THE BUDAPEST TREATY:
CODE OF PRACTICE FOR IDAs

OBLIGATIONS OF THE IDA

PRACTICALITIES

Content

TEST METHODS AND CRITERIA FOR VIABILITY TESTING

The IDA is obliged to test the viability of each deposited mo. Hence the depositor must provide the information necessary to perform the test.

In general the testing of the purity of the deposited cultures is performed simultaneously with the viability test.

Although the identity of the mo is not checked extensively, procedures for purity control commonly bring, to a varying degree, attention to the taxonomic positioning of the mo. **If the IDA notices discrepancies between the identity or the properties of the organism and the description given by the depositor, it is recommended that the IDA notifies the depositor of this fact. The depositor can then check the authenticity of the deposited**

culture.
If during the course of discussions about the identity of the organism the IDA is required to take further action (e.g. carry out the identification of the mo), the depositor may be charged for this additional service.

NB It should be remarked that, according to the Budapest Treaty, the depositor is recommended but not obliged to give the scientific description of the organism or he may give this information later. National laws or restrictions concerning the kinds of mo accepted by the IDA, however, might oblige the depositor to indicate the taxonomic designation of the strain.

Test procedures and criteria for viability vary according to the type of mo. The following principles and minimal criteria are applied to the type of cultures listed below.

For fungi and yeasts

To test the viability of fungi and yeasts IDAs inoculate the organism onto the recommended media and incubate under the recommended conditions. Viability is proven by observation of growth of the organism (i.e. visible increase of cell material).

Purity is verified macroscopically and microscopically.

For bacteria

To test the viability of bacteria IDAs inoculate the organism onto/into the recommended media and incubate under the recommended conditions.
Colony formation or increase in cell number (in case of liquid cultures) must be observed.

Starting from an active culture, the minimal criteria for confirming viability range from 10 - 12 colonies from the original biomass to 1 colony provided that this single colony can be successfully subcultured. The colonies obtained should look "normal" and should be of the type expected for the particular bacterium being deposited.

In cases where only a few colonies are obtained from frozen or freeze-dried preparations and where the IDA does not propagate the material, one of the following options can be taken:

1. request replacement samples from the depositor, repeat the viability test and issue BP/9 if the new samples are cultured successfully. In this case, the date of receipt of the replacement samples is deemed the date of deposit.
2. issue a viability statement (BP/9) but immediately request new samples under *rule 6.2.* of the BT.

Since option 2 can not guarantee the viability of the replacement samples, option 1 is to be preferred.

Examination of purity is usually done macroscopically (colony morphology) and microscopically (cellular morphology). If both cell and colony morphology appear to be the type expected for the bacterium being deposited, then these observations are considered to be sufficient for issuing BP/4 and BP/9.

For plasmid bearing (genetically modified) mo

To test the viability of plasmid bearing (genetically modified) mo, the organism is inoculated

on an appropriate selective medium. Viability is proven by growth of the organism on this selective medium.

To check the purity, the organism is streaked on the appropriate medium with and without selective pressure. In addition, microscopic analysis is recommended.

NB The taxonomic designation and the indicated plasmid size should be verified in case of doubts about the designation indicated by the depositor or if these examinations must be conducted due to other regulations.

For isolated plasmid DNA

The presence of plasmid DNA is proven electrophoretically in an agarose gel. At the same time the approximate amount and the size of the DNA can be estimated.
The viability of the plasmid is proven by the transformation of a suitable host with the plasmid and the subsequent inoculation of this host/plasmid combination onto a selective medium.

NB
- In special cases the restriction pattern of the plasmid DNA can also be determined.
- Other examinations might be necessary in order to comply with other regulations.
- In case it is not available in the public collection the depositor should also supply a suitable host strain.

For bacteriophages

The viability of phages is tested by applying the spot-test or by plating bacteria and phages together in a top layer.
The number of plaque-forming units (pfu) per ml of lysate is determined by the serial dilution method or by the spot titre method. A minimum of 10^7 pfu/ml is required to have a sufficiently safe quantity to store the lysate for the purposes of the BT.

Purity of the phage lysate can be tested by streaking the lysate on an uninoculated agar plate.

NB The depositor should also supply a suitable host strain, if it is not available in the public collection.

For plant cell cultures

When plant cell cultures are deposited in the form of a callus, viability is proven by growth on an appropriate medium. A definite increase of cell mass must be observed.
For samples in the form of a suspension culture, growth in an appropriate medium must result in a definite increase in cell density. Frozen samples are first thawed and transferred to an appropriate medium. The growth of the cells is observed until the cell mass has at least doubled.

The viability test will be deemed negative, if the cell number has not increased considerably (i.e. doubled) after a period of at least two months.

Depositors should be encouraged to give the normal growth characteristics of the culture. This information will assist the IDA make the appropriate determination as to viability.

The purity of the cell culture is checked by microscopic examination. If contamination with

microorganisms is suspected, further tests are to be conducted.

NB
- To test the viability of plant cells several laboratory tests are available (FDA, TTC, reduction). Nevertheless, because a positive result obtained from one of these tests does not guarantee that a cell culture will regrow after cryopreservation, the result of the viability test must be based on the observation of obviously growing cultures.
- Since plant cell cultures may be mixed populations of genetically different cells (ploidy changes, chromosome changes and transposon activation occur in cell cultures), tests must be conducted to determine whether cryopreservation changes the specific characteristics of a cell culture.

For plant viruses

In order to revitalize the virus in desiccated infected leaves the leaves are to be ground with a few drops of inoculation buffer until a green paste is achieved. This paste is to be diluted with inoculation buffer to yield 2-3 ml of final inoculum. The inoculum is to be rubbed onto the leaves of the appropriate propagation host(s) which had been dusted with a sterile abrasive, such as Celite or Carborundum. After a few minutes the inoculated leaves are to be rinsed with tap water.

For plant seeds

Germination is the sole criterion for the determination of seed viability.

Germination can be considered to occur at the initial imbibition stage or at the emergence and development of a seedling. For the purposes of a patent deposit, however, the first sign of radical emergence constitutes germination and hence viability.

For plant seeds, viability should not be confused with storage ability or longevity of the deposit. Although in principle a single seed germinating in a batch of 400 can render the batch viable, it is preferred that 85% of the seeds germinate.
If less than 85% of the seeds germinate, the depositor must be notified that it is unlikely that his deposit will survive the 30 years storage period and that a new deposit will be required at a later stage. This eventuality can be covered under *rule 6.2.*

NB
- If required by the guidelines specified by the International Board for Plant Genetic Resources (IBPGR) or the International Seed Testing Association (ISTA), all seeds tested should be germinated under dormancy breaking procedures.
- Alternative methods for viability testing, e.g. tetrazolium topography, should be used only in exceptional circumstances and then only by suitably trained personnel.

For animal cell cultures

For animal cell cultures a minimum number of 4 x10^6 viable cells per ampoule (or 2 x 10^6 cells for adherent cultures) is required to confirm viability. For an acceptable deposit a good recovery and growth of the cells must be observed. Growth can be measured by counting the cells in a counting chamber. The number of viable cells must increase within one and a half weeks.

The purity of the culture is to be rigorously verified against the presence of bacteria

(mycoplasma, etc.) and fungi.

For animal viruses

Viability tests are performed *in vivo* on eggs and on primary cells.
Many different assays can be used to test the viability of animal viruses, e.g. RT assays.
A minimum number of infective particles corresponding at least to 100 times the minimum detectible level is required.

Viruses are checked for purity against the presence of bacteria (mycoplasma, methylotrophic bacteria, etc.) and fungi.

NB If the most appropriate host cell is not generally available to the public, it must be supplied by the depositor.

Contamination of deposited cultures

Each IDA must refuse the deposit of contaminated cultures.

If the culture transmitted by the depositor is impure, two solutions are possible:

1. The IDA notifies the depositor of its inability to accept the culture (the acceptance of the mo is precluded for scientific reasons) and **requests that he transmits another, pure culture. In this case the date of deposit changes.**

2. **The IDA may offer to the depositor the possibility to purify the culture. In this case the date of deposit remains the date of receipt of the material since the IDA has already disposed of the organism.** To be absolutely sure that the correct culture is deposited, however, the IDA must send a sample of the purified and preserved culture to the depositor with the request to verify the authenticity of the culture. The depositor is to be advised that if he does not confirm or reject the authenticity of the culture in a written statement within a certain time limit (e.g. three months), the culture will be considered to be the correct one (see also 'Responsibility for authenticity and purity of the deposited cultures').
   If the depositor notices that the wrong organism has been isolated, he must make a new deposit and, consequently the deposit date changes.
   The depositor is to be aware that he may be charged for the purification service that the IDA conducts.

The first solution is to be preferred. Therefore depositors should be encouraged to start the deposit procedure in time, thereby avoiding the more cumbersome second option.

Responsibility for authenticity and purity of the deposited cultures

In practice the IDA and the depositor share the responsibility for the purity of the deposited culture.
The depositor must ensure that a pure culture is transmitted to the IDA (if more than one component must be present, the culture is to be recognized as a 'mixed' culture).

The IDA must check the purity of the culture before accepting it and must notify the depositor if any contaminants are found. Also, the IDA must take all the necessary

measures to ensure that the culture remains uncontaminated.

The final responsibility for the authenticity of the culture lies with the depositor. The IDA is not obliged to check the identity or the performance (e.g. product expression) of the culture. Most IDAs are technically not in a position to perform this task. Nevertheless, it is important that the IDA takes such measures as are needed to enable the depositor to fulfil his responsibilities.

If it is the case that the IDA must propagate the material in order to have sufficient material to preserve the mo, **the IDA must send a sample of the propagated material to the depositor and request him to verify the authenticity of the culture.** As before, the depositor should be made aware that if he does not provide a written confirmation or rejection of the authenticity within a certain time limit (e.g. three months), the propagated material is to be regarded as identical to the original deposited culture *(see also 'Contamination of deposited cultures')*. In case the authenticity of the culture has been rejected, the IDA must ask the depositor to transmit a new sample of the culture. According to rule 6.2 the depositor has to add, among other things, a written statement alleging that the mo which is the subject of the new deposit is the same as that which was the subject of the previous deposit.

When a sample of a culture for which the IDA did not receive a confirmation or rejection of authenticity is to be furnished to a third party, the IDA should provide a statement that "the culture has not been checked by the depositor".

Request for information about a deposited culture or the related deposit documents

According to rule 9.2. the IDA should not disclose information that a particular mo has been deposited with it under the BT.

Information about a deposit is given only to the person who, after request, is entitled to obtain a sample of the deposited mo according to rule 11 of the BT.
According to rules 11.4. and 7.6., this person is also entitled to receive the following information:

- the accession number given to the deposit
- a copy of the receipt (international form BP/4)
- an indication of any properties of the mo which are or may be dangerous to health or to the environment
- upon request, an indication of the conditions which the IDA employs for the cultivation and storage of the mo
- upon request, the most recent scientific description and/or proposed taxonomic designation of the deposited mo.

This information is to enable the recipient to handle and analyse the microorganism correctly.

Further information (e.g. concerning the relations between the IDA and the depositor, the delivery of samples or any other kind of file inspection) should not be made available to third parties.

All requests for information from parties other than those mentioned in *rule 11*, should be accompanied by the written permission of the depositor.
The IDA should ensure that the necessary criteria are fulfilled before releasing any information. The IDA should also ensure that any request for information, even from the

depositor, is given in a written statement.

End of the period of storage

The IDA must store the deposited mo for a period of at least 30 years after the date of deposit (or for a period of at least 5 year after the most recent request for the furnishing of a sample of the deposited organism was received by the IDA).

It is advisable that IDAs make suitable arrangements about what to do with the deposited material when this period is over. Such arrangements should be specified in a contract between the IDA and the depositor. Possible arrangements are:

- destruction of the material
- return the material to the depositor
- make the material available to the public

In the case of absence of such arrangements, civil law is applicable.

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THE BUDAPEST TREATY:
CODE OF PRACTICE FOR IDAs

ANNEX 1

LIST OF PARTICIPANTS of the ECCO-WORKSHOP, held in Brussels BCCM™ (OSTC), February 12, 1996, on THE BUDAPEST TREATY: DISCUSSION OF THE RESULTS OF THE QUESTIONNAIRE "Inventory of problems and ad hoc solutions in the framework of the deposit of microorganisms for patent purposes under the Budapest Treaty".

Participants:

Dr. M.C. Agterberg	CBS
Mrs. M. Bosschaerts (reporter)	BCCM™
Mrs. Y. Cerisier	CNCM
Dr. T. Dando	NCIMB
Mr. J. De Brabandere (chairman)	BCCM™
Dr. D. Fritze	DSMZ
Dr. M.D. Garcia	CECT
Dr. B. Holmes	NCTC
Dr. D. Janssens	BCCM™/LMG
Mrs. F. Symoens	BCCM™/IHEM
Prof. Dr. F. Uruburu	CECT
Dr. F. van Asma	CBS

Mrs. M. Vanhoucke	BCCM™/LMBP
Dr. V. Weihs	DSMZ

Excused:

Dr. J. Day	CCAP
Dr. A. Doyle	ECACC
Dr. D. Smith	IMI

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ANNEX 2

WORKING GROUP COORDINATORS FOR COLLECTING/HARMONIZING THE PROCEDURES AND CRITERIA FOR VIABILITY TESTING OF MICROORGANISMS

Type of microorganism	Coordinator	IDA
Fungi and yeasts	Dr. F. van Asma	CBS
Bacteria	Dr. T. Dando	NCIMB
Plasmids	Dr. V. Weihs	DSMZ
Bacteriophages	Dr. F. van Asma	CBS
Plant cell cultures	Dr. D. Fritze	DSMZ
Plant viruses	Dr. D. Fritze	DSMZ
Plant seeds	Dr. T. Dando	NCIMB
Animal cell cultures	Mrs. Y. Cerisier	CNCM
Animal viruses	Mrs. Y. Cerisier	CNCM

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ANNEX 3

LIST OF PARTICIPANTS of the WORKSHOP, held in Veldhoven, August 29, 1996 on THE BUDAPEST TREATY: OPPORTUNITY FOR A CODE OF PRACTICE FOR IDAs?

Participants:

Dr. M.C. Agterberg	CBS
Dr. V. Arunpairojana	TISTR
Mrs. M. Bosschaerts (chair)	BCCM™
Prof. A.-M. Corbisier	BCCM™/MUCL
Dr. T. Dando	NCIMB
Mr. J. De Brabandere	BCCM™
Dr. A. Doyle	ECACC
Dr. D. Fritze	DSMZ
Dr. I. Gandjar	UI Fac. FSI
Dr. B. Holmes	NCTC
Dr. D. Janssens	BCCM™/LMG
Dr. P. Packer	ECACC
Mrs. B. Parodi	ICLC

Dr. R. Roblin	ATCC
Dr. D. Smith	IMI
Dr. G. Stacey	ECACC
Mrs. F. Symoens	BCCM™/IHEM
Dr. M. Uhl	EPO
Prof. Dr. F. Uruburu	CECT
Dr. F. van Asma	CBS
Mrs. M. Vanhoucke	BCCM™/LMBP
Dr. V. Weihs	DSMZ

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THE BUDAPEST TREATY:
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ANNEX 4

LIST OF IDAs WHO EMPHATICALLY EXPRESSED THEIR AGREEMENT WITH THE PRINCIPLES DESCRIBED IN THE CODE OF PRACTICE FOR IDAs (situation on 28 April 1998).

ABC	Advanced Biotechnology Center, Italy
BCCM™	Belgian Coordinated Collections of Microorganisms, Belgium
CBS	Centraalbureau voor Schimmelcultures, The Netherlands
CECT	Colecci\ n EspaÁ ola de Cultivos Tipo, Spain
CGMCC	China General Microbiological Culture Collection Center, China
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany
IMI	CABI Bioscience UK Centre (formerly: International Mycological Institute), United Kingdom
KRIBB	Korean Collection for Type Cultures, Korea
MSCL	Microbial Strain Collection of Latvia, Latvia
NCAIM	National Collection of Agricultural and Industrial Microorganisms, Hungary
NCIMB	National Collections of Industrial and Marine Bacteria Limited, United Kingdom

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