Amanitin ELISA

Management of suspected mushroom poisoning

The only commercial Amanitin ELISA

In Case of Emergency:
Results within 1 hour

Accurate

Highly Sensitive

Excellent Negative Predictive Value

BÜHLMANN

Amanitin ELISA

Assay Performance Data

Intra-assay precision **6.3%**
(n=4 urine samples, range: 3.1-90.8 ng/ml; 20 duplicates, CV range: 2.8-14%)

Inter-assay precision **7.3%**
(n=3 urine samples; range 6.7-81,8 ng/ml; 20 runs; CV range: 5.3-10.6%)

Dilution linearity **97.8%**
(3 urine samples diluted 1:25, 50, 100, 200, 400 & 800; Range: 79.0-119.0%)

Spiking recovery **99.9%**
(n=1 urine sample 4 times spiked and tested; Range: 80.0-127.5%)

Analytical sensitivity **0.22 ng/ml**
(n=20, measured in duplicates)

Functional sensitivity **1.5 ng/ml**
(cut-off: intra-assay CV = 15%)

Specificity

α-Amanitin:	100%	ε-Amanitin:	0.1%
β-Amanitin:	0.1%	Phalloidine:	not detect.
γ-Amanitin:	90%	Phallacidine:	not detect.

Sample type **Urine, Serum, Plasma**

Sample storage and stability
2-8°C: up to 7 days
-20°C: for at least 6 months

Standard Range **1-100 ng/ml**

α-Amanitin

α-Amanitin is the major toxin of the extremely poisonous toadstools, Amanita phalloides (death cap), A. verna (white death cap), A. virosa (destroying angel) and other Amanita species. These mushrooms may also contain other amatoxins such as β- and γ-Amanitin as well as phallotoxins. Amatoxins are also found in Galerina marginata (marginate pholiota), G. autumnalis, G. sulciceps and other Galerina and Lepiota species. A. phalloides, A. verna and A. virosa account for over 90 % of clinically relevant mushroom intoxication cases in Europe & Northern America. Amanita species also occur in Asia and Australia.

Epidemiology

Amanita intoxication has been reported to be lethal in up to 25 % of the cases. Mortality rate in children under 10 years of age is above 50 %.

Clinical Effects and Laboratory Analysis

Amatoxins infiltrate hepatocytes in which their primary action is to inhibit nucleoplasmic RNA polymerase II (which in turn interferes with mRNA synthesis). This results in the arrest of protein synthesis and cellular necrosis ultimately leading to severe acute hepatitis. Amanitin also acts as a direct nephrotoxin. The symptoms of phallatoxic Amanita species poisoning are of delayed onset type. Patients may therefore not primarily associate their symptoms with the ingestion of the wild mushroom. Once symptoms appear, amatoxins can be detected in urine. The detection of α-Amanitin (100 %) and γ-Amanitin (90 %) in urine by means of the BÜHLMANN Amanitin ELISA confirms Amanita Intoxication. Amatoxins are detected in urine samples within 6-60 hours after ingestion, i.e. the time by which clinical symptoms occur.

From internal evaluation data and clinical toxicologic study, capability characteristics may be outlined as follows:

1. The Amanitin ELISA test is an additional tool in an entire diagnostic work-up.
2. A negative result does not exclude a possible Amanitin intoxication.
3. Sample collection must be within 36 hours.*
4. The Amanitin ELISA test detects alpha-Amanitin (100%) and γ-Amanitin (90%). There is no crossreactivity to β-Amanitin.

*F. Butera et. al: Diagnostic Accuracy of Urinary Amanitin in Suspected Mushroom Poisoning: A pilot Study. J. of Toxicology 2004

ELISA

Precoated Microtiter Plate

wash 2x

50 µl Sample (diluted 1:25), Standard or Control+ 50 µl Amanitin-Biotin Conjugate

incubate 30 min at RT (18-28°C) on a plate rotator

wash 3 x

add 100 µl of Enzyme Label

incubate 15 min at RT (18-28°C) on a plate rotator

wash 3 x

add 100 µl TMB Substrate

incubate 15 min at RT (18-28°C) on a plate rotator

add 100 µl Stop Solution

Read at 450 nm

Time to Result: 1 Hour

Diagnostic accuracy of Amanitin analysis in Urine*

Diagnostic Accuracy (%)	SENS	SPEZ	PPV	NPV
All patients included in the study (n=61)				
Urinary amanitin levels ≥ 1.5 ng/ml	70	82.4	43.8	93.3
Urinary amanitin levels ≥ 5.0 ng/ml	60	100	100	92.7
Patients evaluated within 36 hours (n=51)				
Urinary amanitin levels ≥ 1.5 ng/ml	100	87.2	50	100
Urinary amanitin levels ≥ 5.0 ng/ml	100	100	100	100

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