



# AMATOXtest

Rapid detection of the deadliest mushroom toxin

## WARNING

This test is NOT for determining whether a mushroom (or animal or human) is toxic or intoxicated. It is also NOT a test for edibility.

## Storage and Stability

The AMATOXtest should be stored at room temperature (20°C/68°F- 25°C/77°F). Note the shelf life on the kit box. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated envelope until you are ready to use the strips.

## Materials Provided

1. Pouch containing desiccant and test cassettes.
2. Cassette with enclosed test strip
3. Pipette, disposable
4. Eppendorf tube with dried salt crystals enclosed

## Disposal of amatoxin-containing materials.

- Place all used materials (cassette, pipette, eppendorf tube directly in the trash).
- If this test is operated in a facility, follow your facility's safety procedures for disposal of samples and extracts potentially containing or known to contain amatoxins.

## GENERAL DESCRIPTION

The AMATOXtest lateral flow immunoassay (LFIA) is an immunoassay for the qualitative (yes/no) detection of amatoxins. Amatoxins are a group of toxins associated with mushroom poisoning from eating certain wild mushrooms. Some mushrooms that contain amatoxins are in the genera Amanita, Galerina, Lepiota, or Conocybe. Amatoxins are potent RNA polymerase II inhibitors. After ingestion, they can cause gastrointestinal upset and ultimately lead to liver damage and death. This test is suitable for the qualitative detection of amatoxins in mushroom as well as urine samples. For mushroom samples a sample preparation is required. If necessary, positive samples can be confirmed by HPLC, LC/MS, or other conventional methods.

## INTENDED USE

This AMATOXtest kit is a 10-minute LFIA designed for the qualitative determination of amatoxins in mushroom extracts as well as in urine samples. This kit is for research use only, not for therapeutic or diagnostic applications.

## LIMITATIONS OF THE AMATOXTEST LFIA, POSSIBLE TEST INTERFERENCES/SENSITIVITY AND CROSS-REACTIVITY

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded. Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, exposing the test strip to too much humidity for too long prior to sample addition, inadvertently touching the strip membrane with ungloved hands, inaccurately placing the sample not on the sample pad region, inappropriate sample material used, inaccurate sample volumes, too long or too short incubation times prior to reading, extreme temperatures during the test performance (lower than 10°C/50°F or higher than 30°C/86°F). The AMATOXtest provides screening results. As with any analytical technique positive samples requiring some action should be confirmed by an alternative method. This test detects alpha-amanitin and gamma-amanitin at 10 ppb (ng/mL) and beta-amanitin at 2000 ppb (ng/mL) in neutral pH aqueous solutions (The detection limit of beta-amanitin is improved to 200 ppb (ng/mL) in pH solutions around 5). This test is known to cross-react with phallotoxins (phalloidin and phalloidin) at 0.005% (200 ug/mL, ppm). The following mushroom toxins and other cyclic peptide toxins have been tested with this kit using the protocol specified herein with no false positive results. (Muscimol, Ibotenic acid, Psilocybin, Nodularin, Microcystin-LR)

## RESULTS

**Negative Results** – A sample containing amatoxins of less than 10 ppb (ng/mL) will develop 2 distinct lines in the test area. A negative test result can be interpreted as soon as a test line develops, generally within 2-3 minutes. **Positive Results** – The AMATOXtest for amatoxins is designed to screen for amatoxins at levels of approximately 10 ppb (ng/mL) or higher in mushrooms (fresh or dried) or in urine. A sample containing amatoxins of 10 ppb (ng/mL) or higher will develop 1 distinct line, the control line. The absence of a test line should be interpreted as positive for amatoxins. Allow the strip to develop for the full 10 minutes.

# HOW TO USE THE AMATOXtest: Testing a mushroom for amanitin

WEAR GLOVES! While you're unlikely to get sick from simply handling deadly amanitin-containing mushrooms, it's possible, especially if you handle them repeatedly. DISCARD the mushrooms after you've sampled them by collecting them in plastic bags and putting them in the trash. KEEP AWAY from pets, small children, or those who might mistake them for edible mushrooms. The AMATOXtest is **NOT** a test for edible mushrooms. A negative test DOES NOT MEAN THE MUSHROOM IS EDIBLE!

1. Tear off a small piece of the mushroom cap the size of a fingernail clipping (see **Figure 1, left**). Brush off any dirt or foreign material such as leaf or twig fragments.
2. Place this piece of mushroom sample in the provided Eppendorf tube (see **Figure 2, left**).
3. Add enough room temperature (20°C/68°F- 25°C/77°F) water to fill the tube a bit more than halfway.
4. Cap the tube tightly and shake for 30 seconds (see **Figure 3, left**). Proceed with the test as described below, using the mushroom extract instead of urine.

## Testing urine or mushroom extract for amanitin

1. Open the cassette pouch and lay a cassette on a flat surface. Seal the remaining cassettes in the pouch they came in (along with the desiccant pouches) until you are ready to use them.
2. Carefully open the tube containing the urine sample or mushroom extract. You have your gloves on, yes? Good. If you happen to get urine or extract on your skin, flush immediately with lots of water. This stuff could be poisonous. Be careful.
3. Using the provided disposable pipette, draw up some of the urine or mushroom extract from the tube. Avoid solid material such as bits of mushroom.
4. Drip three drops of urine or mushroom extract into the circular opening in the cassette. Don't put it in the rectangular part! You're wearing your gloves, yes? Be careful.
5. Watch the fluid soak into the strip through the rectangular window. Isn't it cool? Watch the video I made for you [\[insert link here\]](#), showing the parts of the kit, how to use them, and, most magically, the development of the positive (see Figure 4, below) and negative (see Figure 5, below) test results. It takes about 10 minutes.



**4. Positive test:** Deadly toxin present.  
Only one red line in window



**5. Negative test:** <10 ng/ml amanitin  
Two red lines in window

This is *amazing!* Amanitin, the toxin that is so deadly that eating just one ***Amanita phalloides*** (*Death Cap*) mushroom can kill you, is only 1 part in 10,000 by weight in a mushroom. This test can detect that deadly toxin in just a tiny piece of a mushroom. Thank Dr. Candace Bever for her hard work and dedication. For more information visit <http://amatoxtest.com>

Let me say it again: **DON'T EAT A MUSHROOM THAT TESTS NEGATIVE!** There are many mushroom toxins, and this test is for only the most deadly. Be careful!