



# Baden-Württemberg

MINISTERIUM FÜR SOZIALES GESUNDHEIT UND INTEGRATION  
LANDESGESUNDHEITSAMT BADEN-WÜRTTEMBERG

Landesgesundheitsamt Baden-Württemberg · Postfach 10 29 42 · 70025 Stuttgart

To all Participants of the 43rd Round Robin  
Test “Microfungi”, October 2022

Datum 25.10.2022  
Name Dr. Guido Fischer  
Durchwahl #49-711 25859-307  
Aktenzeichen R73\_RV43\_2022

## 43<sup>rd</sup> Inter-Laboratory Test “Identification of Indoor- and Food-borne Fungi”

Dear Madam, dear Sir,

thank you very much for your participation in the 43<sup>rd</sup> Inter-laboratory Proficiency Test “Identification of Indoor- and Food-borne Fungi”. The test comprises six pure cultures and, additionally, a mixed sample if ordered explicitly. The participating laboratories have to investigate the samples within 6 weeks.

Please send back your results in electronic form to [Med-Chem@sm.bwl.de](mailto:Med-Chem@sm.bwl.de) until **12<sup>th</sup> of December 2022**.

### **1. Processing and evaluation of the pure cultures:**

Each strain was sent out in single. A retain sample is not delivered any more. The strains are all cultivated on Malt-Extract-Agar (MEA).

- Each correctly identified strain is rated **with 1 point**
- **Four** correctly identified strains (genus and species level !) are needed to pass the Proficiency Test successfully and to attain a **Certificate**.
- The participant who fails the Test will receive **written confirmation of participation** (“Certificate of attendance”).
- **Incorrect spelling of the species name will be evaluated wrong. Please use the taxonomically valid species name (see MYCOBANK database).**

To pass the test successfully, **four** out of six strains must be correctly identified up to species level.

## **2. Analysis and evaluation of the Mixed Sample:**

A mixed sample is attached (if ordered) which comprises **four fungal species** suspended in glycerol. This sample must be analyzed according to the instructions given below. Besides the pure cultures, we regard the analysis of mixed samples as a very important part of the external quality control. The results of the mixed sample are evaluated separately using those of the reference laboratories as a standard reference.

### **2.1 Processing of the mixed sample within 3 days after delivery:**

- Vortex the tube (it contains approx. 1.3 ml Glycerol suspension)
- To get a 10-fold dilution, suspend **0,5 ml Glycerol suspension (original suspension) in 4,5 ml 0,85% NaCL/Tween 80**
- To get a 100-fold dilution, suspend **0,5 ml of the 1:10 suspension with 4,5 ml 0,85% NaCL/Tween 80**
- Plate 100 µl of each suspension (the original, the **10**-fold, and the **100**-fold dilution) on **3 DG-18 agar-plates** and **3 MEA-plates** per step of dilution and incubate the agar plates at 25(± 3)°C. To assess a wide spectrum of species in practice, the combined use of Malt-Extract-Agar (MEA) and Dichloran 18% Glycerol-Agar (DG18-Agar) is necessary.
- The incubation time depends on the type of medium and the species, however at least 7 days of incubation are necessary. In case of slow growing species an incubation time of 10 days may be appropriate for accurate qualitative and quantitative assessment. Quantification of CFUs of different types/species should be start on day three of incubation and thereafter every 2-3 days. Especially in species with strong sporulation, growth of secondary colonies must be taken into consideration.

**Note:** Strongly sporulating species such as *Penicillium* spp. are dispersed even more effective (secondary colonies) when the plates are intensely moved, turned upside down or placed vigorously on the bench. This can affect germination of slowly growing colonies and their accurate quantification.

### **2.2 Evaluation of mixed sample**

The fungi in the mixed sample need to be quantified and identified correctly. This should be done between day 3 and day 10, **especially xerotolerant species should be quantified at day 8 or 9**. Please use the Excel-file (sheet "mixed sample") for documentation. Quantification and evaluation of the results must be carried out according to the DIN ISO 16000-17:2008. The most important criteria are listed below:

- (1.) The quantitative data will be registered primarily from the **DG-18** plates. From the **MEA-plates** only those species are quantified, that do not/hardly grow or sporulate readily on DG-18; e.g. *Acremonium*, *Stachybotrys* and *Chaetomium*.
- (2.) Certain fungi may inhibit the growth of other species. To assess statistically reliable data, the agar plates used for quantification should contain less than 100 cfu and more than 10 cfu. **In practice, cfu-numbers are often below 10 on the plates. Please comment these data by adding "semi-quantitative"**.
- (3.) The most representative results are gained from plates with colonies between 20 and 40 cfu.

- If the results are not plausible, e.g. if very rapidly growing species (i.e. *Chrysonilia*, *Trichoderma*) spread on the plates, this must be documented.
- The result must be given as cfu/sample and it should be mentioned from which medium the cfu were quantified. The mean value is calculated from three replicates, including samples with 0 cfu. The total cfu per ml are calculated as the sum of all species.

The identification must be done to species-level. Although the sample is suspended in approx. **1.3 ml** liquid, the result must be given as **cfu per ml**. By plating 100 µl from the original suspension, the cfu per ml can be calculated by multiplication with factor 10.

### **3. Submission of results from the participants:**

Please **use the Excel-file sent via E-mail**. There are two data sheets, one for pure cultures and one for mixed cultures integrated. Please do not alter any given form or text within the Excel-file (except your results), because otherwise the automatic registration will not work correctly.

#### *Pure cultures:*

In case of an incorrect identification result, we want to crosscheck the identification procedure. Therefore, we need information on the type and manufacturer of the culture media used. In addition, please give the criteria that lead to the identification result (e.g. identification key used, growth rate, biochemical reaction, sequencing data).

#### **Criteria for acceptance of molecular identification results:**

If you use molecular methods for identification, please give information on both the **target gene** analyzed (or primer used) and the **accession No.** of the database used (or strain No., literature). **Results of molecular identification can only be accepted when the above information are given.** These changes are necessary because the public sequence databases are not quality controlled and misidentifications are regularly occurring in routine diagnostics. Please crosscheck the validity of the species name using **MYCOBANK** (<https://www.mycobank.org/>). In the past, we repeatedly found contradictory or incorrect information in the *Index Fungorum*.

### **4. Objections:**

If you suspect that - despite our quality control measures - the pure culture(s) are contaminated, the LGA should be notified within one week. A new culture will be sent as soon as possible. To assure purity of strains and to sort out a possible contamination, test strains are checked again by all reference laboratories.

Objections to the evaluation of this Inter-Laboratory Proficiency Test must be directed to the LGA (Dr. G. Fischer), which will discuss this matter in accordance with the reference laboratories.

Good luck and Sincerely yours,



Dr. Guido Fischer

## **Checklist !!!!**

Please consider:

- Give short information on media, target gene and accession No. in Excel file.
- Mark only those Excel cells in colour (e.g. yellow) that have been used for calculation of results.**
- Do not deliver separate Excel files for pure cultures and mixed sample.
- Do not send pdf files with data.
- Do not change names of data sheets within the Excel file.
- Please use the English Excel file, when certificates should be printed in English.**
- Send Excel file by e-Mail ([Med-Chem@sm.bwl.de](mailto:Med-Chem@sm.bwl.de) ).
- Give cfu numbers as integral numbers.**
- Please use correct spelling of fungal names.

**We are very grateful for all your efforts to facilitate our data assessment.**

Sincerely Yours, the round robin team at LGA  
(M. Beresowski, M. Faisst (IT) und Dr. G. Fischer)

## **Literature for Identification ([new literature](#)):**

### **Basic literature (Genus-level):**

- The **Genera of Hyphomycetes**, CBS Biodiversity Series, K. Seifert, G. Morgan-Jones, W. Gams, B. Kendrick, CBS-KNAW Fungal Biodiversity Centre Utrecht, The Netherlands **2011**, ISBN 978-90-70351-85-4
- **Food- and Indoor Fungi**, CBS Laboratory Manual Series **2010**, R.A. Samson, J. Houbraken, U. Thrane, J.C. Frisvad & B. Andersen, CBS KNAW Fungal Biodiversity Centre Utrecht, The Netherlands, ISBN 978-90-70351-82-3; ISSN 1879-6877
- **Compendium of Soil Fungi** (Domsch, Gams & Anderson) 2nd Edition, IHW-Verlag und Verlagsbuchhandlung, München ISBN 978-3-930167-69-2, <http://www.ihwverlag.de/frameset/pilzbuchset.html>
- **Atlas of clinical fungi**, 2. Edition, G.S. de Hoog, **2010**, ISBN 90-70351-43-9 (3<sup>rd</sup> edition only online)
- **Dematiaceous Hyphomycetes**, M.B.Ellis, CAB Publishing, CAB international Wallingford, Oxon OX10 8 DE, UK, ISBN 0 85198 027 9 oder ISBN 0 85198 618 8 (soft cover) **1971**
- **More Dematiaceous Hyphomycetes**, M.B.Ellis, CAB Publishing, CAB international Wallingford, Oxon OX10 8 DE, UK,, ISBN 0 85198 365 0, **1976**
- The **Genera of Fungi Sporulating in Pure Culture**, J.A. von Arx, Lubrecht&Cramer, ISBN 3-7682-0693-9, A.R. Gantner Verlag K.G., FL-9490 Vaduz, **1981**
- **Cephalosporium-artige Schimmelpilze** (Hyphomyceten), Walter Gams, Baarn/Niederlande, Gustav Fischer Verlag Stuttgart, **1971**, ISBN 3-437-30117-9

### **Monographs:**

#### **Centraalbureau voor Schimmelcultures:**

- [Cephalotrichum](#) and related synnematosous fungi with notes on species from the built environment. J.H.C. Woudenberg, M. Sandoval-Denis, J. Houbraken, K.A. Seifert, and R.A. Samson; **Studies in Mycology 88: 137–159 (2017)**.
- [Scopulariopsis](#) and scopulariopsis-like species from indoor environments. J.H.C. Woudenberg, M. Meijer, J. Houbraken, and R.A. Samson; **Studies in Mycology 88: 1–35 (2017)**.
- Species diversity in *Aspergillus*, *Penicillium* and *Talaromyces*. R.A. Samson, C.M. Visagie, and J. Houbraken (editors), CBS-KNAW Fungal Biodiversity Centre, The Netherlands, **Studies in Mycology 78**, 2014, ISSN 0166-0616
- The genus *Cladosporium* K. Bensch, U. Braun, J.Z. Groenewald, and P.W. Crous, CBS-KNAW Fungal Biodiversity Centre, The Netherlands, **Studies in Mycology 72**, **2012** ISBN 978-90-70351-xx-x
- Phylogenetic and taxonomic studies on the genera *Penicillium* and *Talaromyces*, R.A. Samson, J. Houbraken editors, CBS-KNAW Fungal Biodiversity Centre, The Netherlands, **Studies in Mycology 70**, **2011** ISBN 978-90-70351-87-8
- Taxonomic studies on the genus *Aspergillus*, R.A. Samson, J. Varga, and J.C. Frisvad, CBS-KNAW Fungal Biodiversity Centre, The Netherlands, **Studies in Mycology 69**, **2011** ISBN 978-90-70351-86-1
- *Aspergillus* systematics in the genomic era, Robert A. Samson and Janos Vargas, CBS Fungal Biodiversity Centre, Utrecht, The Netherlands, **Studies in Mycology Nr. 59 (2007)** ISBN/EAN: 978-90-70351-69-4
- *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites, R.A. Samson and J.C. Frisvad, **2004**, Centraalbureau voor Schimmelcultures Utrecht, P.O. Box 85167, 3508 AD Utrecht, **Studies in Mycology 49**, ISBN 90-70351-53-6.
- *HypocrealTrichoderma* (*Ascomycota*, *Hypocreales*, *Hypocreaceae*): species with green ascospores; Priscila Chaverri and Gary J. Samuels, **Studies in Mycology Nr. 49 (2003)**, Centraalbureau voor Schimmelcultures Utrecht, ISBN:90-70351-51-X.
- A revision of *Chrysosporium* and allied genera, C.A.N. van Oorschot, **Studies in Mycology 20**, Centraalbureau voor Schimmelcultures, Utrecht, **1980**.
- A compilation of the *Aspergillii* described since 1965, R.A. Samson, Centraalbureau voor Schimmelcultures, Utrecht, , **Studies in Mycology 18**, **1979**.
- On certain species of *Mucor* with a key to all accepted species and On the genera *Rhizomucor* and *Parasitella*, **Studies in Mycology 17**, M.A.A. Schipper, Centraalbureau voor Schimmelcultures, Utrecht, **1978**.
- *Paecilomyces* and some allied hyphomycetes, **Studies in Mycology Nr. 6**, R.A. Samson, Centraalbureau voor Schimmelcultures, Utrecht, **1974**.

#### **Other Authors:**

- Identification of common *Aspergillus* species. M.A. Klich, Centraalbureau voor Schimmelcultures, Utrecht, **2002**, ISBN 90-70351-46-3
- A Laboratory Guide to Common *Penicillium* Species, John Pitt, Food Science Australia, ISBN: 0 643 04837 5 **direct order or via email:** [John.Pitt@foodscience.afisc.csiro.au](mailto:John.Pitt@foodscience.afisc.csiro.au)
- The Deuteromycetes, Mitosporic Fungi, Classification and Generic Keys. **2000**, E.Kiffer and M.Morelet, Science Publishers Inc., P.O.Box 699, Enfield, NH 03748, ISBN 1-57808-068-1
- The Genus *Aspergillus*, Raper&Fennell **1977**, ISBN 0-88275-109-3
- The Genus *Penicillium*, Pitt, J.I., 1979 Academic Press, ISBN 0-12-557750-7
- *Fusarium* species, an illustrated manual for identification, Nelson, P.E. Tousson, T.A.&Marasas 1983, Pennsylvania state Univ.Press, Univ. Park, London

#### **International and national standards:**

- **VDI 4300 Richtlinie Blatt 10:**  
Messen von Innenraumluftverunreinigungen - Messstrategie bei der Untersuchung von Schimmelpilzen im Innenraum
- **DIN ISO 16000-17:2008:** Innenraumluftverunreinigungen – Teil 17: Nachweis und Zählung von Schimmelpilzen – Kultivierungsverfahren

#### **German guidelines on Indoor Fungi:**

##### **Landesgesundheitsamt Baden-Württemberg:**

- Handlungsempfehlung für die Sanierung von mit Schimmelpilzen befallenen Innenräumen, (Neuaufgabe Februar 2006)
- Schimmelpilze in Innenräumen – Nachweis, Bewertung, Qualitätsmanagement. Abgestimmte Ergebnisprotokolle der Arbeitsgruppe „Analytische Qualitätssicherung im Bereich der Innenraumluftmessung biologischer Schadstoffe“ am Landesgesundheitsamt Baden Württemberg 14.12.2001 (Neuaufgabe Dezember 2004):
- Untersuchungen zum Vorkommen und zur gesundheitlichen Relevanz von Bakterien in Innenräumen - Forschungs- und Entwicklungsvorhaben des Umweltbundesamtes - Förderkennzeichen 205 62 236 - Verbundvorhaben September 2008

##### **Umweltbundesamt:**

- [Leitfaden zur Vorbeugung, Erfassung und Sanierung von Schimmelbefall in Gebäuden, 2017](#)

##### **Berufsgenossenschaft der Bauwirtschaft:**

- Handlungsanleitung zur Gefährdungsbeurteilung nach Biostoffverordnung (BioStoffV) Gesundheitsgefährdungen durch biologische Arbeitsstoffe bei der Gebäudesanierung“ BGI 858

**Species sent out (blue with RV No.); green: „New“ names (cf. Mycobank, 20.10.2022)**

- Acremonium murorum* (3, 15)  
*Acrostalagmus luteoalbus* (24)  
**Akanthomyces** (*Lecanicillium*) *lecanii* (18, 38)  
*Alternaria alternata*  
*Alternaria tenuissima* (20)  
*Aspergillus calidoustus* (22, 28, 33, 38)  
*Aspergillus carbonarius* (24)  
*Aspergillus chevalieri* (*Eurotium chevalieri*) (23)  
*Aspergillus creber* \* (20, 29)  
*Aspergillus clavatus* (34)  
*Aspergillus glaucus* (*E. herbariorum*) (21, 25)  
*Aspergillus fumigatus* (3, 10)  
*Aspergillus japonicus* (32)  
*Aspergillus jensenii* (M 33, 39, M 40) \*  
*Aspergillus montevidensis* (35)  
*Aspergillus nidulans* (*Emericella*) (1, 11, 22, 36)  
*Aspergillus niger* (8)  
*Aspergillus penicillioides* (1, 9, 29)  
*Aspergillus restrictus* (4, 8, 11, 15, 42)  
*Aspergillus sydowii* (5, 13, 14, 19, 30, 41)  
*Aspergillus tamarii* (11,37)  
*Aspergillus terreus* (7, 31, 34)  
*Aspergillus ustus* (4)  
*Aspergillus versicolor* Sektion (2, 11, 20)  
*Aspergillus vitis* (*Eurotium amstelodami*) (2, 9)  
*Aspergillus wentii*  
*Aspergillus westerdijkiae* (25, 31, 40)  
*Aureobasidium pullulans* (2, 7, 12, 23, 27)  
*Aureobasidium melanogenum* (35, 41)  
*Beauveria bassiana* (33, 40)  
*Botrytis cinerea* (7, 34)  
*Botryosporium* sp. (23)  
*Byssosclamyces nivea* (29, 30)  
*B. spectabilis* (*Paecilomyces variotii*) (6, 12, 18)  
*Cadophora fastigiata* (= *Phialophora fast.*) (7)  
*Candida albicans* (38)  
*Cephalotrichum* sp. (*C. microsporium*) (34)  
*Chaetomium elatum* (38, 39)  
*Chaetomium globosum* (3, 10, 13, 40)  
*Chromelosporium* sp. (30)  
*Chrysosporium* sp.  
*Cladosporium cladosporioides* (3)  
*Cladosporium herbarum* (16, 35)  
*Cladosporium sphaerospermum* (5, 13, 24, 32, 39)  
*Curvularia geniculata*  
*Curvularia lunata* (23, 31)  
**Cyphellophora** *europaea* (24, 28, 40) (*Phialophora*)  
**Didymella** *glomerata* (4, 14)  
**Didymella** *macrostoma*  
**Dipodascus** *geotrichum* (20, 25, 42)  
*Epicoccum nigrum* / *italicum* (26, 40)  
*Fusarium solani* Komplex (38)  
*Fusarium sporotrichioides* (30)  
*Gliomastix murorum* (38)  
*Lecanicillium psalliotae* (*Verticillium psalliotae*) (29)  
**Lichtheimia** *corymbifera* (6, 21,35, 42)  
*Scopulariopsis brevicaulis* (1, 10)  
**Memnoniella** *echinata* (26)  
**Microascus** *paisii* (21, 36, 39)  
*Monascus ruber* (31)  
*Mucor circinelloides* (41)  
*Mucor plumbeus* (7, 13, 22, 27,37)  
*Mucor racemosus* (2, 19, 33)  
*Mycotypha microspora* (30)  
*Ochroconis musae* (37)  
*Oidiodendron griseum* (17,37)  
**Parengyodontium** *album* (22, 29, 39)  
*Penicillium brevicompactum* (24, 27, 38)  
*Penicillium camemberti* (22, 36)  
*Penicillium chrysogenum* / *rubens* (41)  
*Penicillium citrinum* (21)  
*Penicillium citreonigrum* (29)  
*Penicillium commune* (14), *P. biforme* (32)  
*Penicillium corylophilum* (8, 21)  
*Penicillium crustosum* (19)  
*Penicillium digitatum* (1, 2, 5, 12, 18)  
*Penicillium expansum* (4, 13, 15)  
*Penicillium glabrum* (6, 10)  
*Penicillium griseofulvum* (18, 26, 37, 38, 42)  
*Penicillium hirsutum* ( 20)  
*Penicillium italicum* ( 27, 33)  
*Penicillium nalgiovense* ( 25)  
*Penicillium olsonii* (4, 9, 23)  
*Penicillium roqueforti* (8, 22)  
*Penicillium solitum* ( 27, 31)  
**Pseudogymnoascus** *pannorum* (5, 12, 16, 25, 34,37M)  
*Purpureocillium lilacinum* (29, 42)  
*Rhizomucor miehei* (34)  
*Rhizopus stolonifer* (1, 10, 26, 31)  
*Rhodotorula minuta*  
**Sarocladium** *kiliense* (36)  
**Sarocladium** *strictum* (27, 36)  
*Scopulariopsis brevicaulis* (28, 33, 42)  
*Scopulariopsis candida* (20)  
*Simplicillium lamellicola* (41)  
*Sporobolomyces salmonicolor* (31)  
*Stachybotrys chartarum* (M 17, M21, M 28, M 24, M 33, M 40)  
*Syncephalastrum racemosum* (3, 15, 32)  
*Talaromyces funiculosus* (18, 25, 32, 38)  
*Talaromyces islandicus* (38)  
*Talaromyces purpurogenus* (3, 15, 35)  
*Talaromyces piceus* (26)  
*Talaromyces rugulosus* (7, 15, 37, 40)  
**Talaromyces** *wortmannii* (35, 39)  
*Trichoderma harzianum*  
*Trichoderma viride* (Komplex) (36, 41)  
*Trichoderma longibrachiatum* Komplex (14, 30)  
*Trichothecium roseum* (33)  
*Tritirachium oryzae* (37)  
*Wallemia sebi* (21,37)  
**Mucor** *moelleri* (24, 28)