Development of a mAb library targeting Batrachochytrium dendrobatidis antigens for the field diagnosis of chytridiomycosis.


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**ABSTRACT**

Fungal diseases are a growing threat to the health of global ecosystems. The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) causes chytridiomycosis, a catastrophic disease of amphibians (frogs, salamanders and caecilians) responsible for the greatest disease-driven loss of biodiversity ever documented. Rapid detection and biosecurity are essential to prevent further extinctions as the global spread of a hyper-virulent lineage of Bd is linked to the international trade in amphibians. The World Organisation for Animal Health has listed Bd as an internationally notifiable disease and yet current diagnostic methods for monitoring occurrence and spread of the fungus are time-consuming and cannot be carried out in the field. DNA-based techniques such as PCR are the most effective detection methods at present, but require transportation of potentially infectious material to diagnostic laboratories manned by skilled scientists and equipped with costly and sophisticated equipment. While results are potentially available in a matter of hours, they typically take several days or even weeks to appear by which time diseased amphibians have been transported from sites of infection. Consequently, there is a pressing need for the development of a more rapid method to diagnose chytridiomycosis. One such method is the lateral-flow antigen capture assay (LFA), a rapid (10 min), cheap, point-of-care test (POCT) that is used in home pregnancy kits, for example. Lateral-flow assays (LFAs) present an opportunity for a more rapid spread of the disease to fungus-free populations.

**METHODS**

**Immunisation of mice and production of anti-Bd mAbs.** BALB/c mice were immunised with lyophilised whole Bd lysate. Hybridoma cell lines specific for Bd were identified via ELISA and grown in T175 culture flasks until supernatant was fully saturated with mAbs.

**ELISA analysis.** mAbs were serial diluted in microtiter plates that were coated overnight with lyophilised Bd or related fungi. Bound mAbs were then detected using horseradish peroxidase (HRP) labelled Goat-anti-mouse antibodies, followed by a tetramethylbenzidine substrate.

**Western blot analysis.** Whole Bd lyse was transferred to a nitrocellulose membrane and probed with mAbs. Bound mAbs were then detected using alkaline phosphatase labeled goat-anti-mouse IgM, followed by a colorimetric substrate.

**INTRODUCTION**

*Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrinovans* (Bs) are important fungal pathogens of amphibians. Only discovered in 1997, Bd is an emerging infectious agent that has been implicated in the extinction of over 200 amphibian species worldwide. Infection causes chytridiomycosis, a panzootic disease that leads to morbidity and mortality by disrupting the skin’s osmoregulatory function and electrolyte balance. Infected populations can crash in just a few short months, and in remote locales, decline often goes unnoticed until it is too late.

Bd is thought to have originated in Asia or Africa, where it benignly cohabits with multiple amphibian species. During the last 100 years, a hyper-virulent strain of Bd has been disseminating across the globe and is now found on all continents. This rapid spread has been linked to the international trade of the African clawed frog, *Xenopus laevis*. As such, the World Organisation for Animal Health (OIE) has listed Bd as a notifiable disease that must be monitored and controlled so to limit its spread and prevent additional extinction events. Despite this, there is still no easy way to diagnose chytridiomycosis in real-time. The current diagnostic gold standard is a skin swab combined with PCR. While highly sensitive, PCR cannot be done on-site and requires skilled scientists working with expensive equipment. This method is highly technical, costly, and has limited uses in containing the spread of disease.

Lateral-flow antigen capture assays (LFAs) present an opportunity for a more rapid and inexpensive diagnostic by capitalising on the high specificity and sensitivity of monoclonal antibodies (mAbs). LFAs function in a similar manner to enzyme immunoassays, where an antigen is captured between two antibodies: one immobilised and one conjugated to a particulate label. These assays are simple to perform and are often done by individuals with no prior experience (e.g. home pregnancy kits). They can be completed on-site and results can be had in as little as 10 minutes. LFAs have been developed for several fungal diseases of humans, including cryptococcal meningitis and invasive aspergillosis. The goal of the present study is to develop a library of monoclonal antibodies that can identify Bd, for implementation into an LFA for the rapid, on-site diagnosis of chytridiomycosis. This will allow for more efficient reporting and better containment of the disease.