

Index

Index		2
Forewo	ord	3
Emerge	4	
Option	5	
-	analysis	6
1)	Fresh material, preparation residues	6
2)	Cooking residues, remnants from cans, jars, etc.	6
3)	Dry material	6
4)	Gastric juices, vomit	7
5)	Stools	8
•	cal Test methods	9
1)	ELISA test for amanitin	9
2)	Newspaper-Test according to Wieland	10
3)	Orellanin-Test according to Pöder und Moser	11
4)	Haemagglutination-Test according to Lefèvre	12
Spore t		13
1)	Table A – hyaline, inamyloid spores	14
2)	Table B – hyaline, inamyloid spores	15
3)	Table C – hyaline, inamyloid spores	16
4)	Table D – amyloid Spores	17
5)́	Table E – dextrinoid Spores	18
6 <u>)</u>	Table F – brown spores	19
7)	Table G – brown spores	20
8)	Table H – black spores	21
9)	Table I – spores, basidia, asci	22
10)	Table K – Cystidia	23
Identifi	cation tips	24
1)	Spores	24
2)	Spore powder	24
3)	Basidia	25
4)	Cheilocystidia	25
5)	Pleurocystidia	25
6)	Chrysocystidia	25
7)	Marginal cells	25
8)	Cap cuticle	25
	cal Reactions	27
1)	FeSO ₄	27
2)	KOH 20%	27
3)	NH ₃	27
4)	Schäffer-reaction	28
5)	Further staining- & Test methods	28
	Cresyl Blue / Toluidin Blue O	28
	 Patent-Blue V 	28
6)	Primordial hyphae	29
7)	Siderophile Granulation	30
Brief po	ortraits of the 18 syndromes	31
The syl	ndromes, their toxins and their target organs	33
Append	dix: Table of first symptoms and latency periods	34

Foreword

Fungal collection inspectors do not have microscopical skills or medical knowledge, so that emergency experts, with the help of microscopy, and especially spore analysis, are needed to determine which of the **18 poisoning-syndromes** conforms to initial symptoms and latency period

The purpose of this course is to further interdisciplinary co-operation between medicine and mycology, and to demonstrate possibilities and limitations of mycological emergency diagnoses.

Often one must be satisfied by only circumstantial evidence, even when knowledgeable mycologists are involved.

We thank Prof. Heinz Clémençon, Le Mont-sur-Lausanne, and Harry Andersson, Braunschweig, for valuable references. We thank Ray Tantram, Surrey, UK for the English translation and some comments.

Wittenbach, February 2008

Fichtenstrasse 26
CH-9300 Wittenbach SG
Switzerland
rene.flammer@freesurf.ch

Dr. med. R. Flammer

Schaffhausen, February 2008

Thomas Flammer

Pfarrweg 3
CH-8200 Schaffhausen
Switzerland
thomas.flammer@freesurf.ch

www.giftpilze.ch

Further reading – Bibliography with 149 references

Flammer R, Horak E, Giftpilze – Pilzgifte, Schwabe Basel 2003, ISBN 3-7965-2008-1

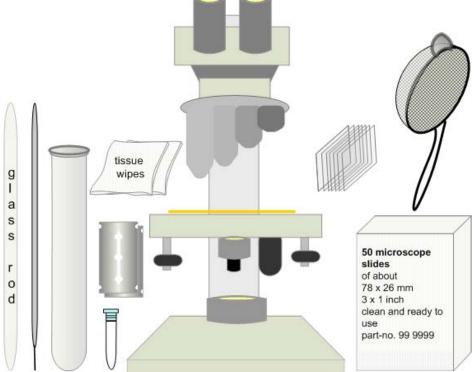


Emergency kit

Substance	Formula		Application
Caustic potash	КОН	3 - 5 %	For tissue maceration and softening dry material
Melzer Reagent	dist. water	20 ml	Testing for amyloid or dextrinoid
	Potassium iodide	1.5 g	behaviour
	Iodine	0.5 g	
	Chloral hydrate	22 g	
Hydrochloric acid	HCI	30 %	For the newspaper test
Iron chloride	FeCl ₃ .6H ₂ O	0.6 g	Orellanin-determination
	HCI 0.5 mol	20 ml	
Immersion oil			Magnification 1 : 1000
Ethanol		70 %	Cleaning optical components

- Glass slide
- Cover slips
- Paper tissues
- Glass rod
- Rubber gloves

- Scalpel, razor blades
- Coffee sieve
- Microscope with x100 objective und graduated eyepiece
- Glass test tubes for working with ether
- Plastic reagent tubes for other tasks
- Literature for following up measurements



Information on Institutes that carry out the ELISA test (Amanitin presence in urine, serum, plasma).

Country Telephone / emergency numbers

Switzerland Toxicology Centre Zurich from Switzerland 145

from outside Switzerland +41 44 251 51 51

corresponding

emergency numbers



5) Stools

Visible fragments present

- Stool soaked with 10 times its quantity of water, and filtered through a coarse sieve.
- Look for fragments in the residue.
- · Wash fragments in hot water.
- Alternative: Degrease in coffee sieve with washing-up liquid from a spray bottle, with the sieve
 placed above a large vessel to avoid loss of material from foaming during the washing process.
- Examine under the microscope.

Visible fragments missing

- Add 5 ml ether to 5 ml of the filtrate (degreasing) and shake well.
- Pipette off the ether layer.
- Centrifuge the aqueous phase for 10 Minutes at 7000 rpm.
- Decant supernatant liquid down to the sediment.
- Examine sediment microscopically after lightly drying a drop over a flame.

Care, use only glass vessels when working with ether!!

Comment

These methods are very time-consuming and mainly have a place in forensic medicine.

Fungal fragments and spores remain present in stools for up to 5 days following a fungal meal. Theoretically one could examine stools for determining Orellanus-syndrome. However the number of similar Cortinarius spores negates this as a confirmation character.

Confirmation of Orellanus-syndrome is by the test according to Pöder und Moser and detection of the toxin in kidney needle biopsy tissue. This is possible for several weeks following a fungal meal, using thin-layer chromatography (page 11).



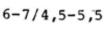
Cortinarius rubellus

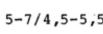
1) Table A - hyaline, inamyloid spores

5,5	5-7/4,5-5,5
-,-	0 11 110 010







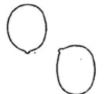


Scale / Notes

10 µm ,,,,,,,,











5-7/5-6

2

4-6/3-4

3



5-6/3-4,5







6

7

8

6-8/4-5

7-9/4-5



5-6,5/3-4



5-7/3-4,5







10

11

12



9-12/7-8

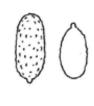


6-8/4-5









15

16

- Albatrellus subrubescens Albatrellus ovinus
- Clitocybe rivulosa Clitocybe phyllophilla (cerussata)
- Pluteus atricapillus
- Clitocybe geotropa
- Omphalotus olearius Hydnum repandum Clitocybe dealbata

- Collybia fusipes
- 7 Calocybe gambosa
- 8 Tricholoma albobrunneum Tricholoma saponaceum Tricholoma portentosum
- 9 Lentinus edodes
- 10 Tricholoma fulvum Clitocybe clavipes Sparassis crispa Clitocybe suaveolens

- 11 Tricholoma equestre
- 12 Hygrophorus marzuolus
- 13 Tricholoma tigrinum
- 14 Amanita pantherina
- 15 Lepista nebularis
- 16 Lepista nuda

Brief portraits of the 18 syndromes

This working booklet is no substitute for more comprehensive works of reference. It is a brochure for use in emergencies Page 34 summarises what a fungus-expert needs to know. The division into individual syndromes is partly artificial as nature often resists our efforts to define tidy boundaries in attempts to classify matters. Seamless transitions and exceptions must always be borne in mind. Experience has shown, however, that summaries in tabular form provide an essential and useful rough screening function within the framework of a rapid initial orientation. Symbols: basically all the syndromes can cause serious, even if not fatal outcomes. All depends on quantity of toxin, therapeutic latency time (time from first symptoms to the beginning of treatment), age, etc. The crosses should therefore be seen a rough guide, in which the probability of a fatal outcome or permanent damage to organs correlates to the number of crosses shown.

Phalloides-Syndrome † † † † Latency > 4 hours. Victims suffering vomiting and diarrhoea are always frightened of potential amanitin-poisonings. Resolving this question quickly is of the utmost priority. Do not rely on statements by the patient, whose mycological knowledge is unlikely to be helpful. Do not forget that small *Lepiota*-species and especially *Galerina marginata* (wood chippings in woodland and garden) also contain amanitin. Take into account unusual timings in poisoning episodes (deep-frozen and dried fungi).

European toxic fungi		North America
Amanita phalloides	Lepiota brunneoincarnata	Amanita bisporigera
Amanita verna	Lepiota josserandii	Amanita ocreata
Amanita virosa	Lepiota brunneolilacea	
Galerina marginata	7 further, difficult to differentiate	
Galerina autumnalis	species	

Acromelalgy-Syndrome † Pain and swelling in the hands and feet 1-2 days following consumption of *Clitocybe amoenolens* und *Clitocybe acromelalga* (Japan). Rare.

Coprinus-Syndrome † Inkcaps and alcohol are not compatible. Acute symptoms can occur up to 4 days after a fungus meal very shortly after any alcohol is consumed. Very infrequently an Antabuse reaction can occur after (generous?) partaking of *Coprinus comatus?* Coprine has also been found in *Boletus torosus*, whilst *Boletus luridus* is toxin-free.

Equestre-Syndrome † † † Muscle disintegration after eating *Tricholoma equestre*. Toxicologically many questions remain open, as apparently poisonings occur only infrequently. Individual factors? Variable toxin content in the fungi? Critical stimulation threshold exceeded by generous quantities and repeated consumption? Rare. Latency > 24 hours.

Fungal allergy † Often difficult to separate from indigestibility and gastrointestinal early-syndrome. Indicators are skin rashes, asthma, mucus membrane swellings and circulatory collapse, also constitutional tendency to allergies with corresponding case histories.

Gastrointestinal Early-syndrome † † Several dozen toxin-containing fungi cause vomiting and diarrhoea, most of which show short latency periods of less than 4 hours.

The most frequently found to	USA, Tropics & Subtropics	
Agaricus xanthoderma	Omphalotus olearius 1)	Chlorophyllum molybdites
Boletus satanas	Paxillus involutus	
Entoloma sinuatum	Ramaria formosa	
Hebeloma sinapizans	Ramaria pallida	
Hypholoma fasciculare	Russula (hot species)	
Lactarius (hot species)	Tricholoma saponaceum	
Macrolepiota venenata 1)	Tricholoma tigrinum 1)	¹⁾ Unknown or rare in Great Britain