Baden-Württemberg
MINISTERIUM FÜR SOZIALES GSUNDHEIT UND INTEGRATION
LANDESGESUNDHEITSAMT BADEN-WÜRTTEMBERG

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

To:
Participants of the 44th Round robin test
"Identification of microfungi"

Datum 20.02.2023
Name Dr. Guido Fischer
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Aktenzeichen R73 RV44_2023

**44th Inter-Laboratory Test "Identification of Indoor- and Food-borne Fungi"**

Dear Madam, dear Sir,

thank you very much for participating in the 44th Inter-laboratory Proficiency Test "Identification of Indoor- and Food-borne Fungi". The participating laboratories have to investigate the six pure cultures and the mixed samples within 6 weeks.
Please send back your results to **Med-Chem@sm.bwl.de** by using the pre-formatted Excel-file, which was send to you via email. Your results have to be submitted until **14th of April 2023** only electronically.

Submission of your results:
We will register and analyse your results electronically by uploading your Excel file into the Laboratory Information System (LIMS). You received a newly formatted Excel file via email, which is already named correctly including your participant number.

1. Fill in the names of the strains **A to F** (in that order) and give only the most important criteria für identification;
2. Fill in the names of the four species in the mixed sample in **alphabetical** order;
3. Add the cfu numbers (three per step of dilution!) for each species and mark those cells in yellow, from which you calculated the amount of cfu per species and the total amount per ml.
4. There are two sheet within the file: Pure cultures and Mixed culture.
5. Please, do not alter the layout or format of the tables, cells, and sheets. Otherwise, we cannot import your data!

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

We send a single slant per strain to reduce cost for postage. Except strain A (DG-18) all strains are inoculated on Malt-Extract-Agar (MEA). Strain B needs be identified only on the level of a complex/section.

- 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
- The participant who fails the Test will receive **written confirmation of participation** ("Certificate of attendance").
- **Incorrect** spelling of the species name will be recorded as wrong. Please use the taxonomically valid species name (see list in the annex or use *MycoBank*).

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

Please consider, that for a correct identification both genus and species name are required. However, during the last years fungal taxonomy has become more complex due to sequence data. **In cases, where correct identification of species within a complex is difficult by morphology only, we accept the identification on complex or section level (e.g. in case of *Aspergillus versicolor* Section).**

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

In addition to the pure cultures, a mixed sample is attached (if ordered) which comprises **four** fungal **species** suspended in glycerol-NaCl-water. 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 (containing 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 dilution step, A) the original suspension, B) the **10**-fold, and C) 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, 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, at least 7 days of incubation are necessary. In some cases an incubation time of 10 days may be appropriate to make an accurate qualitative and quantitative assessment. On the third day of incubation and thereafter every 2-3 days, the number of CFUs should be counted and differentiated. Especially in species with strong sporulation growth of secondary colonies must be taken into consideration.

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

## 2.2 Evaluation of mixed sample

The fungi in the mixed sample must be quantified and identified correctly. This is done between day 2 and day 10, **especially xerotolerant species should be quantified at day 8 or 9**. Please use only the Excel-file for documentation.
Quantification and evaluation of the results must be done 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 for single species to be quantified. Even in practice, cfu-numbers of species 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.
- (4.) If the results are not plausible, e.g. when very rapidly growing species (i.e. *Chrysonilia, Trichoderma*) appear on the plates, this must be documented and the results should adequately be considered for quantification.
- (5.) The final result is given as cfu/ml. It should be indicated by "yellow cells" from which medium the cfu have been quantified. Only the data from the yellow cells will be scanned by the LIMS-System as your results. The mean value is calculated from three replicates, including samples with 0 cfu. The total cfu per ml are calculated as the sum of the four species found.

The identification must be done to the 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 our pre-formatted Excel-file** to submit your results to the LGA via **Med-Chem@rps.bwl.de**.

*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 media used. Please give – in short – the most important criteria that lead to the identification result (e.g. identification key used, growth rate, biochemical reaction, sequence data).

**Criteria for acceptance of identification results**:
If you use molecular methods for identification (PCR and sequencing), please give information both on the **target gene** analyzed (or primer used) and the **accession No.** of the database used (or strain No., literature). These strict rules are necessary because the public sequence databases are not quality controlled and misidentifications are regularly occurring in routine diagnostics. We need these data at least to objectify your molecular identification.
**Please crosscheck the validity of the species name using MYCOBANK (www.mycobank.org).**

## 4. Objections to the results:

If a participant suspects that - despite our quality control measures - the pure culture(s) are contaminated, the LGA should be notified within one week. A new culture will then be posted immediately.
To assure for purity and sort out a possible contamination, test strains sent out simultaneously will be checked by all reference laboratories.
Objections to the evaluation of the 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!

Sincerely yours,

Dr. Guido Fischer

## Literature for Identification (note 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 Ultrecht, 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 (3rd 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 synnematous 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. Frsivad, 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.
- *Hypocrea*/*Trichoderma* (*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 Autors:**


- 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
- 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:


- **DIN ISO 16000-17:2008**: Innenraumluftverunreinigungen – Teil 17: Nachweis und Zählung von Schimmelpilzen – Kultivierungsverfahren

**Annex**: Species sent out during the RV in blue (No. of RV); new names in **green**.

*Acremonium murorum* (3, 15)
*Acrostalagmus luteoalbus* (24)
***Akanthomyces*** *(Lecanicillium) lecanii* (18, 38, 43)
*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, 43)
*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)
*Byssochlamys nivea* (29, 30)
*B. spectabilis (Paecilomyces variotii)* (6, 12, 18)
*Cadophora fastigiata (= Phialophora fast.)* (7)
*Candida albicans* (38)
*Cephalotrichum sp. (C. microsporum)* (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, 43)
***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, 43)
***Microascus paisii*** (21, 36, 39, 43)
*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, 43)
*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)