
	+ Avoid using bleach solutions for disinfection because doing so can degrade *Bsal* DNA in swab samples (resulting in false‐negative tests; Cashins et al. 2008).
		- 1. **Avoiding PCR inhibitors in samples.** Foreign material, such as dirt or plant matter, can contain materials that inhibit the PCR reaction, which can result in a false‐negative test result (i.e., animal is infected with *Bsal*, but it is not detected by the PCR test).
* Prior to skin swabbing, efforts should be made to manually remove heavy skin contamination. Animals may be gently rinsed with clean water prior to sampling, but vigorous washing should be avoided because of the potential to also rinse off *Bsal*-infected skin cells or organisms.
* If rinsing is necessary, it is best if the water does not originate from the animal’s enclosure or environment.
* Laboratories that perform PCR for *Bsal* should always use exogenous internal positive controls to detect PCR inhibitors (Hyatt et al. 2007).
	+ - 1. **Storage of skin swab samples.** Storage of swabs after sample collection is an important consideration. Swabs can be stored air-dried or in 70% ethanol. Be sure to check in advance with the Participating Laboratory to which samples will be sent; individual laboratories may have preferences about sample storage conditions.

For air-dried swabs, the major concern is high temperature extremes. DNA on air-dried skin swabs has been experimentally proved to be remarkably stable. Hyatt et al. (2007) demonstrated that PCR sensitivity was unaffected by storage of skin swabs for up to 18 months at room temperature (23°C). However, exposure of swabs to high temperatures (>38°C) for as little as seven days can result in decreased recovery of pathogen DNA, thus increasing the possibility of false-negative results in animals with low-level *Bsal* infections (Van Sluys et al. 2008). Therefore, it is recommended that air‐dried skin swab samples be stored at the lowest temperature possible (Skerratt et al. 2008). The following are general guidelines:

* Store samples at 25°C (refrigerator) or lower.
* Samples should be frozen (–20°C or below) if sample analysis is not performed within six months of sample collection.
* See alternatives to low temperature storage (i.e., where refrigeration may not be possible) in Pessier and Mendelson (2017).
	+ - 1. **Shipment of swabs to the laboratory.**
* Ideally, ship swabs by overnight or 2‐day courier service (e.g., Federal Express; Canada Post Xpresspost, UPS, Purolator, etc.).
* Consider using cold packs to guard against high temperature extremes.
* Samples that have been previously frozen should be sent on dry ice to prevent freeze–thaw cycles.