General instructions for use

How to use Dehydrated Culture Media

Granular culture media

Merck dehydrated culture media are, with just a few exceptions, produced in granular form.
The individual ingredients of each batch are ground and mixed together to produce a homogeneous, finely powdered mixture, which is then subjected to the granulation process. Here the powder mixture binds together to form small granules.

Granular culture media offer several advantages when compared with the conventional powder culture media:

- Considerably less dust is formed when handling the media, and the dangers of allergic reactions and inhalation of toxic substances are thus largely eliminated.
- Better flow properties: the media do not adhere to the walls of vessels or apparatus and are thus easier to weigh out.
- Better wetting of the granulate with water reduces the time required for suspending and dissolving the media, hence preventing the formation of hard to dissolve lumps.
- Homogeneous distribution of the package contents is ensured even after long storage, the components do not therefore separate out.
- Longer shelf life due to
  - lower water content
  - the constant homogeneous distribution of the contents.

Some culture media take the form of very fine granules as a result of their composition. The advantages listed above also apply here, particularly as regards the flow properties and the homogeneous distribution of the contents.

Storage

Wherever possible, dehydrated culture media should be stored in a dry, dark place at a temperature of about +15°C to +25°C; the containers should always be well sealed and tightly reclosed after use to prevent the entry of moisture. Absorption of water leads to pH shifts and eventually clumping. If the pH changes after prolonged storage, this can be corrected (see section entitled "pH adjustment"). Dehydrated culture media that have formed clumps and/or have changed colour, however, must be discarded, as the ingredients may have undergone chemical changes.

Under ideal conditions, most of the dehydrated culture media in original sealed bottles or after first opening can generally be stored for at least five years after their manufacture (see expiry date on the label) without any deterioration.

Preparation

Culture media are for professional use only!
Preparation see also under literature!

Dissolving the dehydrated culture medium

Dehydrated culture media should always be prepared with clean, freshly distilled or completely demineralized, pH-neutral water as per Ph Eur or ISO.

Rinse the clean containers to be used (usually conical flasks) thoroughly with completely demineralized water to remove any traces of other substances (e.g. detergents). The volume of the vessels should be at least double the anticipated volume of the solution to be prepared and should be sufficiently large that the reconstituted culture

media can be thoroughly shaken. If possible, do not use more than 1-2 litres per vessel. Weigh out the desired quantity of the dehydrated culture medium, saturate it with a small volume of water and shake vigorously until a homogeneous suspension is obtained. Add the remaining water, taking care to rinse down any material adhering to the walls of the vessel.

All culture media are heat-sensitive!
Do not heat them any longer than absolutely necessary!

Culture media without agar-agar or gelatin can usually be dissolved in cold water or require only gentle heating. Use should be made of this fact to ensure that the medium is prepared under mild conditions.

Culture media containing agar or gelatin, by contrast, must be heated in order to dissolve them completely. Heating should be carried out in a boiling water bath or free-flowing steam (e.g. in a steam pot or autoclave without overpressure). Culture media that are not subsequently autoclaved must be checked for complete dissolution; this is achieved when the viscous solution flows smoothly and when no agar particles can be seen sticking to the walls of the vessel after shaking.

In the case of some culture media turbidity is essential (e.g. bismuth sulfite agar). The insoluble components should then be distributed as finely as possible to ensure that the turbidity is homogeneous.

If in the case of agar- or gelatin-containing media a volume of more than two litres per vessel is required, the medium should be dissolved under mild conditions in the following manner:

- make a slurry by mixing the dehydrated culture medium with about 1/10 of the total volume of water specified, allow to swell for 15 minutes
- bring the rest of the water to the boil
- stir the slurry into the boiling water
- heat until the medium is completely dissolved.

pH adjustment

The pH value of reconstituted Merck dehydrated culture media prepared with neutral water should be identical to the prescribed values at a temperature of 25°C. It is advisable, however (particularly when older material is being used), to check the pH and correct it if necessary.

The pH value depends very much on the composition of the culture medium, the temperature at which the pH is measured, and the treatment that the culture medium has been subjected to during reconstitution (dissolving, sterilization). The pH should therefore be measured after sterilization; it is best determined with a pH-meter (taking care to compensate for temperature when calibrating the electrode) or with special indicator test strips pH 4.0-7.0 (Merck, Cat. No. 9542) and pH 6.5-10.0 (Merck, Cat. No. 9543).

pH measurement and correction of the pH should be carried out at 25°C with solid as well as liquid culture media. The pH should be adjusted to the specified value as necessary. The pH should be corrected by adding 1 N or 1/10 N hydrochloric acid or sodium hydroxide solution to a sample of known volume taken from the reconstituted culture medium (e.g. 50 ml). The volume of acid or alkali added to the sample can then be used to calculate the quantity necessary to adjust the pH of the remaining prepared culture medium. It therefore should be kept liquid during the pH measurement of the sample, in order to be adjusted if necessary.

How to correct the pH:

1. Sterilize the reconstituted culture medium

2. Remove a sample under sterile conditions and rapidly cool to 25°C

3. Measure the pH of this sample and if necessary adjust to the correct value by titration

4. Add the volume of hydrochloric acid or sodium hydroxide solution that has been calculated from the titration to the reconstituted culture medium under sterile conditions. (The hydrochloric acid and sodium hydroxide can be sterilized by filtration through a glass filter or a special membrane filter).

Sterilization

Before sterilization is carried out, the culture medium should be divided into smaller portions, for example poured into the containers that are to be used in the test (but not Petri dishes).

If not otherwise stated in the directions, sterilization should be performed in an autoclave at 121°C for 15 minutes. This does not include the times required for heating up and cooling, which depend on the apparatus and the volume of medium used. This information must be obtained from the manufacturer of the autoclave. Complete sterility can be guaranteed only when the steam chamber and the vessels are completely degassed. This is achieved by passing a larger amount of free-flowing steam through the autoclave with the valve open at the beginning of the heating-up phase. After sterilization and repressurization the vessels should be removed from the autoclave immediately and cooled rapidly (e.g. to the temperature used for pouring) to minimize exposure of the culture medium to heat. The vessels should be cooled under cold, running water.

High temperatures and prolonged heating result in a deterioration in the quality of the culture medium.

Autoclaving is the most reliable way of sterilizing culture media. If an autoclave is not available, the culture media can be heated in a household pressure cooker as an emergency solution.

The safety regulations for the operation of autoclaves as well as the safety instructions supplied by the manufacturer in the package insert should be strictly followed.

Pouring the plates

Commercially available, sterile Petri dishes should be used.

Take care that no condensing water is formed in the cover.

The culture media should be poured into plates at about 45-55°C to avoid the formation of condensed water in the lids of the Petri dishes. The medium should be swirled before pouring to ensure that it is evenly mixed. Air bubbles in the plates can be removed by briefly fanning them with the luminous flame of a Bunsen burner.

Wet agar surfaces promote microbial swarming and colony liquefaction; these can be dried by heating in the incubator at 30-40°C before inoculation is performed. The base of the Petri dish is inverted and placed on the edge of the lid.

Drying takes about 20-30 minutes or somewhat less if an incubator with circulating air is used.

The thickness of the medium must be at least 3 mm.

Mark the plates with the name and preparation date and/or expiry date.

Slanted agar tubes

In certain areas of application in microbiology it has proved itself appropriate to prepare surface cultures in culture tubes (e.g. for strain management). A large culture-medium surface is required for this purpose, which can be obtained using the “slanted agar” method. Here the tubes, filled with the sterilized, still liquid agar culture medium, are placed in the rack in such a way that the surface is about 3 cm in length over a layer of the same depth. The culture medium is then allowed to solidify in this position.

Acidic culture media

Agar culture media with a pH value below 6.0 must be prepared under very mild conditions, as agar-agar is hydrolyzed when heated in an acidic medium and the gel stability of the culture medium thus declines. Reliquefaction should therefore be avoided wherever possible. If it is impossible to prepare the medium under appropriate conditions or reliquefaction cannot be avoided, agar-agar (Merck, Cat. No. 1.01614) can be added to the culture medium before dissolution. Approximately 5.0 g/litre is generally sufficient for this purpose.

Using the plates

Allow the plates to warm to room temperature.

Internal quality control

Self-prepared culture media should be quality tested with specific test organisms.
Prepared plates must be controlled for sterility by incubation.

Special laboratory equipment/instruments required

Autoclave
pH-meter /glass electrode
Petrimat
Incubator
Laminar-flow cabinet
Water bath
Refrigerator
Bunsen burner
Homogenizer
Filtration unit
Drigalski spatula
Stirrer
Syringes, dispensers
Hot-air sterilizing ovens
Sterilizing containers
Staining equipment
Inoculation loops and needles
Anaerobic chamber or jars
Balances

Glassware and plasticware required

Erlenmeyer flasks (1-5 litre size)
Glass pipettes
Disposable polypropylene tubes
Disposable plastic Petri dishes
Pipetting unit
Disposable plastic transfer pipettes
Durham tubes
Tubes
Flasks
Measuring cylinders
Disposable gloves
Swabs

Reagents required

Ethanol
Disinfectants
Dehydrated culture media
Staining solutions

Use of instruments

The manufacturers' instructions given in the package inserts of the instruments used for the media preparation, inoculation, incubation, interpretation etc must be observed.

ISO standards

Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media
Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory (ISO/TR 11133-1: 2000)
Part 2: Practical guidelines on performance testing of culture media (ISO/TR 11133-2: 2003)

Composition of the culture media

Some of the basic constituents of the culture media are natural products and their properties may therefore vary from batch to batch. In order to obtain reproducible results when cultivating microorganisms, these variations must be corrected in certain cases by adjusting the amounts of substances used in the manufacture of the dehydrated culture media. Therefore "typical" compositions of the dehydrated culture media are specified in the declarations.

Quality control of the culture media

As with all our products for microbiological applications, dehydrated culture media undergo stringent quality controls from the raw materials to the finished product. With these controls we wish to ensure that in spite of the variations that always occur with natural substances (as we discussed in the previous section) we can provide the

user with dehydrated culture media of as high and consistent a quality as possible. The strains that our laboratories used for testing in quality control and their reactions are listed in the descriptions of the individual culture media. These define the microbiological properties of the individual culture medium concerned.

Many culture media are furthermore tested quantitatively by colony counts of bacteria or yeast cultures. These media are inoculated by the spiral plater method with cell suspensions of approximately 10^4 cfu/ml. After incubation and colony count the recovery rate related to a reference medium (e.g. CASO Agar) is given as a percentage figure.

In the case of non-selective culture media numbers of nearly 100 % can be expected, while for the individual results 70% has been set as the lower limit with respect to accuracy of the method. The recovery rates on selective media are naturally lower, even for the target organisms. Bacteria that should be suppressed on purpose are massively inoculated in order to recognize the inhibitor effect by means of as low a recovery rate as possible.

Quality control: for Merck a basic precondition

Merck has always taken great pains to ensure that its products are of a high quality. In the middle of the 19th century, Merck's founder, Heinrich Emanuel Merck, wrote in a letter to a customer that he would always vouch for the purity of his products and was prepared to cover his customers against any disadvantage arising from an impure product. Microbiological products can above all be standardized only if the manufacturer takes great pains with quality control.

The biological origin of most of the ingredients of culture media makes it very difficult for raw materials of a consistent quality to be obtained for every batch. The intensive physical, chemical and biological testing of both raw materials and finished products ensures that high-quality, reliable products are dispatched from our company and are received by customers.

With numerous specialized laboratories in the fields of pharmaceutical, chemical analytical and diagnostic research in addition to a quality control centre, Merck is in an excellent position to set up optimal quality-control measures in the production of microbiological products.

The following problem areas in the production chain are carefully controlled:
- The choice of the raw materials
- The standardization of the products
- The final inspection of the products

A certificate of analysis containing the typical characteristics of each listed item documents these control areas and is available upon request by the customer.

Merck not only pays attention to the quality of its products, but also does its utmost to meet and fulfil all its customers' requirements. Today, quality at Merck also means prompt delivery, excellent technical support, immediate information, safety, user-friendly handling, environmental conservation and much much more: In a nutshell: comprehensive service.

Every Merck employee is trained to practice quality for the benefit of our customers.

Everything we do is done according to the maxim:

Merck creates quality. Quality is our commitment!

Potential sources for errors during preparation and handling

Clumping of dehydrated culture media

Humidity was too high during storage

Container was left open too long

Container was not tightly reclosed after first opening

Dehydrated culture medium was too old

pH shift

Water was not neutral

Container was not tightly reclosed after first opening

Culture medium was overheated during preparation

Dehydrated culture medium was too old

Residues of rinsing solutions

Turbidity, precipitation

Turbidity of the prepared culture medium should be considered as an error only if it appears in the culture vessel (e.g. Petri dish, test tube etc.). Any turbidity observed in the vessel used for preparation due to the presence of a considerably thicker layer of culture medium is of no consequence. Precipitates that settle out to form sediments indicate, however, that an error has been made. Exception: obligatorily turbid culture media!

Water was not adequately demineralized

Vessel used for preparation was not clean

pH value was incorrect (see "pH adjustment")

Culture medium was overheated during preparation

In the case of self-mixed culture media: the basic ingredients contained precipitating impurities

Caused by the sample material

Loss of water of the prepared culture medium due to evaporation

Solidification point too high

Important when sample material or heat-sensitive substances are to be mixed into the culture media when they are still fluid

Too much dehydrated culture medium was weighed out

Agar-agar not suitable

Gel strength too low

Insufficient dehydrated culture medium was weighed out

Dehydrated culture medium was not completely dissolved

Culture medium was overheated, possibly at a low pH value, during preparation (see "pH adjustment")
Vessel was not swirled before pouring the plates

In the case of self-mixed culture media: unsuitable or too little agar-agar

Acidic culture medium was not prepared under mild conditions (see "Acidic culture media")

Colour deviation

In the case of culture media containing indicators, the pH was incorrect (see "pH adjustment")

Culture medium was overheated during preparation: culture medium dark, coloured pigments destroyed, sugar caramelized

Vessel used for preparation was not clean

Ready-to-use culture medium contaminated

Drying of the plates in a contaminated (spores) incubator

Inadequate sterilization

Storage in a contaminated place (refrigerator)

Contaminated after sterilization, e.g. due to errors while pouring the plates, contaminated Petri dishes

Addition of unsterile supplements

Growth too poor

Residues of growth-inhibiting substances present in the vessels used for preparation culture (e.g. detergent), in the water used (e.g. substances from the air), in the sample material

Microorganisms in the sample material already damaged

Shift in the culture medium pH

pH shift caused by acid (or basic) sample material

In the case of culture medium bases: additives dosed incorrectly

Culture medium was overheated during preparation

In the case of pour-plates: temperature was too high when sample was mixed in

Growth too strong

Culture medium was overheated during preparation, causing destruction of selective inhibitors

In the case of culture medium bases: additives dosed incorrectly

Culture medium was inoculated with too much sample material

Colonies liquefy or swarm

Surface of the culture medium was too moist

Surface of the culture medium was inoculated with too much sample material

Culture medium was overheated during preparation, causing destruction of inhibitors

Atypical growth

Culture medium prepared incorrectly, dehydrated culture medium was too old

Prepared culture medium was too old or unfit for use

Wrong conditions were used for cultivation

Residues of foreign substances present in the vessel used for preparation or culture (e.g. detergents), in the water used, in the sample material

Lethally injured cells damaged by the sample material

Storage of prepared culture media

Prepared culture media remain stable only for a limited period of time. If not otherwise specified, they can be kept for several months under appropriate storage conditions.

The media should be stored at 4-12°C for longer periods of time. Agar culture media must not be stored below 0°C as this destroys their gel structure. The media can generally be stored at room temperature for 1-2 weeks; they must always, however, be protected from light.

If agar culture media are to be stored in Petri dishes for a longer period of time, they must be prevented from drying out by sealing each Petri dish separately with adhesive tape along the join between the lid and base, or else by packing several dishes into airtight plastic bags. Liquid media in test tubes or flasks should also be provided with airtight seals. Loss of water can result in precipitation and crystallization of certain substances in the culture media. Also cracks can be caused in the culture media plates due to shrinking.

In the case of culture media that contain unstable additives, it is often better to store the made-up medium base and then add and mix in the additives as required.

The culture media should be warmed to the required incubation temperature several hours before use by placing them in the incubator.

Safe disposal of dehydrated culture media (waste)
Waste Disposal Act and European Waste Directive

Within the EU, disposal issues are regulated by an EU Directive that has to be incorporated into national law of the Member States. The Directive defines waste and the objects to be protected. Any disposal practice must be in compliance with local, state and federal laws and regulations. Check with local and state regulations before disposing of waste.

See also MSDS: www.merck-chemicals.com

Safe disposal of cultures

DIN Standard 58956 Part 4[1] and the recommendations of the German Health Authority[2] give information on the desinfection of microbiological cultures and the cleaning or disposal of microbially contaminated material, in particular regarding the handling of substances proven to be or suspected of being contaminated with pathogenic microorganisms. According to these recommendations, all materials must wherever possible be disinfected by heat treatment before cleaning or disposal are carried out. A chemical disinfection should only be carried out in exceptional cases. Local and national regulations must be followed.

A thermic disinfection of cultures in disposable vessels, in particular plastic, can be simply and effectively carried out by autoclaving them (approx. 30 min at 121 °C) in plastic bags with a high melting point[3]. When the microorganisms have been killed, the plates or the plastic bags and their contents can be thrown away. If suitable incinerators are available, the cultures can also be killed and destroyed by burning. Cultures in reusable glass vessels (e.g. conical flasks, culture test tubes) must first be killed in the autoclave (approx. 30 min at 121 °C). Also slightly contaminated glass vessels or heat-stable equipment are firstly autoclaved (134 °C for about 20 min) or sterilized in a hot-air cabinet (180 °C for at least 30 min). The vessels and equipment can subsequently be cleaned. Any necessary sterilization can then take place -- also with autoclaving or in a hot-air cabinet.

Chemical disinfection is carried out with appropriate disinfectants. The active ingredients in chemical agents are usually only effective against vegetative microorganisms but not against bacteria spores. Certain bacteria and certain viruses are more resistant to certain active substances than are other microorganisms. In chemical disinfection all objects must be thoroughly wetted with the disinfectant. Therefore adherent air bubbles must be removed. For an adequate cover of a culture medium with disinfectant in a Petri dish with a diameter of 9 cm, 10 ml of disinfectant is necessary. The disinfectant should be allowed to act for at least 6 hours (e.g. overnight).

It is recommended to use disinfectants that have been tested by the German Health Agency according to §10 of the German Epidemic Disease Act of 18 December 1979[4] or adopted into the list of tested and efficacy-approved disinfectants drawn up by the German Association for Hygiene and Microbiology[5].

Literature

[1] DIN Deutsches Institut für Normung e.V.: Medizinisch-mikrobiologische Laboratorien. Anforderungen an die Entsorgung. -- DIN 58956, Teil 4.

[2] Bundesgesundheitsamt: Durchführung der Sterilisation. -- **Bundesgesundhbl. 22**; 193-200 (1979).
Bundesgesundheitsamt: Durchführung der Desinfektion. -- **Bundesgesundhbl., 23**; 356-364 (1980).
Bundesgesundheitsamt: Anforderungen der Hygiene an die Abfallentsorgung. -- **Bundesgesundhbl., 26**; 24-25 (1983).

[3] Special bags for waste disposal by C.A. Greiner & Söhne Co., 7440 Nürtingen, or Sarstedt Co., 5223 Nümbrecht.

[4] List of the disinfectants and methods tested and approved by the German Health Agency, 10th edition -- **Bundesgesundhbl**. **30**; 279-290 (1987). Available on request from the German Health Agency, Robert Koch Institute A.-Verw. 1000 Berling 65, Nordufer 20.

[5] Cf. list of disinfectants tested for their bactericidal properties according to the “Guidelines for the testing of chemical disinfectants” and approved as effective by the German Association for Hygiene and Microbiology. Status: 31 July 1981, mhp-Verlag GmbH, Wilhelmstrasse 42, 6200 Wiesbaden.

Abbreviations

In this book the following abbreviations will be used for organisations and standards.

APHA	American Public Health Association
ATCC	American Type Culture Collection
BGA	Umweltbundesamt, Berlin German Public Health Authority
DAB	Deutsches Arzneibuch (German Pharmacopoeia)
DEV	Deutsches Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German Methods for the Examination of Water, Waste Water and Sludge)
DIN	Deutsches Institut für Normung e.V.* (German Institute of Standardization)
Eiprod.-Verordng.	Eiprodukte-Verordnung (German Egg Product Regulations)
EP	European Pharmacopoeia
FIL-IDF	Fédération Internationale de Laiterie International Dairy Federation (Internationaler Milchwirtschaftsverband)
FIBG	Deutsches Fleischbeschaugesetz (German Meat Inspection Law)
ISO	International Organization for Standardization
LMBG	Lebensmittel- und Bedarfsgegenständegesetz (German Food and Consumer Goods Law)
Merkblätter-Packmittel	Arbeitsgruppen des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merckblätter für die Prüfung von Packmitteln (Institute for Food Technology and Packing, Technical University of Munich: Instruction Leaflets for the Examination of Packaging Materials)
Methodenbuch-Milch	Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch) (Methodology Handbook for Agricultural Experiments and Studies)
NCCLS	National Committee of Clinical Laboratory Standards
USP	United States Pharmacopoeia
WHO	World Health Organization

* DIN norm standards and standards "Amtliche Sammlung von Untersuchungsverfahren acc. to § 35 LMBG" can be obtained from Beuth-Verlag, D-10787 Berlin 30, 6 Burggrafenstr. Phone 030/2601

Collection of microorganisms

Abbreviations	Addresses	Important microorganisms
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen Gesellschaft für Biotechnologische Forschung mbH Mascheroder Weg 1 B D-38124 Braunschweig, Germany	Apathogenic funghi and bacteria
ATCC	American Type Culture Collection 12 301 Parklawn Drive Rockville, Maryland 20 852, USA	All types
NCIB	National Collection of Industrial Bacteria 135 Abbey Road Aberdeen, AB9 8DG, Scotland	Apathogenic bacteria
NCTC	National Collection of Type Cultures Central Public Health Laboratory Colindale Ave. London NW9 5HT, Great Britain	Bacteria
NRRL	Northern Regional Research Laboratory ARS Culture Collection US Department of Agriculture 1815 North University Street Peoria, Illinois 61604, USA	Agriculture strains
DBDR	Division of Bacteriology and Dairy Research Science Service Ottawa, Canada	Funghi, bacteria
NCYC	National Collection of Yeast Cultures Agricultural Council Food Research Institute Colney Lane Norwich NR4 7UA, Great Britain	Yeasts
MRL	Mycological Reference Laboratory London School of Hygiene and Tropical Medicine Keppel Street London WC 1E 7HT, Great Britain	Pathogenic funghi
CBS	Centraalbureau voor Schimmelcultures Baarn Oosterstraat 1, P.O. Box 273 3740 Baarn, The Netherlands	Funghi, actinomyces

More information about specific strains and the above listed organizations can be obtained by:
International Center for Information and Distribution of Type Cultures, 19 Avenue Cesar Roux, 1000 Lausanne, Switzerland.

Collection, handling and processing of Clinical Specimens

See Manual of Clinical Microbiology, fifth edition, 1991, American Society for Microbiology, Washington,D.C.
Section I: Specimen Collection and Handling

Additional instructions for users

- Do not use contaminated plates. Dispose of according to national disposal guidelines/directives.
- Any decision to perform an enrichment to be made only by a skilled and authorized person.
- Concerning the enrichment of a sample follow the instructions of the manufacturer of the culture medium.
- Additional biochemical/serological tests must be performed after the isolation step in order to guarantee the diagnostic result.
- Interpretation of the diagnostic result to be made only by a skilled and authorized person.
- National guidelines for the transport and storage of microbiological samples / specimen must be strictly followed.
- National guidelines for handling samples / specimen of human or biological origin must be strictly followed.
- Samples / specimens must be clearly marked with the name of the patient according to the national guidelines.
- Contaminated as well as unused media must be disposed of according to the local or national guidelines.
- A quality assurance programm should be implemented and performed in the laboratory according to the existing standards (e.g. Good Laboratory Practice).
- Medical standards must be observed.
- Standards of clinical microbiology must be followed.
- Microbiological examinations must be performed only by trained staff.
- http:/// For MSDS, warnings and precautions see our website www.merck-chemicals.com