Media operating handbook
Preparation, use and storage

Biokar diagnostics

CONTENTS

USE OF DEHYDRATED MEDIA

Dehydrated media receipt and storage....page 4
Dehydrated media preparation steps
1 – Dissolution....page 5
2 – Sterilization....page 6
3 – Supplement preparation and addition....page 7
4 – pH measurement and adjustment....page 7
Media distribution
1 – Preparation in Petri dishes....page 8
2 – Preparation of agar slant tubes....page 9
Prepared media storage....page 9

USE OF READY-TO-USE MEDIA

Preparation of ready-to-melt media (bottles or tubes)....page 10
Deaeration/regeneration of culture media....page 10
Preparation of complete media....page 11
Media storage....page 11

INOCULATION METHODS

Inoculation of liquid media....page 12
Inoculation of agar media
I – Detection technique
1 – Surface plating in Petri dishes....page 13
2 – Surface plating on agar slants....page 13
II – Enumeration technique
1 – Pour plate technique in Petri dishes....page 14
2 – Pour plate technique in tubes....page 14
3 – Surface spreading....page 15
4 – Membrane filtration....page 15

PRECAUTIONS OF USE

Decontamination and destruction of media after use....page 16
Chemical risk prevention....page 17
Troubleshooting....pages 18-19

LABELLING

CLP regulation....page 20
Labelling information....page 21

Bibliography....page 22

The specific preparation technique for each medium must be followed in order to maintain the product's properties. Please read the technical data sheets and material safety data sheets before use. These documents can be consulted directly on our website.

Dehydrated media receipt and storage

Upon receipt of the culture medium :

1 - Register the medium along with the date of reception.

2 - Store the medium according to the recommendations on the label. The storage temperature is generally between 2°C and 30°C; however some media may need to be stored in a cold room at between 2°C and 8°C.
Culture media are usually hygroscopic, heat and light sensitive and susceptible to mold contamination. Store shielded from light, humidity and any sources of heat such as autoclaves, incubator etc.

BIOKAR Diagnostics medium can be prepared and inoculated until the expiration date indicated on the label. In this case, after a suitable incubation time, the results can be read beyond the expiration date.

Upon the first opening of the product :

1 - Check the expiration date.

2 - Note the date on which the product was opened.

3 - Check the appearance of the medium. Do not use the medium if the powder does not look normal (change of texture or colour).

4 - After use, ensure that the container is closed properly and store it in the appropriate storage area.

In optimal storage conditions, dehydrated media can be kept for 3 to 5 years in their original packaging.

Dehydrated media preparation steps

1 - Dissolution

1 - Weigh out the appropriate amount of medium, using the personal protective equipment (PPE) stated in the material safety data sheets.

2 - Gradually add the amount of water necessary for reconstitution (stated on the label and technical data sheet).

For faster dissolution, preheat the water used for reconstitution to around 50°C (unless otherwise specified on the technical data sheet).

3 - Stir slowly and regularly to dissolve the components and the agar homogeneously.

4 - Bring to a boil (without overheating) the media containing the agar before distributing into tubes or flasks. The agar is fully dissolved when the viscous solution is free of any agar particles sticking to the sides of the recipient.
For agars usually producing precipitates, homogenize the resulting suspension before distributing.
For liquid media, clear solutions are obtained without heating before autoclaving. Except in the case of certain broths such as selenite cystine broth which requires heating for a short time (see technical data sheet and label).

5 - Distribute the appropriate amount of medium into flasks or tubes according to use.

2 - Sterilization

The prepared bottles and tubes are sterilized for the amount of time and at the temperature specific to each culture medium. Some media do not require autoclaving. The specific characteristics of each medium are given in the technical data sheet and on the label.

1 - Generally, culture media distributed into bottles or tubes are sterilized by autoclaving at 121°C ± 3°C for 15 minutes.
For information, the sterilization cycle must be suitable for volumes larger than 1000 mL.

After autoclaving, the medium should be cooled rapidly to prevent overheating. Wear heat protection gloves and glasses for removing the medium from the autoclave. Risk of severe burns.

2 - Let sit on a heat-resistant surface at ambient temperature for a short time (i.e. 2 minutes)*.

3 - Cool the molten medium in a water bath at a temperature between 44°C and 47°C*.

** Follow the cooling steps to prevent thermal shock.*
Risk of explosion and glass smashing.

1.

2.

3.

After sterilization, the medium must be handled in aseptic conditions, in order to protect it from outside contamination.

3 - Supplement preparation and addition

Some media need to be completed by adding a selective supplement or an enrichment supplement. The supplements may be in lyophilisate form or ready-to-use liquid or tablet form.

1 - Lyophilisate supplements are first of all reconstituted by aseptic addition of the required amount of sterile distilled water or diluent (see technical data sheet).

For some supplement such as freeze-dried rabbit plasma, sterile distilled water preheated at 44°C can be used.

2 - Turn end-over-end several times to ensure complete dissolution, avoid frothing the solution.

3 - Add the supplements to a medium maintained between 44°C and 47°C.

4 - Homogenize by turning the recipient upside down several times.

4 - pH measurement and adjustment

The media are adjusted to the pH of use determined for the complete medium (with supplement) ready for inoculation, after autoclaving and cooling to 25°C (NF EN ISO 11133 : 07-2014). Follow the medium preparation instructions carefully to prevent any variation in pH.

1 - Measure the pH using a pH-meter at a temperature of 25°C.

2 - If necessary, adjust the pH. This can generally be done using a sterile sodium hydroxide solution at 40 g/L (c(NaOH) 1 mol/L) or a sterile hydrochloric acid solution at 36.5 g/L (c(HCl) 1 mol/L).

Media distribution

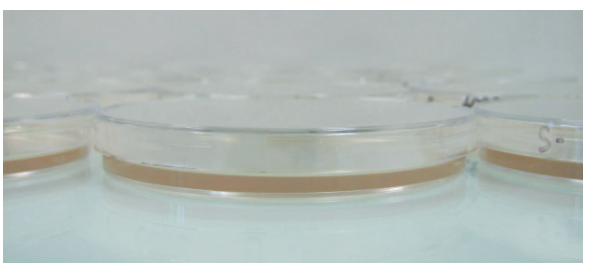
The prepared media are divided into tubes, Petri dishes or any other recipient suitable for the protocol used. After distribution in the relevant containers and cooling, liquid culture media can be inoculated or stored directly. Agar culture media need to be solidified before use.

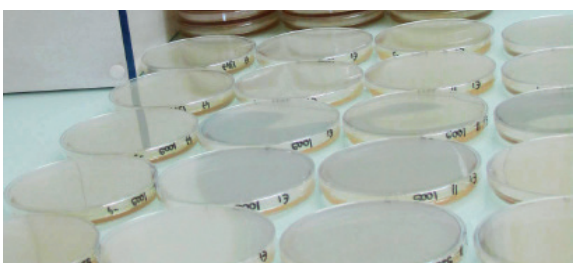
1 - Preparation in Petri dishes

1 - Pour the molten agar culture medium into the Petri dishes to a thickness of 3 mm for 90 mm diameter dishes and to 5 mm for 55 mm diameter dishes (therefore 18 mL to 20 mL agar).
If the dishes are stored or incubated beyond 48 hours or if incubation temperature is above 40°C, a greater amount of medium may be required.

Pour the medium at a temperature of between 44°C and 47°C, to prevent drops of condensation forming in the lids.

2 - Leave the agar medium to cool and solidify by placing the plates with lids on place on a cool and flat surface or under a laminar flow.





2 - Preparation of agar slant tubes

1 - After distribution into tubes and autoclaving, tilt the tubes so as to obtain an oblique slope and a 3 cm pellet (if performing stab inoculation in the pellet).

2 - Let cool and solidify at ambient temperature.

Prepared media storage

The shelf life of media prepared in a laboratory is given for information purposes in the technical data sheet (generally between 2 and 4 weeks for the Petri dishes and between 3 and 6 months for tubes). It is determined according to standard laboratory conditions and must be approved in-house by the user.

Media prepared in a laboratory must be stored in conditions which prevent changes in their composition: away from light, desiccation and if necessary in a refrigerator at 2-8°C.

Preparation of ready-to-melt media (bottles and tubes)

1 - Unscrew the bottle or tube caps before heating to enable pressure exchange.

2 - Melt the medium in a 50°C water bath before increasing to 95°C. Remove the medium as soon as it has melted to prevent overheating.

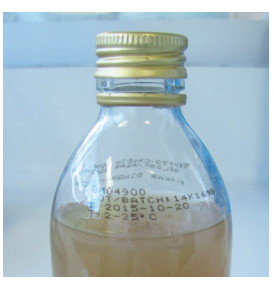
⚠ *Wear heat protection gloves and glasses.*
Risk of severe burns.

3 - Let sit on a heat-resistant surface at ambient temperature for a short time (i.e. 2 minutes)*.

4 - Cool the molten medium in a water bath set at a temperature between 44°C and 47°C*.

⚠ ** Follow the cooling steps to prevent thermal shock.*
Risk of explosion and glass smashing.

1.



2.

95 °C

3.

4.

44-47 °C

Deaeration/regeneration of culture media

Before use, it is recommended for some media to provide correct air/oxygen content (especially for anaerobe cultures) and to regenerate media having already been autoclaved for a minimum duration in order to maintain their quality.

Heat the culture medium in boiling water or under steam flow for 15 minutes, with the caps unscrewed slightly. After heating, the tops must be screwed back on and the media cooled rapidly to temperature of use.

⚠ *Follow the instructions described in the previous paragraph.*

Preparation of complete media

Using the liquid medium or reliquefied agar medium :

1 - Add, if necessary, the supplement(s) to the medium and mix carefully.

2 - Check the pH of the complete medium.

3 - Distribute according to the protocol:
- Agar media in Petri dishes or in tubes,
- Liquid media in tubes, bottles or bags for mixing.

For more details concerning these steps, please refer to pages 7 and 8: «*Dehydrated culture media preparation – supplement addition*» ; «*supplement measurement and adjustment*» and «*media distribution*».

Media storage

1 - Follow the instructions on the label and technical data sheet for the storage conditions and expiry date for ready-to-use media.

BIOKAR Diagnostics medium can be prepared and inoculated until the expiration date indicated on the label. In this case, after a suitable incubation time, the results can be read beyond the expiration date.

2 - For media prepared in the laboratory, the shelf life is given in the technical data sheets as an indication (generally between 2 and 4 weeks for Petri dishes and 3 and 6 months for tubes). It is determined according to standard laboratory conditions and must follow an internal validation by the user.

Media prepared in a laboratory must be stored in conditions which prevent changes in their composition: away from light, desiccation and if necessary in a refrigerator at 2-8°C.

Before use :

1 - Let the culture media return to ambient temperature.

2 - Identify the used product (medium name, sample, dilution etc.).

Inoculation of liquid media

Liquid culture media are used for enrichments and sterility tests.

1 - Inoculate a volume of medium with the appropriate amount of sample.

If Durham tubes are used, remove air bubbles by turning upside down.

2 - Slightly loosen the container cap and incubate in the appropriate conditions according to the followed protocol.

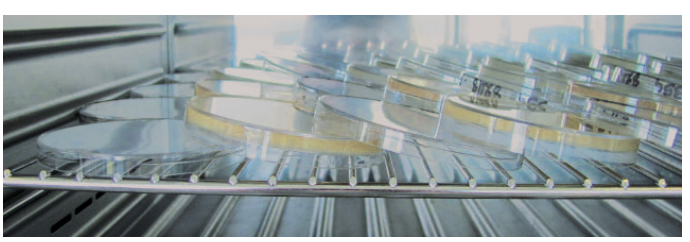
3 - Examine for microbial growth.

Inoculation of agar media

Before surface plating in a solid culture medium, Petri dishes are dried.

Place the Petri dishes in an oven set at between 25°C and 50°C or under a laminar flow hood, preferably without their lid, the agar surface facing downwards, until the droplets disappear from the surface of the medium.

Do not let the medium dry out.



I - Detection technique

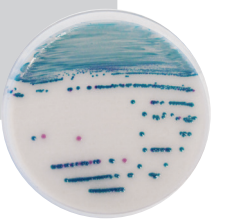
1 - Surface plating in Petri dishes

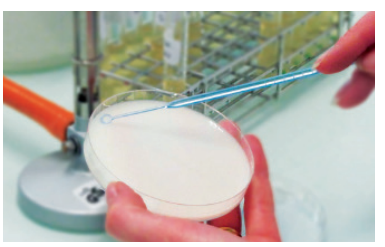
Isolation is used to separate colonies from one another in order to produce pure cultures from a microorganism or mixed flora :

1 - In aseptic conditions, put a drop of the suspension (around 10 µL). Spread using a loop at the surface of the medium, according to the selected isolation method.

2 - Incubate the Petri dishes, lid down, in the relevant conditions.

3 - Examine the colonies isolated.





2 - Surface plating on agar slants

Surface plating in tubes is used to observe the aspects typical of colonies.
Using agar slant tubes with pellet allows the observation of characteristics in anaerobiosis.

1 - Using a loop (10 µL) take a sample to be inoculated (isolated colony, bacterial culture, stock suspension etc.).

2 - Inoculate by ascending zigzags, taking care to not damage the agar. For agars with pellet, make a central stab before surface plating.

3 - Incubate in the conditions according to the desired protocol.

4 - Examine the isolated colonies.

II - Enumeration technique

1 - Pour plate technique in Petri dishes

The pour plate technique is used to enumerate microorganisms for certain types of samples. The method enables the growth and formation of smaller colonies. The pour plate technique is usually carried out in Petri dishes :

1 - In aseptic conditions, place 1 mL of stock suspension and its dilutions, if necessary, at the bottom of an empty Petri dish.

2 - Add 15 mL to 20 mL of agar medium maintained at a temperature of between 44°C and 47°C .

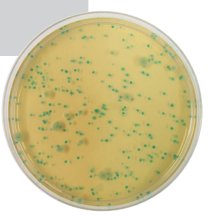
3 - Mix the sample and the culture medium by swirling the Petri dish.

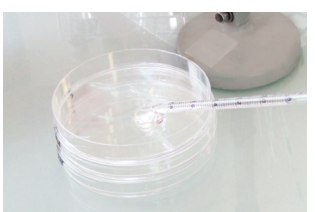
4 - Let solidify by placing the closed dishes on a cool and flat surface.

5 - If necessary, add 5 mL medium in a double layer and let solidify.

6 - Incubate the Petri dishes, lid down, in the relevant conditions.

7 - Count the colonies and define the number of microorganisms in the stock suspension.





2 - Pour plate technique in tubes

The pour plate technique can also be used in an agar medium in tubes :

1- After melting, cooling the medium at 44-47°C, add 1 mL (or other volume according to the protocol used) of stock suspension and its dilutions if necessary.

2 - Mix the sample and culture medium by turning upside down.

3 - Let solidify.

4 - Incubate the tubes in the appropriate conditions.

5 - Count the colonies and determine the number of microorganisms in the stock suspension.

3 - Surface spreading

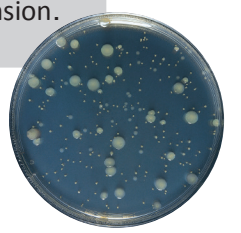
Surface spreading is used to count microorganisms :

1 - In aseptic conditions, place 0.1 mL (for example) of stock suspension and its decimal dilutions, if necessary, on the surface of a Petri dish.

2 - Spread using a spreader or loop until the suspension is fully absorbed.

3 - Incubate the Petri dishes in the relevant conditions.

4 - Count the colonies and determine the number of microorganisms in the stock suspension.



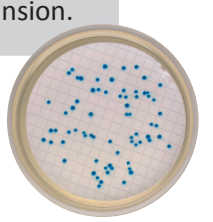
4 - Membrane filtration

The membrane filtration method is used to analyze large volumes of liquid samples such as samples of water and other beverages :

1 - In aseptic conditions, filter a volume of sample through a membrane which retains the sought microorganisms (of a nominal retention threshold of around 0.45 µm diameter).

2 - Using sterile forceps, place the membrane at the surface of the medium, ensuring that no air bubbles form between the membrane and the medium. The membrane must be placed with the contaminated side (squared) upwards.
For anaerobic microorganisms, the membrane can also be placed contaminated side down in an empty Petri dish, before being covered with the liquefied medium.

3 - Count the colonies and determine the number of microorganisms in the stock suspension.



Decontamination and destruction of media after use

Once used, the culture media are likely to contain a large number of potentially dangerous microorganisms. Consequently, contaminated media must be destroyed using thorough and safe methods.

Before cleaning glassware or removing waste, the media should be destroyed by the appropriate thermal treatment. Cultures in Petri dishes, tubes or bottles should be destroyed by autoclaving for one hour, at a minimum temperature of 121°C, in plastic bags with high melting point or by incineration.

Waste should be eliminated according to the legislation in effect in each country.

Chemical risk prevention

Most products do not carry a specific risk, except for risks relating to the use of powders. To avoid inhaling fine particles which may irritate the respiratory system, it is recommended wearing a protective mask suited to such use.

Some products however contain toxic substances and they must be handled with special care.

Before use, the measures described in the material safety data sheets available for all commercialized products should be read.

In all cases, it is essential to use the adequate personal protective equipment (PPE).




Best practices for the control of chemical risks :

- *Inventory of all products*
- *Read and analyze the MSDS for each product*
- *Identify any incompatibilities between chemical products*
- *Display risks in storage areas (see INRS website)*
- *Ensure rooms are well ventilated*
- *Store only small quantities at work stations*
- *Wear PPE suited to the risks during handling*
- *Inform and train the personnel concerned*

Troubleshooting : main potential anomalies during preparation, storage and use of dehydrated culture media

Anomalies	Check points
Formation of clumps in the dehydrated culture medium	Ambient humidity too high Recipient left open for too long Recipient not closed properly Expiry date exceeded
pH incorrect	Recipient stored in incorrect conditions Expiry date exceeded Weighing error Reconstitution water not tested properly Glassware (flasks or tubes) not washed or rinsed properly Medium overheated or melted several times Autoclave not set properly
Turbidity Precipitation	Expiry date exceeded Weighing error Glassware (flasks or tubes) not washed or rinsed properly Water not demineralized properly pH incorrect Homogenization incomplete Overheating during preparation or autoclaving Excessive water evaporation during preparation or storage
Color changes	Dehydrated medium degraded Expiry date exceeded Weighing error pH incorrect Homogenization incomplete Overheating during preparation or in the autoclave Excessive water evaporation during preparation or storage
Abnormal gel strength	Dehydrated medium degraded Expiry date exceeded Weighing error pH incorrect Homogenization incomplete Medium overheated or melted several times (especially in the case of acid media) Autoclave not set properly

ANOMALIES	CHECK POINTS
Prepared medium shown to be contaminated	Dehydrated medium degraded Expiry date exceeded Incorrect sterilization Recontamination of tubes or bottles after autoclaving Petri dishes contaminated
Loss of medium properties (productivity, selectivity)	Dehydrated medium degraded Expiry date exceeded Glassware not rinsed properly, possibly containing toxic residues (detergents, antiseptics, other inhibitors) Reconstitution water not demineralized properly pH incorrect Additives incorrectly dosed Medium overheated or melted several times Autoclave not set properly Pouring temperature too high Medium inoculated with an inadequate quantity of sample Petri dishes not dried properly Incorrect incubation conditions

CLP REGULATION (CLASSIFICATION, LABELLING, PACKAGING)

The European CLP regulation (EC no. 1272/2008 of 16 December 2008), resulting from the recommendations by the General Harmonized System (GHS) of classification and labelling of chemicals, instates new rules to improve protection of human health and of the environment, through an international harmonized system providing information on hazards.
Risks relating to the use of products classified as hazardous (mixture or substance) are described in the material safety data sheets (MSDS) and on the labels.

The hazard warning and precautions include :

- Hazard pictogram
- Warning statement indicating the level of hazard («HAZARD» or «WARNING»)
- Hazard statement (H phrase)
- Precautionary statement (P phrase)

Signal pictograms :

For more information, see regulation EC no. 1272/2008 of 16 December 2008.

Physical hazards

Oxidizing

Flammable

Explosive

Compressed gas

Health hazards

Toxic

Aspiration hazard, CMR*, Toxic

Irritant, Sensitization, Toxic

Physical and health hazards

Corrosive for metals, Corrosive for the skin/eyes

Environmental hazards

Toxic

* Carcinogenic, Mutagenic, Reprotoxic

Labelling information

Symbols table

	: Product reference
	: Manufacturer
	: Batch code
	: Use-by
	: Temperature limits
	: Keep away from light
	: Keep dry
	: *In vitro* use
	: See instructions for use

1 - NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

2 - NF EN ISO 7218/A1. October 2013. Microbiology of food – General requirements and recommendations - Amendment 1.

3 - NF EN ISO 6887-1. September 1999. Microbiology of food- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

4 - NF T 90-461. July 2001. Water quality – Microbiology – Quality control of culture media. Amended by amendment A1 in June 2005 and amendment A2 in May 2007.

5 - Corry JEL, Curtis GDW and Baird RM (eds) 2012, Handbook of Culture Media for Food and Water Microbiology. 3rd edition. Royal Society of Chemistry, UK.

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