EMbaRC

European Consortium of Microbial Resource Centres

Grant agreement number: **228310**

Seventh Framework Programme

Capacities

Research Infrastructures

Combination of Collaborative Project and Coordination and Support Actions

Deliverable D.2.36 (formerly D.NA1.1.1)

Title: **Standard operating procedures and methods used for culture, characterization/control, and preservation by partners**

Due date of deliverable: M38

Actual date of submission: M44

Start date of the project: 1st February 2009 **Duration:** 44 months

Organisation name of the lead beneficiary: IP

Version of this document: V1.0

Dissemination level: PU

PU	**Public**	**X**
PP	Restricted to **other programme participants** (including the Commission)	
RE	Restricted to a **group defined by the Consortium** (including the Commission)	

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Part I
Acquisition

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Document properties	
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Workpackage	WP2 (NA1)
Deliverable	D.2.36 (formerly D.NA1.1.1)
Title	Standard operating procedures and methods used for culture, characterization/control, and preservation by partners
Version number	V1.0
Authors	Bégaud E., Bizet C. and Smith D.
Abstract	This presents the recommended methods for acquisition of bacteria, plasmids, viruses and fungi.
Validation process	Document prepared by Institut Pasteur in collaboration with CABI and BCCM and submitted to the Executive Committee for agreement.

Revision table			
Date	***Version***	***Revised by***	***Main changes***
07/02/2012	09.01.2012	CABI	Style, language and content

Contents

Abbreviation key

BRC	Biological Resource Centre
CABI	International Culture Collection, United Kingdom
CABRI	Common Access to Biological Resources and information
CBD	Convention on Biological Biodiversity
DNA	Desoxyribo Nucleic Acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
EMbaRC	European Consortium of Microbial Resource Centres
EMBL	European Molecular Biology Laboratory
GenBank	NIH genetic sequence database
MTA	Material Transfer Agreement
NIH	National Institut of Health

Guideline for acquisition

of Biological material

1 Background and Objectives / Introduction

In this guideline, the term 'biological material' and the related information is limited to bacteria, fungi, yeasts, plasmids and viruses.

The BRC implements a procedure for the receipt and storage appropriate to the type of biological materials. All parcels that contain unknown or hazardous biological materials must be opened in a suitable containment laboratory or appropriate microbiological safety cabinet with local facilities for the safe handling and disposal of biological materials. Safe procedures must be laid down and documented.

The BRC checks pathogen lists before accepting the biological material to ensure the material received is within the BRC authorised risk group level. If applicable, the depositor has to provide proof that prior informed consent to collect and deposit the biological material has been obtained or reasonable efforts have been taken to do so. Conditions of deposit have to be determined and agreed e.g. laid down in a material transfer agreement (MTA) to meet the Convention on Biological Diversity.

A risk assessment must be carried out on the biological material to determine any potential to cause harm to staff, public and environment.

Biological material is received directly into a laboratory where a laid down procedure is followed to ascertain whether the biological material can be handled safely i.e. the appropriate containment level is in place. A unique accession number is allocated to the biological material, which is never reassigned if the biological material is later discarded.

The biological material is obtained from scientists who document the history of the isolation (for strains), the construction (for plasmids), the maintenance, the preservation, the properties and the bibliography. Total numbers accessed per year is at the discretion of the Curator.

The BRC defines its acquisition policy in a publicly available document.

It is necessary to have a clearly defined acquisition policy on which the acceptance of new biological material offered to the collection is based.

Biological material for deposit has to meet the acquisition criteria of the BRC, according to its specialist expertise.

Acquisition criteria considered are:

- that the biological material is not a duplication in the collection or that over represented in other culture collections (except for type strains);
- it has known unique properties of value to the collection;

- it is representative of a systematic or ecological group not currently in the collection;
- it is of potential interest in the long-term;
- the facilities are available to handle the biological material safely and it can be maintained in the laboratory;
- plasmids must be able to replicate in a bacterial host that can be handled by the BRC (expertise, risk class, …);
- it is subject of a publication and is in accordance with the criteria mentioned above.

1 Accession

Details are recorded in the *BRC database* and a unique accession number is allocated to the biological material which is never reassigned if the organism is later discarded. The viability, purity, identity/authenticity, stability, growth requirements, and methods of maintenance and/or preservation of the biological material must be determined and the information recorded. These records are kept in a database and the accession form is kept to provide a hard copy of the data.

The biological material received shall have the following information:

- name (where one can be applied), other identifier or cell culture description;
- depositors name and address;
- source, substrate or host from which the biological material was isolated or derived (where identified) (not for plasmids);
- geographical location of isolation (the minimum requirement is the country of origin) (not for plasmids);
- for plasmids: the person/institute who/that constructed the plasmid, host properties, plasmid features;
- depositors number or other accession number(s) in the own culture collection or in other culture collections, if deposited elsewhere;
- assigned unique accession number;
- culture medium and growth conditions where appropriate to the biological material supplied;
- preservation or storage conditions where known;
- hazard status.

1.1 Viability and purity

The collection should have separate procedures on the aspects of preparation and storage of media at its disposal.

a) Viability – Bacteria / Fungi

The culture should be grown on the correct medium or host. Check that the growth characteristics have not altered.

It is recommended to always assess growth and sporulation both before and after preservation so that

comparison can be made during storage to determine if there is any deterioration.

b) Purity – Bacteria / Fungi

Careful microscopic examination must be carried out to ensure that the culture is not mixed. Cultures from single spore isolations can be prepared to give a better chance of them being pure. Bacteria are streaked on agar to identify contaminant colonies.

c) Viability and purity – Plasmids

If the plasmid arrives as pure DNA, the appropriate host bacterium is first transformed with this DNA.

The host/plasmid combination is streaked on sterile, selective solid medium described by the depositor.

After incubation for a minimum of12 hours at the appropriate temperature, the host/plasmid colonies are examined visually and/or microscopically.

The following criteria have to be checked:

- viability
  - the host/plasmid combination must be recoverable under specific selection for the plasmid.
- morphology / purity
  - the texture, the size and the opacity of the colonies should be in accordance with the characteristics of the host bacterium;
  - homogeneity of the colonies is required;
  - growth of (slow growing) contaminants (e.g. other bacteria, fungi) is not allowed.

If the visual examination does not fulfill the criteria described above, the collection contacts the depositor requesting new material

1.2 Stability

Pre- and post-preservation comparisons should be made, if possible and relevant. Morphology, pathogenicity, assay properties, and biochemical properties should be checked where appropriate. All observations must be recorded and retained for future reference.

1.3 Names

Fungi

Fungus species have to be kept under the name of the whole fungus, the holomorph (i.e. teleomorph, sexual state; perfect state) and as far as possible this has been followed in the Index Fungorum and Mycobank. The major synonyms are listed in alphabetic order with reference to the names CABI Europe-UK lists the strains under. The names are given without the authority a new policy following the

recommendations for the harmonising of nomenclatural codes (Hawksworth *et al.* 1994; Korf, 1996).

Bacteria

Bacteria names are confirmed by bacteriology department and are consistent with the Approved List of bacteria names, in **International Journal of Systematic and Evolutionary Microbiology**.
See also nomenclature Up-To-Date on the DSMZ Web site:

http://www.dsmz.de/microorganisms/main.php.

Plasmids

Name as published or given by the depositor or BRC.

1.4 Quality Control of Identity / Authenticity

Bacteria/Fungi

The identities of cultures are confirmed upon deposit by the BRC team of expert biosystematists, most of whom are world authorities in this field using methodologies as comparison of morphological characteristicsin keys and species descriptions, molecular sequencing etc …. Strains are checked again by these experts after preservation and during maintenance procedures to ensure that the quality is maintained.

Plasmids

Authenticity check

1. Plasmid DNA is prepared, based on a published plasmid isolation procedure.

2. The plasmid length should be verified by one of the following methods:
a) determination of the molecular weight of the covalently closed circle (ccc) DNA (Meyers et al. (1976), Southern (1979)); it is necessary to use at least two ccc plasmid standards with known molecular weights close to the plasmid length to be determined.

b) analysis of the restriction site pattern by agarose gel electrophoresis after single and (when possible) double restriction enzyme digestion.

3. The following criteria have to be checked:

- in case 2a) has been carried out:

- the plasmid size estimated from the molecular weight of the ccc DNA must match the depositor's data.

- in case 2b) has been carried out:

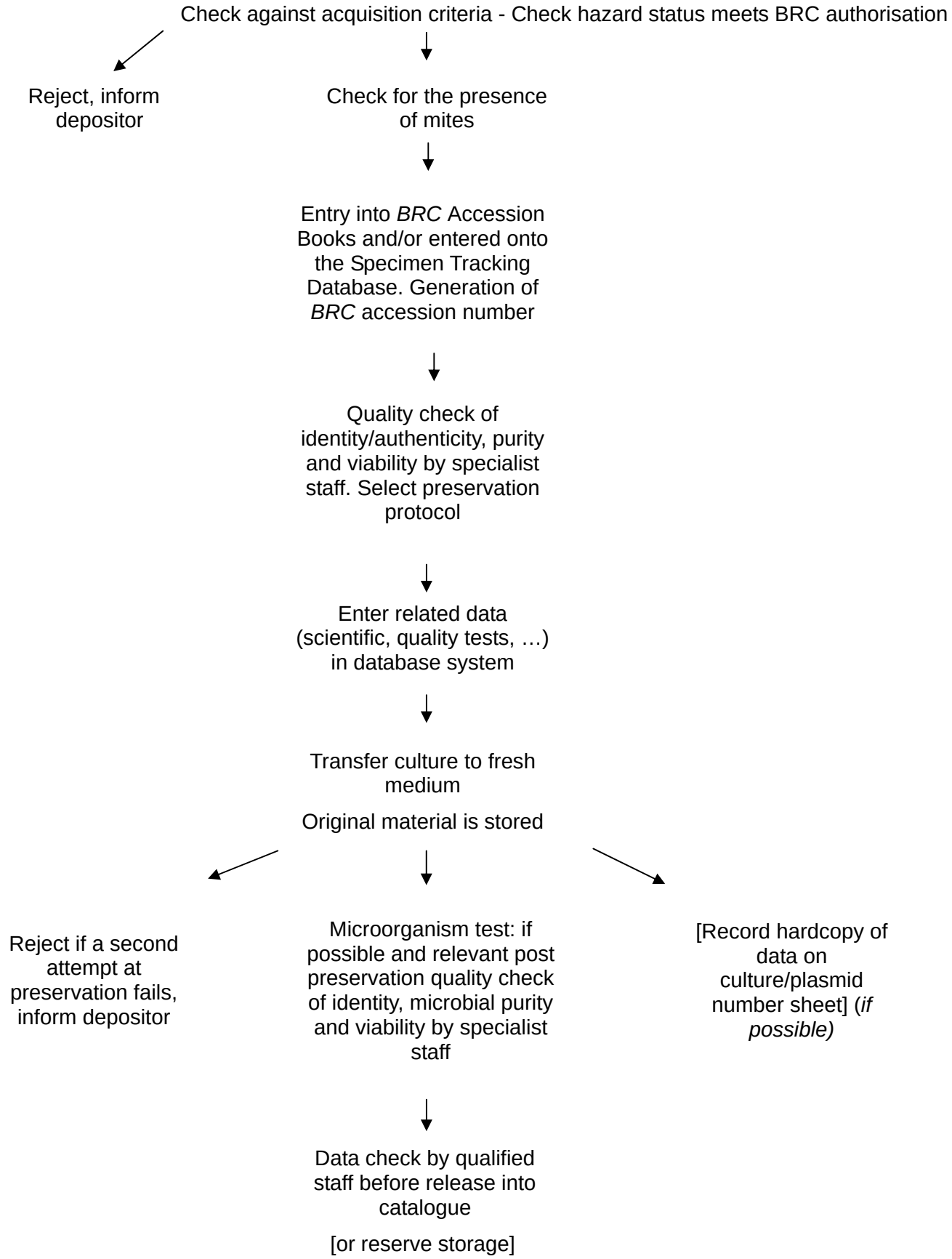
- the plasmid size estimated from linearized DNA must match the depositor's data;

- where two or more restriction sites are known, the presence and the location of at least two restriction sites must be confirmed.

If the criteria described above are not fulfilled, the collection contacts the depositor requesting new material

2 Accession flow diagram (example)

Strain/Plasmid offered to the collection or requested by the collection



4. *Example : Accession forms to accompany deposits*

4.1. Bacteria

Accession number:

Accession date:

ACCESSION FORM TO BE COMPLETED BY DEPOSITOR OF BACTERIA STRAIN. PLEASE PRINT OR TYPE.

1- Scientific name of the strain:

.....

2- Strain number or designation used by the depositor:

.....

Other accession numbers in own or other culture collections:

.....

3- Is this the type strain of this organism?

.....

If this strain has been designated in the literature as the type strain, please cite reference:

.....

.....

4- Origin of the strain (please give as much information as possible):

- source of isolation: .....
- geographical area: .....
- isolated by: ……
- date of isolation: .....

5- Information relating to the Convention on Biological Biodiversity (CBD)

- Country of origin and /or geographical location (no organisms can be accepted without this information):

a) Country: …… ……Geographical area of sampling (locality):……

b) Sampling agreement ("Prior informed consent" issued by competent authority):

- Are acquisition, transfer, and utilization of this strain controlled by the agreements of this authority? ☐ YES ☐ NO

- If YES, give the details of any agreed benefit sharing or other form of agreement between parties involved: ……

- And the name and address of the person or organization who issued the « Prior Informed Consent »: ……

6- History of culture since isolation:

ID collection < depositor < < < <

< < < < <

7- Properties of the strain

- Production of: .....

- Degradation of: .....

- Control of: .....

- New taxon: .....

- Other: .....

8- Maintenance

- Medium (give formula): .....

.....

.....

- Temperature: .....

- pH: .....

- Incubation time: .....

- Oxygen relationship:
  - ( ) aerobic
  - ( ) microaerophilic
  - ( ) anaerobic
  - ( ) facultative anaerobic

- Special conditions: .....

.....

9- Preservation

- by freeze-drying ( )
- by freezing in liquid nitrogen ( )
- other:

Please specify recommended conditions (growth, medium, suspending, fluid, cryoprotectant...)

10- Pathogenicity of the strain

- It is pathogenic for humans: YES ( ) NO ( ) UNKNOWN ( )
- It is pathogenic for animals: YES ( ) NO ( ) UNKNOWN ( )
  animal species:……
- It is pathogenic for plants YES ( ) NO ( ) UNKNOWN ( )
  plant species:……
- It is dangerous for any other raison? Please specify: .....

.....

11 - Molecular identification:

- which gene(s) : …….

- which primer(s) : ……

- which parameters for gene amplification : ……..

12 - References (please enclose one of each if available):

.....

.....

.....

NOTE

I agree to deposit this culture in the public BRC collection. I authorize the BRC to catalogue the strain data and to distribute samples to third parties under the general conditions of the BRC Material Transfer Agreement and any other conditions if applicable.

Name of depositor: Signature of depositor:

E-mail:

Address of depositor: Date:

4.2. Plasmids or transposon

Accession number:

Accession date:

Other accession numbers in own or other culture collections:

ACCESSION FORM TO BE COMPLETED BY DEPOSITOR OF PLASMID OR TRANSPOSON. PLEASE PRINT OR TYPE.

Plasmid/Transposon designation:

.....

Scientific name of recommended host strain:

.....

Plasmid/Transposon isolated or constructed by:

.....

If you did not isolate or construct this plasmid or transposon, indicate from whom you received it:

BRC < depositor < < < <

< < < < <

Is the distribution of this plasmid or transposon general or restricted?

.....

If restricted, which restrictions would you place on its distribution?

.....

Plasmid/Transposon properties: (see CABRI minimum data set and recommended data set for plasmids)

Origin:

☐ natural:

natural host:

.....

☐ recombinant:

construction / parental clone(s):

.....

Incompatibility group:

.....

Molecular weight:

.....

Size:

.....

Cloned gene(s) + EMBL/GENBANK accession number:

.....

Replication origin(s):

.....

Promoter(s):

.....

Ribosome binding site(s):

.....

Terminator(s):

.....

Markers (resistance to antibiotics, heavy metals, bacteriocin production, metabolic characters, etc ...):

.....

Host range:

.....

Comments (further information: plasmid applications, ability to the mobilized, transfer proficiency, copy number, etc...):

.....

.....

Cloning sites:

.....

Other restriction sites:

.....

Provide map or sequence file if possible:

Original reference:

.....

Other references:

.....

Host strain properties :

Genetic description of the host:

.....

Literature references of the host:

.....

Source and references:

.....

Auxotrophies:

.....

Resistance/susceptibility:

.....

Is the host strain pathogenic for humans, animals or plants?

.....

Culture conditions:.....

Other information:

Culture conditions:

.....

Stability: .....

Elements of quality control:

.....

Selective media:

.....

Agreement for deposit in the public collection

Name of the depositor: ……

Institution: ……

Address:……
……

Tel.: ……..

Fax: ……

E-mail: ……

I agree to deposit this plasmid in the public Plasmid Collection of the BRC. I authorize the BRC to catalogue the data and to distribute batches within the restrictions mentioned in the attached Material Transfer Agreement.

Name of depositor Signature of depositor:

E-mail:

Address of depositor: Date:

4.3. Viruses

Collection accession number:

Accession date:

ACCESSION FORM TO BE COMPLETED BY DEPOSITOR OF A VIRUS STRAIN

1- Full scientific strain name:

.....

2- Strain number or designation used by the depositor:

.....

Equivalences (i.e strain reference number in other official collections):

.....

3- If the strain has been described in the literature, thank you to giving references associated (include 1 copy each)

.....

.....

.....

.....

4- Origin of the strain (please give as much information as possible):

- source of isolation:.....
- isolated by (author's name): .....
- geographical area (country, town or other):.....
- date of isolation: .....

5- History of culture since isolation:

BRC < depositor < < < <

< < < < <

6- Strain properties:

.....

.....

7- Class of pathogenicity of the strain: …… (if possible, please provide the official documentations)

- Is it pathogenic for humans? YES ( ) NO ( )

- Is it pathogenic for animals? YES ( ) NO ( )

- Is it pathogenic for plants? YES ( ) NO ( )

- Other: ……

- Comments: ……

.....

.....

8- Growth conditions

- Cell lines: ……

- Medium (please attached the formula with this form if possible):

……..

- Other type of support for growth (brain of young mice, etc):

……..

- Corresponding protocol (to be attached with this form if possible):……

……

- Temperature: ……

- Incubation time: ……

- Atmosphere ie: CO_2 – percentage: ……..

- Special conditions: ……

- Title of protocols for maintenance of the cellular growth: if possible, to be attached with this form

.....

9- Method for the strain characterisation: if possible, to be attached with this form

- Titles of the standard operating procedures

a) ……

b) ……

c) ……

d) ……

10 - Molecular identification:

- which gene(s): ……

- which primers: ……

- which amplification conditions: ……

11 – Preservation

- by freeze drying YES ( ) NO ( )

- freezing at -80°C YES ( ) NO ( )

- freezing at -150°C YES ( ) NO ( )

- others:

.....

- Cryoprotectant used:……..

NOTE : ***I understand that subcultures of the deposited strain will be distributed to the scientific community for a fee to cover expenses of the BRCn.***

Name of depositor: Date:

E-mail:

Address of depositor:

Signature of depositor:

4.4. Fungi

	NAME & AUTHORITY: PRINCIPAL SYNOMYS/NAME CHANGE: IDENTIFIED BY:	BRC ACCESSION Nº BRC ACCESSION DATE: TYPE OF DEPOSIT:

DEPOSITOR: PLEASE FILL IN BELOW AS MUCH AS POSSIBLE

NAME OF ISOLATE: DATE SENT TO BRC: NAME & ADDRESS OF DEPOSITOR: ISOLATE DESIGNATION: PREVIOUS HISTORY (Other collection/owners/isolate designation): OTHER COLLECTIONS WHERE HELD: (Give Collection Number and Acronym):
ISOLATED FROM: (Substratum/Genus & Species of Organism): ANATOMICAL PART/SUBSTRATUM PART: GEOGRAPHICAL LOCATION: ISOLATED BY: DATE OF ISOLATION : ISOLATED METHOD: (Soil Plate, Damp Chamber, Surface Sterilisation, etc.):
SPECIAL FEATURES & USAGE (Metabolic products, Culture derived from Type, etc.): REFERENCES (Journal, Volume, Page, Year) Attach copies/reprints if possible):

RECOMMENDATIONS FOR MAINTENANCE & PRESERVATION	FOR BRC USE ONLY
Growth medium: Incubation temperature: Incubation time: Light requirements/pH etc.: Period between transfer: **Please tick appropriate method** Freeze-drying — Water storage Liquid nitrogen storage — Soil storage Oil storage — Silica gel storage Other - please specify	

PERMISSION TO DEPOSIT IN CABI COLLECTIONS AND DISTRIBUTE TO THIRD PARTIES UNDER THE CONVENTION ON BIOLOGICAL DIVERSITY

Was the organism collected after December 1993?……Yes □ or No □

If the answer to the above question is YES please answer the following questions and provide the requested information.

From whom did you receive prior informed consent to collect the material?

Land owner:

National Authority:

To the best of your knowledge do the above have the authority to grant this permission?

Yes □ or No □

Do you have the authority to deposit them in the CABI living and dead dried collections for further use and distribution? Yes □ or No □

If so under what terms:

References

- CABRI-guidelines: http://www.cabri.org/guidelines/micro-organisms/M204.html
- OECD-guidelines: http://www.oecd.org/dataoecd/7/13/GGTSPU-styx2.bba.de-7664-3281383-DAT/38777417.pdf
- WFCC-guidelines: http://www.wfcc.info/guidelines/

Significance of this deliverable

The OECD Biological resource Centre (BRC) initiative introduced the term of BRCs referring to a specific definition and distributed a mechanism for their development and coordination in a global network.

A key role of EMbaRC was to help in the process of transition of collections to BRCs. Implementing this protocol will take the first step of the process.

Part II
Preservation

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Version number	V1.0
Authors	Bégaud E., Bizet C. Ryan M. J. and Smith D.
Abstract	This document will present recommended methods of preservation for bacteria, fungi, plasmids and yeasts.
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07.02.2012		D. Smith	Style, language and content
14.02.2012		M. J. Ryan	Style, language and content
24.08.2012		E. Lang	Liquid drying (4.3.3)

Contents

Abbreviation key

BRC	Biological Resource Centre
CABI	CAB International, Culture Collection, United Kingdom
DNA	Desoxyribo Nucleic Acid

Guideline for Growth, Preservation and Storage of Biological material

In this guideline, the term ‘biological material’ and the related information is limited to bacteria, fungi, yeasts, plasmids and viruses.

1. Introduction

Microorganisms are ubiquitous, including species able to grow in a wide variety of environments utilising a vast array of natural and synthetic substrates. Some are host specific or have as yet unidentified growth requirements and therefore cannot be grown in-vitro. Generally, microorganisms grow best on media that are formulated from the natural materials from which they were isolated.

However, methods of storage and maintenance that allow growth and reproduction may allow the organism to change and adapt to laboratory conditions. To enhance the long-term stability of microorganisms the selection of variants from within the population, strain deterioration and contamination should be avoided. BRC’s aim is to reduce the risk of change during growth and preservation through the application of optimised protocols.

States and Propagules amenable to Preservation

i. Filamentous fungi and yeast

Although vegetative hyphae may be amenable to cryopreservation, generally they are not easily preserved. Fungi produce several structures that enable them to survive adverse conditions such as conidia, spores sclerotia, asci and thickened fruiting structures. These structures withstand partial desiccation and lack of nutrients and these properties make them amenable to preservation.

ii. Bacteria

Like fungi, some bacteria also form spores but generally their vegetative cells are more robust than the hyphae of fungi and can withstand the stresses that they may encounter during cryopreservation.

Plasmids and host/plasmid combinations

Plasmids have been identified in virtually all species of prokaryotes and also in some fungal strains. Although not essential for the survival of their host, they may encode a wide variety of genetic determinants that increase survival in adverse environmental conditions or that bestows a competitive advantage over other micro-organisms occupying the same ecological niche.The relative ease with which plasmids may be extracted, purified and stored either within a host or as naked DNA makes them one of the preferred 'tools' of genetic engineers. The current explosion in the number of man-made 'recombinant' plasmids represents a challenge to their safe-keeping and the maintenance of detailed and reliable information.

2. Preservation

Biological material should be preserved by at least two methods and/or as master cell banks and as stocks for distribution.

The BRC selects the preservation methods according to recommendations from the depositor and/or previous experience. The BRC documents the preservation procedures to ensure they are reproducible and that key parameters of the process are recorded and monitored.

3. Storage of preserved biological material

The biological material is stored under environmental conditions that assure the retention and stability of its properties. Where useful, parameters are monitored before preservation and at specified intervals post-preservation. Stocks of preserved biological material are replenished at suitable intervals, dependent on the preservation method and storage conditions used. Details of the inventory control, lead times and re-stocking practices are documented.

A duplicate collection has to be maintained, preferably on another site as a 'disaster' protection measure and to avoid accidental loss.

4. Preservation and Storage techniques

Introduction

BRC's utilise a combination of preservation techniques to ensure viability of the collected organisms and stores each one by a minimum of two techniques, of which at least one

should result in a reduction or suspension of metabolism. At least one method should be a long-term method such as: freeze-drying or cryopreservation below -80°C (ideally -139°C for bacteria, fungi and virus, below -70°C for host/plasmid combinations and below -20°C for DNA.

There are many more basic techniques that can be used as an addition to the longer tem methods of storage and the simplest are adaptations of growth on a*gar including* low temperature storage in refrigerators or cold rooms (~5^{o}C), storage under a layer of mineral oil and agar blocks in water. Other methods rely on desiccation or low temperature to reduce or suspend metabolism.

Maintenance/Storage

The simplest method of maintenance is by serial transfer from stale (spent) media to fresh solid or liquid media and subsequent storage by the most suitable conditions for the individual strain. Microorganisms can be maintained for several years by this method although some strains require special conditions (which should be listed in the 'growth conditions' fields in the BRC database). Successful maintenance depends on transfer from 'optimally growing' well-developed parts of the culture.

However, if possible this method of maintenance should be avoided because of:

- risk of variation, loss of pathogenicity or other physiological or morphological characteristics through strain drift of as a result of transfer of genetic variants.
- risk of contamination by air-borne spores or mite carried infections;
- the requirement of constant specialist supervision to ensure that the microorganism is not replaced by a Check hazard status contaminant or subcultured from an atypical sector.

The time period between transfers varies from organism to organism, for bacteria 24-36 hours to several days and for fungi, some every 2-4 weeks, the majority every 2-4 months, though others may survive for 12 months without transfer. Details of transfer periods are given in the *BRC* database and on the accession forms.

The use of cold storage can slow the rate of metabolism and thus increase the intervals between transfers. Storage at 4-7°C in a refrigerator or cold room can extend the transfer interval to 4-6 months from the average period of 2-4 months. Storage in a freezer (~-20°C) will allow many fungi to survive 4-5 years between transfers, though freeze damage may occur. Details of transfer periods are given in the BRC database and on the strain accession forms.

The disadvantages of serial transfer are not overcome by slowing the growth rate, although it is thought that the rate at which variation may occur is similarly reduced. There are

instances where long-term preservation techniques fail with some organism types and maintenance by continuous growth becomes the only option. In such cases growth conditions are carefully controlled and monitored and the time between transfers to fresh media is particular critical and optimised for each cell type. This requires a significant, dedicated staff resource.

Special consideration should be given to Plasmids and Bacterial hosts. For Plasmids, each deposit is maintained by two different preservation methods either preservation of the plasmid DNA (e.g. cryopreserved below -20°C, precipitated under ethanol) or preservation of the host/plasmid combination (cryopreservation below -70°C, using glycerol as cryoprotectant).

For Bacterial hosts, preservation should be by cryopreservation below -70°C, using glycerol as cryoprotectant, in a master stock and a distribution stock.

4.1. Storage under oil

This method is used for filamentous fungi, yeasts and filamentous bacteria where mature healthy cultures grown on agar slants in universal bottles are covered with mineral oil. The oil prevents dehydration and slows down metabolic activity and growth through reduced oxygen tension. If the oil is too deep the fungus may not receive sufficient oxygen to survive. Alternatively, if mycelium becomes exposed or agar is present on the sides of the container, moisture will evaporate and the culture will desiccate.

Retrieval from oil is by removal of a small section of the colony on a mounted needle, ensuring that excess oil is drained away. The fungus is then streaked onto a suitable agar medium. Several replicates should be established as growth rate can be compromised.

The fungal mycelium can normally recover when it is re-isolated from the edge of the colony on the first agar plate and transferred to fresh media. Central inoculation on an agar slope, may give better results, as excess oil can drain down the slope allowing the fungus to grow towards the top. There is an added risk of personal contamination by spattering of oil containing fungus when sterilising inoculation needles in the Bunsen flame.

4.2. Storage in water

This method is suitable for fungi. Storage of colonised agar blocks in water extends storage for periods of up to 2-3 years for *Phytophthora* and *Pythium* strains). These cultures may show some deterioration in pathogenicity but the majority is able to infect their host. Viability deteriorates rapidly after 2 years storage.

Growth may sometimes occur during storage in water. This will be reduced if the spores or hyphae are removed from the surface of agar and no nutrient medium is transferred.

4.3. Drying methods

Methods that utilise drying are commonly used for the preservation of many microorganisms. Freeze drying is a preferred method, but BRC's may employ other methods in a secondary capacity.

4.3.1. The silica gel method

The silica gel method has proved to be very successful for sporulating fungi which can be stored, in a morphologically stable state for 7-18 years. Methodology is simple, spore suspensions are inoculated to bottles containing non-indicator silica gel. Recovery is obtained, by transferring a few granules of silica get to a suitable nutrient media (as noted in the BRC database).

4.3.2. Storage in Soil or Sand

Storage in sterile sand or soil van be used to preserves microorganism, particularly those originally isolated from soil or that produce Conidia such as Conidia. Methodology is very simple, an inoculum is added to a vial containing sterile sand or soil, which is allowed to colonise before storage. Resuscitation involves sprinkling a few granules onto a suitable culture medium prescribed in the BRC database.

4.3.3. Liquid drying

Liquid drying applied as an alternative to freeze drying (see 4.4) has proved successful for the preservation of bacteria and yeasts. The processes and equipment are similar to freeze drying, with the modification that the first steps, the freezing and primary drying, are substituted by alternative principles. During freeze drying, the physical separation of water from solids (concentrated cell components) is achieved by "collection" of the water in ice crystals. This formation of ice crystals which potentially may damage a part of the cells is avoided by two alternative processes. One is known as liquid drying. A mild underpressure of 20 - 80 hPa, preventing extended evaporative cooling, is applied to the samples maintained at a temperature of 20°C. Thus most of the water is withdrawn without freezing of the preserved material. Activated charcoal as a good thermal conductor is part of the predried carrier material and of the suspending medium. Charchoal is said to be additionally effective as a protecting agent. Protective additives such as glutamate, trehalose or *meso*-inositol to the cell suspension may improve survival furthermore. The second drying phase is carried out as described for freeze drying. This process may be adopted to the

preservation of microorganisms sensitive to ambient oxygen concentration and is routinely carried out at the DSMZ for such organisms.

The second, still unnamed, alternative, is to drop a small volume of cell suspension onto predried skim milk pellets thick enough to absorb the water of the suspension completely and more or less instantly. By this way the cells are dehydrated via the water absorption by the skim milk and are incorporated into its matrix before the samples are subjected to a sole drying process conducted with a freeze-drying machine. Since drying is conducted with the constricted ampoules mounted to a manifold and the amount of free water per sample is very low it is assumed that evaporative cooling is not strong enough to freeze the samples. This method is time-saving because the freeze drying machine has to be loaded only once. It is routinely applied at the DSMZ for marine bacteria among others.

4.4. ***Freeze drying***

Freeze drying can be used to preserve bacteria, yeasts and spore producing filamentous fungi and is used in nearly all BRC's. Two freeze-drying processes can be employed, two stage centrifugal / (spin) freeze-drying and shelf freeze-drying with the former more commonly used.

The freeze-drying process consists of a freezing phase, followed by two drying phases. During the freezing phase, water crystallizes and the sample consisting of microorganisms and lyoprotectant(s) is converted into a solid physical state. The freezing can occur inside the freeze-dryer (in case of a shelf freeze-dryer) or externally in a freezer. During the subsequent first 'primary' drying phase, formed ice crystals are sublimated by lowering the pressure, removing around 90% of the water. During the second drying phase, the residual

water, appearing as a non frozen liquid entrapped in the dried matrix, is removed by isothermal desorption.

Controlled freezing at 1°C min^{-1} has been found to enable the freeze-drying of microorganisms that do not normally survive the above method. By using such a pre-freezing procedure some delicate or non-sporulating fungi can be preserved on the shelf freeze-drier.

The most critical points of the freeze-drying process are the selection of a suitable suspending medium, application of optimal cooling rates, maintenance of the solid state during drying, retention of a residual water content after drying of between 1 and 2% for fungi &, 1-5% for bacteria and the avoidance of re-hydration and contact with oxygen during processing and storage.

Suspending medium (lyoprotectant)

Many solutions and chemical mixtures have been used to protect organisms during freeze-drying. Skimmed milk and inositol has been used successfully for many years for fungi and a mixture of sucrose and peptone for bacteria. Furthermore, successful use of other chemicals such as trehalose and several other protectants have been reported. The use of protectants that avoid the use of Bovine products essential where BRC clients require the use of agents that do not carry a risk of TSE or BSE contamination.

Cooling rate – see Cryopreservation

Monitoring the frozen state

To achieve high survival levels, it is important that the temperature of the frozen material is kept below -15°C until the water content is reduced to <5%. A number of microorganisms have shown poor recoveries from freeze-drying because the lowest temperature reached with evaporative cooling in some freeze-drying machines is only -12°C. The freezing point of the cell cytoplasm may be well below this temperature for many species. When suspended in a lyoprotectant consisting of proteins and sugars, the suspension will form a solid glassy matrix during freezing when freezing temperature is below the glass transition temperature (Tg). Below Tg; the glassy matrix is a stable structure that completely entraps the bacterial cell and protect them during freezing and subsequent drying. Therefore it is important to keep the suspension solid during freezing and drying. The Tg is dependent on the water content left in the suspension. During drying, the Tg will raise and the temperature of the suspension is allowed to raise too as long as it stays under the Tg.

Residual moisture

It has been found that the residual moisture of the freeze-dried material must be prevented from falling below 1%. The removal of structural water may cause irreparable damage. Water of hydration can be removed, affecting the binding of molecules, and thus the cell structure will become unstable. If insufficient water is removed initial viability and stability may be good but rapid deterioration during storage occurs. Water content above 10% by dry weight is damaging to fungi. For bacteria, it is generally agreed that residual moisture levels below 1% (m/m) cause more viability losses because structural water is removed. On the other hand survival of freeze-dried bacterial cultures with too much residual water will decrease rapidly during storage. The maximal residual moisture limit for optimal storage of samples consisting of lyoprotectants and microorganisms is around 5%.

Storage conditions

Once dried, ampoules can be stored at room temperature, provided the residual moisture is low enough (<10%). Storage within the temperature range of -70 to +4°C may reduce the rate of deterioration that can occur, but the lower temperatures appear unwarranted, as excellent results have been recorded for storage at 4°C. In addition stored samples should not come into contact with oxygen, water or light as this will cause deterioration. Heat sealed ampoules or vials with reduced pressure or back filled with an inert gas will prevent this.

Re-hydration

Fungi

This is an important factor in the recovery freeze-dried microorganisms. Factors include the length of time allowed for re-hydration and whether additives should be added to aid recovery. For fungi, sterile distilled water is used, most samples can be left for 30 minutes post rehydration although more sensitive fungi may require up to24 h in 0.1% peptone. For bacteria, recovery is best achieved using broth suspensions or growth media. Typically, the same recovery volume should be used as the fill volume before freeze-drying.

4.5. Cryopreservation

Cryopreservation is considered to be the best method for the stable, long-term storage of microorganisms and is widely used by all major BRC's. In order to develop an optimised

protocol, there are various criteria that must be addressed. It is important that cultures are in a good physiological state; cryoprotection should be used and an optimum cooling protocol employed. Storage temperature should be at -135^{o}C or below in either a mechanical freezer or liquid nitrogen tank.

Pre-growth conditions for cryopreservation

Bacteria are harvested after growing for 16-72 hours under optimal conditions. For fungi,,well sporulating fungi survive best, but mycelial forms can also be successfully preserved. . For cryopreservation recalcitrant microorganisms, cells may be preconditioned prior to cryopreservation Spore or bacterial suspensions are easily prepared, but for mycelial forms, freezing intact colonies grown in broth shake cultures, agar plugs cut from growing colonies on agar or growing cultures in the ampoule or vial avoids such damage. It may be necessary to incubate freshly cut agar blocks from colonies to allow the severed hyphae to grow and seal.

Cryoprotection

Injuries during freezing are caused by either the formation of ice crystals or as a result of 'concentration' effects which can result in changes in pH or electrical conductivity and may also have an effect on protein stability. The major damage is caused by disruption of membranes caused by the ice crystals and dehydration and shrinkage of the cell caused by solute concentration.

By consequence, it is important to minimise the effects of cryoinjury using Chemical substances (cryoprotectants). Some organisms may produce these naturally such as Trehalose but generally they are added to the solution in which cells are suspended. There have been many chemicals used as cryoprotectants for fungi and bacteria and these include glycerol, trehalose and DMSO

In summary, cryoprotection is achieved by:

- non-critical volume loss by the reduction of ice formation;
- an increase in viscosity which slows down ice crystal growth and formation and solute effects;
- reduction of the rate of diffusion of water caused by the increase of solutes.

Cooling

It is important to use a cooling rate that induces least stress on the cell. Slow cooling rates (-1°C min-1 has proved best) are generally more effective for most microorganisms particularly in the presence of a cryoprotectant. It is normally at the faster rates of cooling

that the water in the cell cytoplasm freezes, forming ice crystals and inflicting greatest damage on the cell. Induction of vitrification, production of an amorphous glass like structure of frozen water is often beneficial. At slow rates of cooling loss of water may occur due to freeze concentration of solutes with the subsequent shrinkage of the cell.

Cells are usually cooled to temperatures between -50 and -80°C before being placed in the liquid nitrogen vessel. This results in rapid cooling to the storage temperature at a time when the cell solutions are normally already immobilised. BRC should record details of optimised cooling regimes in their Technical Operating Procedures.

Storage temperature

A wide range of sub-zero storage temperatures are available and have been used for microorganisms. Storage temperatures of -20 to -40°C can be used in the short-term but are inadequate for long-term storage as viability diminishes with time and properties are lost or change.

Freezer storage at -80°C has been used extensively for both fungi and bacteria, particularly in Japan, where storage of fungi and bacteria has been proven successfully. It is, however, a temperature where ice structure can change and inflict damage on the stored cells. Temperatures below -140°C (below Tg of water) are preferred and are available both in freezers (-135 to -150°C) and in or above liquid nitrogen. Vapour phase storage in liquid nitrogen vessels does allow fluctuation of temperature and where cultures are kept entirely in the vapour phase, storage at -170°C to -190°C is quite common.

Storage in the liquid phase will keep the samples at the ultra-low temperature of the liquid, but may increase the risk of cross contamination.

Storage period and some influences on stability in storage

Storage periods in liquid nitrogen are estimated at infinity so long as the storage temperature is below the temperature which will allow any change, which is -135°C. Where isolates have died during cryopreservation, generally, it has been where storage temperatures have been allowed to rise above -135°C for long periods and following storage periods in excess of 12 years. When temperatures of -196°C are maintained, possible causes for deterioration are thought to be due to such problems as deleterious levels of background radiation.

Cultures of all organism types held by BRCs that have been frozen and stored at temperatures below -140°C can remain viable and stable for extremely long periods of time.

Thawing

It is generally accepted that rapid thawing gives best recovery of microorganisms. Rapid thawing avoids re-crystallisation of ice which could cause mechanical damage. A warming rate of c. + $200°C\ min^{-1}$ can be achieved in a water bath at +37°C or in a programmable cooler using a thawing programme.

Recovery

Microorganisms should be inoculated onto an appropriate nutrient source and stored in optimal conditions. This is strain specific and should be detailed on the BRC database

5. Selection of Preservation techniques

There are many factors that influence the selection of preservation technique for any particular organism. These include staffing levels, logistics, cost, the taxonomy of the organism to be preserved and its importance.

5.1. Recommended methods of preservation for Taxonomic Groups of Fungi

Chromista (Straminiples)

These fungi are best stored in liquid nitrogen using a cooling rate of c. -10°C min^{-1}, although some strains do not survive the freezing stages. In these cases storage under mineral oil may be satisfactory for periods up to six months. Representatives of the *Oomycota* survive longer periods under oil ~4 years. It is not advisable to leave cultures for such a long storage periods before subculture because of the risk of loss of irreplaceable strains. Alternatively, cultures can be kept viable in water storage and transferred every 2 years; these two techniques are not so successful for retaining particular properties of the fungi, but can be used if stability is not a priority or as a back up to liquid nitrogen storage. Dring methods are not suitable.

Zygomycotina

Liquid nitrogen storage is recommended for the preservation of fungi of the *Zygomycota* such as *Mucor*, *Rhizopus* and similar genera. Most isolates can be successfully freeze-dried and remain viable for many years, in excess of 25 years.

Not all survive dehydration, particularly in silica gel, for example *Coemansia*, *Martensiomyces*, *Conidiobolus*, *Entomophthora*, *Piptocephalis* and *Syzygites*. Of these genera, only *Piptocephalis* and *Coemansia* strains have been freeze-dried successfully.

Ascomycotina

The majority of *Ascomycetes* including common fungi such as Penicillium and Aspergillus can be grown in culture can be freeze-dried or cryopreserved in liquid nitrogen. The majority of genera and species that do not sporulate well in culture survive dehydration techniques poorly. However, most of these have survived long-term storage in liquid nitrogen. Fewer species survive in silica gel although healthy heavily sporulating strains generally survive well for periods over 8 years and several strains have survived 18 years, for example, *Neurospora crassa*, *Aspergillus nidulans* and *Penicillium griseoroseum*.

Basidiomycotina

Basidiomycota, apart from the yeasts, generally grow only as mycelium in culture and therefore present problems in preservation. Usually such fungi can only be preserved by serial transfer on agar with or without oil, or stored in liquid nitrogen. Those fungi producing thick walled hyphae can be freeze-dried but their viabilities are usually low. However, basidiospores harvested from fungi growing in their natural environment can usually be freeze-dried, and will survive other preservation techniques better than the mycelium does.

Mycelium of wood inhabiting *Basidiomycetes* can be grown and maintained on wood chips. The best technique for preservation of all *Basidiomycetes* is cryopreservation below -140°C.

Rusts

Rusts do not normally grow in culture, but living collections can be maintained in good condition in liquid nitrogen on the host or harvested spores. *Ustilaginales* produce very disappointing cultures and survive best in liquid nitrogen though it is possible to keep them by other means. If the spores can be harvested successfully some survive freeze-drying quite well.

5.2. *Recommended methods of preservation for Yeasts*

The single celled vegetative yeasts survive freeze-drying well. Most species survive silica gel storage but recovery of cells is usually very low. The best technique is again in or above liquid nitrogen. Those that produce ascospores behave similarly to the filamentous *Ascomycetes*. *Basidiomycete* yeasts in general survive cryopreservation best but can be freeze-dried. Silica gel storage is less successful.

5.3. Recommended methods of preservation for Lichen Forming Fungi

These fungi tend to produce mycelium only in culture. They do not survive for long periods by serial transfer leaving only cryopreservation as the method for successful preservation of this group. They respond well to cooling rate of -10 to -20°C min^{-1} in 10% v/v glycerol and storage in or above liquid nitrogen.

5.4. Recommended methods of preservation for Bacteria

Most bacteria survive freeze-drying well. However, there are several genera that recover in very small numbers or do not recover at all after freeze-drying. These organisms can be cryopreserved in or above liquid nitrogen (Example: *Helicobacter*, strains with plasmids, delicate / fastidious or recalcitrant strains like *Campylobacter*, *Aliivibrio*, *Vibrio*, *Flavobacterium* ….)

5.5. Recommended methods of preservation for Plasmids

Plasmids can be preserved under 2 formats: as isolated DNA and as plasmid-carrying culture.

Isolated plasmid DNA can e.g.:

- be precipitated under ethanol and cryopreserved below -20°C;
- be spotted on filter paper and stored at room temperature;
- be evaporated and stored at room temperature.

Plasmid-carrying cultures can be:

- cryopreserved below -70°C, using glycerol as cryoprotectant;
- cryopreserved in the vapour phase of liquid nitrogen.

Plasmids containing genes that may tend to destabilize the physical and/or functional integrity (either by insertion, deletion or point mutation) should preferably be deposited, maintained, tested and delivered as pure DNA.

Summary

Special storage conditions and recommended methods are included in the *BRC* database record for each strain and this includes recommendations for storage time before represervation.

6. Inventory Control

Freeze-dried and cryopreserved stocks

Generally a defined number of ampoules is prepared for each strain; one can be used to check recovery. Stocks are replenished when, for example, 5 ampoules remain. An ampoule is opened from the oldest batch prepared i.e. the closest to the date of accession.

To ensure a minimum number of sub-cultures from the original biological material, the BRC uses master (or seed) and distribution stocks.

The BRC produces the master stock from the original biological material. This master stock shall be used to generate the distribution stock. The BRC uses the distribution stock to supply biological materials.

Longevity in liquid nitrogen is considered close to infinity. Stocks are replenished when only (*X*) ampoules remain in stock.

Stock control is incredinly important, some collection stipulate the freeze dried should not be distributed when they over 10 years old. Automated ‘Bar code’ driven stock control can allow BRC managers to control and monitor stock control.

Oil stored strains

Oils are retained as back-up to strains only in liquid nitrogen, in this case new stocks should be prepared from the oldest stock when necessary, and others are retained to provide information on storage. The latter are restocked when the cultures are examined but not more often than every two years.

References

Source literature

- CABRI-guidelines: http://www.cabri.org/guidelines/micro-organisms/M204.html
- OECD-guidelines: http://www.oecd.org/dataoecd/7/13/GGTSPU-styx2.bba.de-7664-3281383- DAT/38777417.pdf
- WFCC-guidelines: http://www.wfcc.info/guidelines/
- Alexander G.A. 1981 Improved lyophilisation procedure for storage of reference cultures and clinical isolates. J. Clin. Microbiol. 13:805-806
- Burlacu, E., C. David, and T. Vasilescu. 1989. Comparative evaluation of three in vacuo dessication procedures on bacterial cultures. Arch. Roum. Path.Exp. Microbiol. 48: 65-78.
- Dando, T.R., and L.J. Bousfield, 1991. Maintenance of industrial and marine bacteria and bacteriophages. A Manual of Laboratory Methods. Academic Press Limited, London, UK.
- (Heckly, 1978) Perservation of Microorganisms. Advances in Applied Microbiology 24: 1-51.
- Malik, K.A. and Hoffmann, P, 1993. Long-term preservation of yeast cultures by liquid drying. World J. Microbiol. Biotechnol. 9: 372-376.
- Smith, D & Ryan, M.J. (2011) Implementing best practices and validation of cryopreservation techniques for microorganisms The Scientific World Journal *In Press*
- Smith, D., Ryan, M.J. & Day, J.G. (2001). The UKNCC Biological Resource: Properties, Maintenance and Management. 382 pages. UKNCC Secretariat, Egham, Surrey, UK. ISBN 0954028503.
- Spring, S., 2006. Preservation of thermophilic microorganisms. Methods in Microbiology, 35: 349-368.
- Ryan, M.J., Ritchie, B, & Smith, D. (2012). Maintenance and storage of fungal plant pathogens. In Fungal Plant Pathogens – Principles and protocols (eds, C. Lane,

Beales, K. Hughes) CAB International, Wallingford, Oxon.

- Ryan, M.J. & Smith, D (2007) Cryopreservation and Freeze drying of Fungi employing centrifugal and shelf freeze-drying. In: Cryopreservation and Freeze Drying Protocols. Second Edition (Eds Day, J.G. & Stacey G.N) pp127-140. Humana Press N.J.

Significance of this deliverable

The OECD Biological resource Centre (BRC) initiative introduced the term of BRCs referring to a specific definition and distributed a mechanism for their development and coordination in a global network.

A key role of EMbaRC was to help in the process of transition of collections to BRCs. Implementing this protocol will take the first step of the process.

Part III
Control

EMbaRC

European Consortium of Microbial Resource Centres

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Contents

Abbreviation key

BRC	Biological Resource Centre
DNA	Desoxyribo Nucleic Acid
RNA	Ribonucleic Acid

Guideline for Control of Biological Material

1 Preparation and sterilisation of culture media and reagents

Accurate preparation of culture media is one of the fundamental steps in the growth and maintenance of living biological materials and their replicable parts and must be given special attention. The BRC shall define standards for all preparations; media formulae must be documented and procedures must be in place for making changes to procedures and for their approval and adoption. Media batches must be clearly labelled and expiry dates defined and clearly indicated.

Supplies of materials for use must reach high standards and must not be contaminated.

Media should occasionally be performance / quality tested using appropriate standard test strains and procedures.

2 Authentication

Authentication includes the processes by which microorganisms, viruses, metazoan cells, DNA, RNA and related macromolecules (biomaterials) are characterized and identified. Included are tests for viability and contaminants, plus one or more morphological, biochemical, physiological and/or genetic criteria specific for the genus, species, strain or molecule in question.

Before being considered “authentic” the culture holding is often characterized further by the depositor(s), originating investigator(s) or other members of the scientific community. Pertinent data are published and/or donated to a biological resource centre and incorporated into the centre’s databases. In addition to the critical data required to support claims of authenticity, other details include recovery from the cryopreserved or lyophilized state, viability, growth characteristics, and comparative data with other related holdings (see example accession forms in the document ”Guideline for Acquisition of Biological Material). BRC’s may wish to undertake further authentication tests to support the claims of the depositor or add to the data set of necessary.

3 Validation of methods and procedures

The BRC shall document all methods and procedures used in validation. The validation of quality check, characterisation and preservation methods can be carried out by using one of the following approaches:

- performing blind tests;
- performing tests with appropriate standard test or assay strains
- comparing the results of the same method performed at different times;
- comparing results obtained with different methods;
- comparing the results obtained for the same method performed by different persons.

The results of method and procedure validation shall be recorded.

4 Quality checks

Methods for purity, viability and identity tests

Recommended Minimum Authentication/Characterization

Bacteriology	Confirm viability, colony plus cell morphology, genomic and phenotypic, biochemical characteristics.
Cell Biology	Verify viability plus species, and test for bacterial, fungal or mycoplasmal contaminants.
Molecular Biology	Confirm viability by growth with appropriate antibiotic & document recovery, molecular identification & verification by an appropriate method including restriction endonuclease mapping, direct sequencing.
Mycology	Verify viability and purity by cultivation and microscopic examination; identify species or higher rank by morphology, authenticate species or genus using molecular markers (when they are available), plus genomic or biochemical characteristics.
Protistology	Verify viability, test for contaminants and confirm identity using microscopic examination and/or zymogram patterns.
Virology	Verify recoverability and typical CPE by cultivation using recommended substrate plus identity by reaction with specific antisera or other reagent where feasible.

Annexes

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