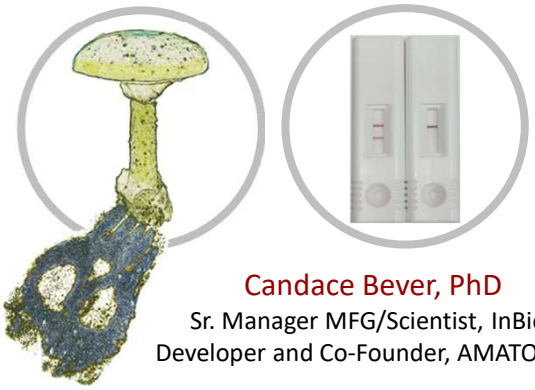
Rapid, portable detection of amatoxins



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CPCS Statewide Grands Rounds

June 15, 2026

Disclaimer: Opinions are my own and do not represent the views of InBios or AMATOXtest.

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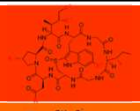
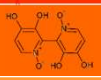
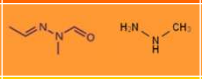
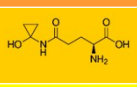
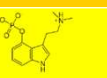
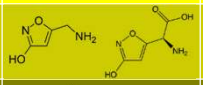
Mushroom toxins

CLASS & MUSHROOMS	CHEMICAL STRUCTURE	REPRESENTATIVES
CLASS A *– AMATOXINS* *Amanita spp.* *Galerina spp.* *Lepiota spp.*		
CLASS B *– ORELLANINE* *Corinarius spp.*		
CLASS C *– MONOMETHYLHYDRAZINE* *Gyromitra spp.* *Helvella ssp.* (suspect)		
CLASS D *– COPRINE* *Coprinopsis spp.* "Inky cap"		
CLASS E *– PSILOCYBIN/PSILOCIN* *Psilocybe spp.* "magic mushrooms"		
CLASS F *– IBOTENIC ACID/MUSCIMOL* *Amanita muscaria* *Amanita pantherina*		
CLASS G *– MUSCARINE* *Clitocybe spp.* *Inocybe spp.*		
CLASS H *– unknown toxins* *Agaricus spp.* *Chlorophyllum molybdites*		

Images from iNaturalist

Image adapted from 'Diagnosis and Treatment of Mushroom Poisoning on the Basis of Symptoms and Mushrooms' by Kit Scates.

2

Mushroom poisonings

Mushroom poisonings are difficult to diagnose

- Early symptoms resemble GI illness
- Mushroom identification is often impossible or unavailable
- Survival relies of early detection and early intervention
- Dogs cannot communicate
- Humans mistakenly identify deadly mushrooms as edible ones

No definitive diagnostic exists.

3

Mushroom poisonings

Amatoxin-containing mushroom poisonings

- Long delay between ingestion and when symptoms appear; typically 6-24 hours after ingestion
- Amatoxins cause severe liver failure
- Amatoxin-containing mushrooms cause the majority of fatal mushroom poisonings worldwide

# of human mushroom exposures/yr (US Poison Control Center, 2008-2025)	Treated in health care facility	Outcome: Death
6,000	2,000	0-7
30-60 (cyclopeptides)	25-45	0-4

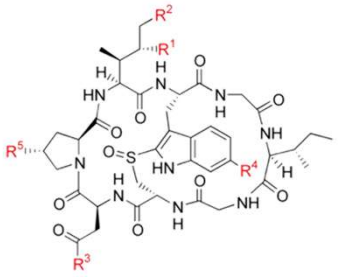
https://aapcc.org/annual-reports

Ruling out Amanita toxicosis would be a great first step

4

What are amatoxins?

- Class of toxins
- Most well-studied are
  - α-, β-, and γ-amanitin
- RNA polymerase II inhibitor
  ⟹ cell death
- LD_{50} humans ≈ 0.1 mg/kg (6 mg/60 kg)
  0.2mg/g toxin/fresh mushroom (20mg/100g)
  ⟹ 1 large mushroom contains a lethal dose
- Cannot be destroyed by cooking, drying, or freezing
- Produced by some mushroom species



Name	R^1	R^2	R^3	R^4	R^5
α-Amanitin	OH	OH	NH_2	OH	OH
β-Amanitin	OH	OH	OH	OH	OH
γ-Amanitin	H	OH	NH_2	OH	OH
ε-Amanitin	H	OH	OH	OH	OH
Amanullin	H	H	NH_2	OH	OH
Amanullinic acid	H	H	OH	OH	OH
Amaninamide	OH	OH	NH_2	H	OH
Amanin	OH	OH	OH	H	OH
Proamanullin	H	H	NH_2	OH	H

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What mushrooms make amatoxins?


Amanita phalloides
Death cap


Amanita ocreata
Western destroying angel


Amanita bisporigera
Eastern NA destroying angel

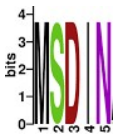

Galerina marginata
Deadly galerina


Galerina badipes


Conocybe filaris


Lepiota subincarnata

Images are own and from iNaturalist

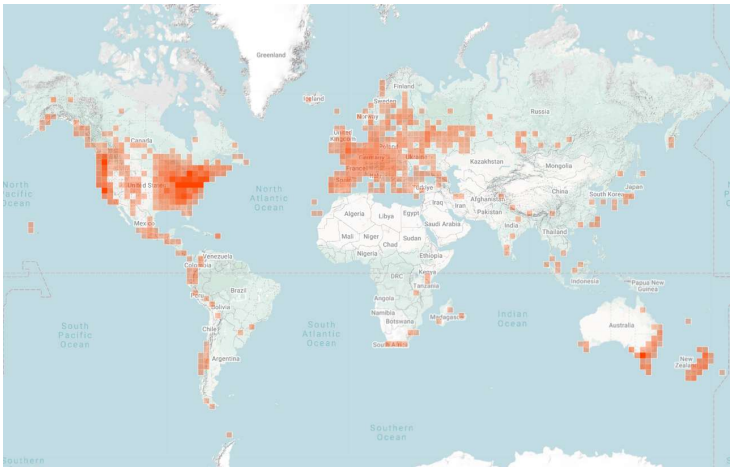


MSDINATRLP**IWGIGCNP**CIGDDVTTLLTRALC

Walton. 2018. *The cyclic peptide toxins of Amanita* and other poisonous mushrooms
Hallen, et al. 2007. *PNAS*. 104(48):19097-19101.

6

Amatoxin-containing mushrooms are found worldwide



iNaturalist observations 2026

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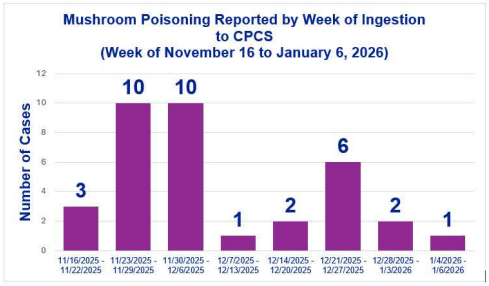
Current events

Figure 1: Case count over ongoing Death Cap mushroom poisoning outbreak



cdph.ca.gov/Programs/OPA/Pages/CAHAN/Increase-in-mushroom-poisonings-in-California

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Amanitin detection in human poisonings

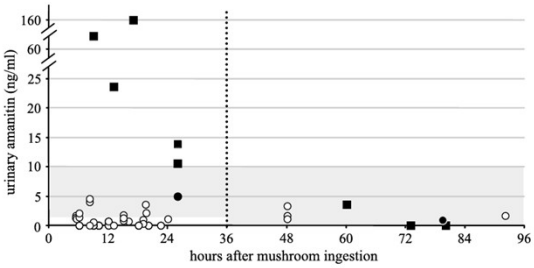

Figure 1. Time-plotted urinary amanitin levels in the study population (n=61). In 20 cases amanitin levels overlap visible symbols (values below 1.5 ng/ml in urine samples collected 6 to 24 hours after mushroom ingestion). Closed symbols indicate amatoxin poisoned patients (n=10), open symbols patients with other diagnosis (n=51). Squares identify cases of documented acute hepatitis or liver damage (n=8). The gray area indicates results that required, for clinical purposes, individualized evaluation based on history and clinical findings.

Lower detectable concentrations of amanitin in confirmed (closed symbols) cases.

Butera, et al. 2004. J. of Toxicology, 42(6):901-912.

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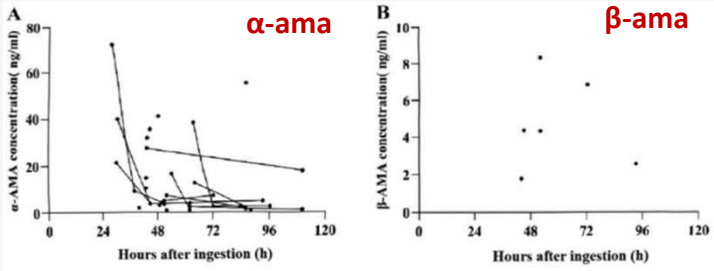
Amanitin detection in human poisonings

Figure 5. Scatter plots of urinary toxin concentrations in patients with *A. exitialis* poisoning: (**A**) α-AMA; (**B**) β-AMA; (**C**) PCD; (**D**) PSC. The black dots in the figure represent the concentration of toxins detected in the urine, and the test data connected by the same line pertain to the same patient.



Amanitin detection **early** is critical. Concentrations drop quickly.

Chen et al 2025, *Toxins*, *17*(12), 576; https://doi.org/10.3390/toxins17120576

12

Amatoxin math

- 1 mushroom contains ~10mg of amatoxins
- Urinary output volume ~1,500mL / day (5 days = 7,500mL)
- 40-80% of amatoxins are excreted in urine*

  → 4mg toxin / 7,500mL urine = 533ng/mL (average over 5 days)

  - Previous studies showed: Urinary α-amantin levels range from 100-2,000ng/mL in the first 24hrs
  - Ratio of toxins – α:β:γ 51:41:8 – maybe detecting only 51% of what is harmful?
  - Is a great majority excreted (via urine and feces) in the first 24 hrs before symptoms? Or going into organs (liver, kidneys) and bile?
  - What is the amount a person ate?

*Kayes and Ho 2024, Int J Mol Sci, Dec 4;25(23):13028
Faulstich et al 1985, *Arch Toxicol*, 56:190-194

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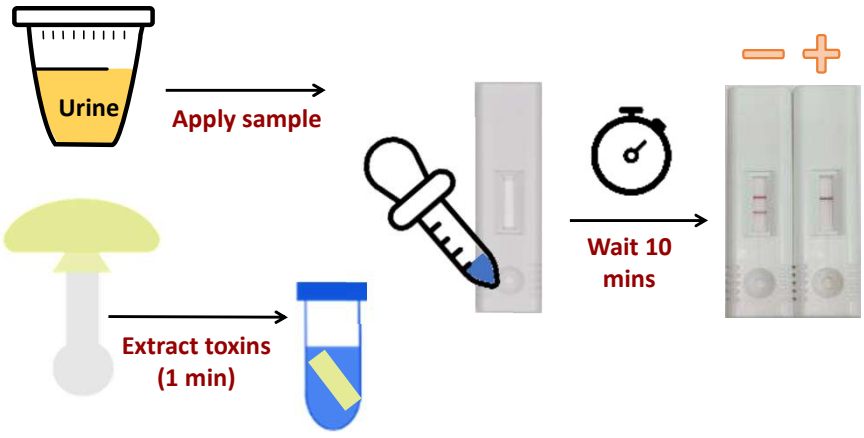
How the AMATOXtest works



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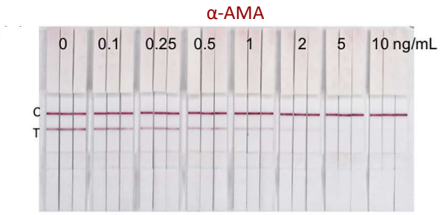
Analytical and Clinical Sensitivity
CANINE

α-AMA



Easy to read by **eye**

Clearly indicates presence of **10 ng/mL** of α-AMA

UCDAVIS VETERINARY MEDICINE
California Animal Health & Food Safety Laboratory System

PET EMERGENCY & SPECIALTY CENTER OF MARIN

Bever, et al. 2020. *PLoS ONE*. 15(4): e0231781.;
Bever, et al. 2020. *Toxins*. 12(2):123.

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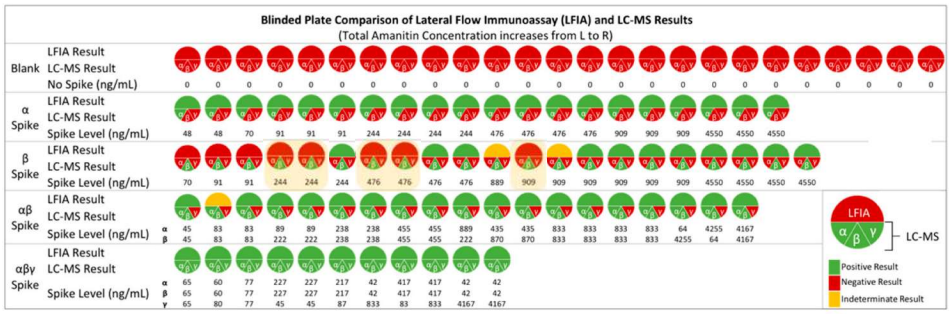
Fortified urine sampling

- **Human urine** (fortified 42-4,550 ng/mL)

Based on data from: Jaeger, et al. 1993.



⟹ 5 False negatives

Bever, et al. 2020. *Toxins*. 12(2):123.

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Summary of urine testing

Diagnostic Parameter	Fortified Human Urine	Intoxicated Dog Urine
# of samples	*n = 96*	*n = 38*
True positive	60	8
True negative	28	22
False positive	0	0
False negative	5	8
Sensitivity	92.3%	50%
Specificity	100%	100%
Efficiency	94.6%	78.9%

Bever, et al. 2020. *Toxins*. 12(2):123.

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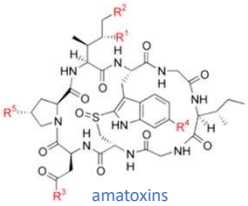
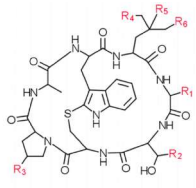
Cross-reactivity



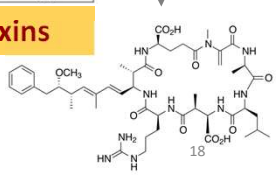
amatoxins



phallotoxins

Toxin	Concentration tested (µg/mL)	LFIA result (n=3)	Cross Reactivity (%)
α-amanitin	0.01	+ + +	100
γ-amanitin	0.01	+ + +	100
β-amanitin	2	+ + +	0.5
	4	+ + +	
	8	+ + +	
phalloidin	200	+ + +	0.005
	20	– – –	
	2	– – –	
phallacidin	200	+ + +	0.005
	20	+ – –	
	2	– – –	
psilocybin	100	– – –	nd
microcystin-LR	20	– – –	nd
nodularin	10	– – –	nd
ibotenic acid	200	– – –	nd
muscimol	200	– – –	nd

Assay is selective for **amatoxins**



Bever, et al. 2020. *PLoS ONE*. 15(4): e0231781.

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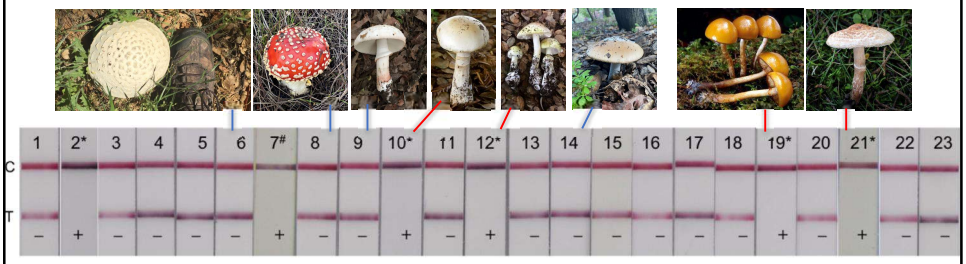
Mushroom testing

- Tested with 110 mushroom specimens, 96 species



1) *Amanita augusta*
2) ***A. bisporigera****
3) *A. calyptratoides*
4) *A. constricta*
5) *A. gemmata*
6) *A. magniverrucata*
7) ***A. marmorata#***
8) *A. muscaria*
9) *A. novinupta*
10) ***A. ocreata****
11) *A. pantherina*
12) ***A. phalloides****
13) *A. protecta*
14) *A. velosa*
15) *Agaricus californicus*
16) *Ag. xanthodermus*
17) *Boletus edulis*
18) *Cantharellus californicus*
19) ***Galerina marginata****
20) *G. sideroides*
21) ***Lepiota subincarnata****
22) *Pholiotina utricystidiata*
23) *Volvariella volvacea*
.... + 87 additional negatives

Bever, et al. 2020. *PLoS ONE*. 15(4): e0231781.

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Regulatory considerations

Test type	Description	Requirements
RUO (research use only	Experimental assay used in research settings.	Cannot be marketed with clinical claims.
LDT (lab-developed test)	Developed and performed by a CLIA lab.	Analytically and clinically validated internally, performed within that lab, lab must adhere to CLIA oversight.
IVD* (in vitro diagnostic) FDA-cleared/ approved	Diagnostic assay marketed for a specific disease.	Reviewed by FDA, for evidence of safety, effectiveness, and clinical performance. Manufacturing complies with FDA Quality System.

*Manufacturing under Quality Management System costs at least $500K/year, performing clinical and analytical validation costs at least $1M. Limited sample availability for clinical validation.

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Regulatory considerations

- Challenges for an FDA-cleared amatoxin diagnostic:
  - Rare disease (amanita toxicosis or general mushroom poisoning)
  - Difficult specimen acquisition
  - Limited commercial market
- LDT (LC/MS) is a viable, practical solution if intending to report the findings as a clinical diagnostic.
- An RUO assay can be integrated alongside other testing (GI symptoms, AST/ALT, INR, history) to inform clinical thinking.
  - An RUO assay can serve as a secondary screening tool, rather than a primary diagnostic metric.

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Practical considerations

- Patients report to the ED around 6-96 hours after ingestion
- Patients cannot identify the number of amatoxin-containing mushrooms they consumed
- Physicians observe general symptoms of GI distress and might not suspect mushroom poisoning
- Urine collection: earlier sampling is most reliable to catch a true positive
- Test availability
  - Send sample to LDT/central lab, rush-order RUO tests; either option would lead to delayed results

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Diagnostic Test Manufacturer Considerations

- **Intended Use**: defined properly and accurately
  - What analyte, what specimen, what symptoms, for what purpose
- **Manufactured** under a formal quality management system
  - US: ISO 13485 or EU: CE-IVDR

  → Every design decision must be documented and traceable
- **Design Controls**: verify and validate that each batch of tests is consistent
- **Risk Management**: clinical consequence of a FN or FP are understood and mitigated
- **Analytical/Clinical Performance**: remains stable over the product's shelf life
- **Perform Post Market Surveillance**

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Thank you for your attention!

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