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## A Llama Can Tell

Nancy McGuire

In the ongoing effort to improve biohazard detection, the newest recruits are big and woolly, and they have been known to spit. Luckily (for them and us), only their antibodies need to report to the suspected biohazard area. Researchers from the Naval Research Laboratory and the Southwest Foundation for Biomedical Research (SFBR, San Antonio, TX) found that the disease-detecting antibodies that a llama produces naturally in its bloodstream are ideally suited for building versatile and stable biological detectors.



**Llama**

Photo courtesy of USDA.

Antibodies, an important part of the immune system, recognize and react to substances, called "antigens," that are foreign to the body. Antibodies have "binding sites" into which a specific antigen fits like a key fits a lock. Over the course of a lifetime, an animal builds up a collection of antibodies, each one customized to recognize a specific antigen. Animal-derived antibodies have been incorporated into commercially available test kits that detect a wide variety of biological substances using a method known as "immunoassay."

To test for several antigens at once, an assortment of antibodies can be deposited onto panels. Immunoassay panels made from llama antibodies are effective at detecting and identifying several types of viruses and chemical toxins, including some of the most virulent killers around. The antibodies in these panels may be re-used many times, in contrast to conventional one-use immunoassay tests.

Ellen Goldman (NRL) and her colleagues used the messenger RNA antibody coding sequences extracted from llama blood as the starting point for an antibody "library." Why llamas? Along with their cousins the camels, the molecular structures of llama antibody proteins contain single-domain antibodies (sdAbs), the smallest known fragments capable of binding antigens. Sharks have similar antibodies, but have you ever tried drawing blood from a shark?

Llama sdAbs can bind antigens at temperatures in excess of 90 °C (just below where water starts to boil), in contrast with existing environmental diagnostic assays, which are useless after they are heated above 60–70 °C. Thus, assays based on sdAbs can be stored without refrigeration and remain stable for long periods of time. In addition, assays based on sdAbs can be regenerated and used over and over because the proteins re-fold themselves after being denatured.

The sdAbs isolated from llama blood can be selected in the laboratory to recognize a variety of specific antigens. Thus, there is no need to inject a healthy animal with harmful substances. For toxins or viruses that kill their host too quickly for the immune system to react, generating antibodies in the lab is the only workable solution.

Goldman's group selected various sdAbs in their library to recognize cholera toxin, ricin (a potent toxin derived from castor beans), and staphylococcal enterotoxin B (what makes you sick when you have food poisoning). In Andrew Hayhurst's laboratory at SFBR, virus-binding sdAbs were selected for live vaccinia virus (a

stand-in for smallpox virus). The researchers are continuing to test their methods for finding binders for SARS coronavirus and the Marburg hemorrhagic fever virus.

The researchers were able to select and isolate their specific antibodies using inexpensive and portable techniques, and the process took much less time than conventional antibody production methods. They tested their antibodies using the toxin of interest and compared the response to that for an irrelevant substance such as ovalbumin. In many cases, the signal for the virus or toxin of interest was over 100 times stronger than the signal for the irrelevant substance. The researchers tested various methods and substrates to see which conditions produced the clearest response and the fewest errors.

Goldman's group is confident that sdAbs "are likely to be high-performance yet low-maintenance substitutes for any antibody-based biosensor."

**Reference:**

Goldman, Ellen R., et al. *Analytical Chemistry* 2006; 78(24), 8245–8255.

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