Coffee, black, decaf and a little llama on the side

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Three llamas and two camels have provided a way to tell whether your waiter swapped regular coffee for decaf in your after-dinner cup. Using the heat-resistant antibodies these camels and llamas make, researchers at Washington University School of Medicine in St. Louis are developing a quick test for caffeine that works even with hot beverages.

The researchers plan to adapt their technology to a simple test ("dipstick") that can be used to check for caffeine in a variety of drinks. Their research will appear in the June 1 issue of the American Chemical Society's journal *Analytical Chemistry*.

Caffeine can cause restlessness, irritability, dehydration or heart arrythmias, and those who are highly sensitive to caffeine can feel its stimulant effects for as long as 20 hours. In addition, some medicines adversely interact with caffeine.

"We believe our test would be the first consumer test for caffeine and would be beneficial for anyone wishing to avoid caffeine for health or personal reasons," says senior author Jack H. Ladenson, Ph.D., the Oree M. Carroll and Lillian B. Ladenson Professor of Clinical Chemistry and director of the Division of Laboratory Medicine.

Interestingly, the key to the caffeine test comes from llamas and camels -- pack animals that have transported caffeinated commodities such as coffee, tea and cocoa for centuries. These camelids happen to be among the few creatures whose immune systems can produce antibodies that

aren't destroyed at the high temperatures common to brewed beverages.

The researchers reasoned that if they could create heat-resistant camelid antibodies that reacted to caffeine, they could potentially build a durable assay suitable for use almost anywhere. They gave intramuscular injections of a caffeine-linked protein to three llamas and two camels to elicit an immune response to caffeine. They found that blood from the animals contained antibodies that were heat-stable and reactive to caffeine.

The most stable version of the caffeine-specific antibody, which came from a llama named Very Senorita, recovered 90 percent of its activity after exposure to 194 degrees Fahrenheit (90 degrees centigrade) -- about the temperature of a really hot cup of coffee. A similar antibody produced from mice broke down at 158 degrees Fahrenheit.

A lab test using the caffeine-specific antibody accurately measured the amount of caffeine in coffee and cola drinks. The antibody cross-reacted very little with theophylline or theobromine, the caffeine-like compounds in teas, so the caffeine content of teas could be measured without interference from these substances.

"Now that we've isolated the sequence of this stable anti-caffeine antibody, we can produce copies in the lab to develop a convenient caffeine test--we don't need to rely on the animals," Ladenson says. "And unlike other methods for measuring caffeine, which require large and expensive laboratory equipment, this test is potentially adaptable to a format that people could carry with them,"

An eight-ounce cup of regular coffee has about 80 to 280 milligrams of caffeine, and a similar-sized cup of black tea can vary from 60 to 100 milligrams, depending on brewing time. Cola drinks, both diet and regular, typically contain between 30 to 50 milligrams of caffeine in

each 12-ounce can, while high-caffeine sodas can have as much as 80 milligrams per can. In comparison, decaffeinated coffee generally has only about 5 milligrams of caffeine.

The caffeine test has shown accuracy that compares well with measurements made with sophisticated equipment. Ladenson and his colleagues are currently working to develop a portable, point-of-consumption test.

Ladenson's laboratory is well-known for having developed the first practical blood test for troponin I, a protein that is released into the blood after a heart attack. This test, along with a test for a form of the enzyme creatine kinase that was also developed in Ladenson's lab, is widely used to determine whether patients with certain symptoms have had a heart attack.

Source: Washington University School of Medicine