

Wilkins Chalgren Agar – DM235

Introduction

MAST® Wilkins Chalgren Agar is a medium for the isolation, cultivation and susceptibility testing of anaerobic organisms.

MAST® culture media is supplied in a dehydrated powder form, allowing the end-user to prepare a suitable medium for bacterial & fungal culture. It is suitable to be prepared in a variety of receptacles and at volumes that conform to the end-users desired purpose. The culture of bacterial and fungal species are essential for routine laboratory purposes.

FOR IN VITRO USE ONLY
NOT FOR USE IN DIAGNOSIS OF HUMAN DISEASE

Intended Purpose

Wilkins Chalgren Agar dehydrated culture media is intended to produce a medium for the growth of anaerobes. When prepared in accordance with the instructions for use, it is recommended for the antibiotic susceptibility testing of anaerobes.

Wilkins Chalgren Agar is intended to be used in conjunction with other *in vitro* tests to aid the detection of bacterial pathogens from all types of clinical specimens. It is intended to be used by professional, trained clinical laboratory users for *in vitro* use and is not intended for use in the diagnosis of disease or other conditions in humans or as the basis of treatment or case management decisions.

Principle of the test

Culture media remains the gold standard for the growth and isolation of viable bacterial and fungal cells. Plates are inoculated with the target organism or specimen by surface plating. The four-quadrant streak method can be used to obtain single colonies. Plates should be incubated under the appropriate atmospheric conditions and temperature for the target organism(s). Interpretation of primary cultures following incubation requires significant skill on behalf of the operator in the determination of additional procedures required. This determination is reliant upon growth characteristics of the microorganism including such as morphology and observing changes in the media surrounding the colonies.

These methods should be used in conjunction with other *in vitro* devices in the aid of diagnosis.

Once prepared a single culture media plate is only for single use and cannot be re-used.

Components

MAST® culture media is supplied in a dehydrated form for reconstitution by the end-user. The formulation of the product is described in Table 1.

Table 1. Formulation of DM235*

Material	Concentration in medium
Casein hydrolysate, Enzymic	10.0 g/L
Pancreatic digest of gelatin	10.0 g/L
Yeast extract	5.0 g/L
Glucose	1.0 g/L
Sodium chloride	5.0 g/L
L-arginine hydrochloride	1.0 g/L
Sodium pyruvate	1.0 g/L
Haemin	0.005 g/L
Menadione	0.0005 g/L
Agar	12.0 g/L

*Formulation may change to meet performance criteria.

The formulation is illustrative of the DM235 product range. The product is manufactured within an ISO:9001 and ISO:13485 environment. Inter-batch variation is expected to be minimal with no direct impact on the product.

Stability and storage

The expiry date applies to unopened containers of MAST® dehydrated culture media when stored in the primary container and in accordance with the manufacturer's instructions. The expiry date and batch number are indicated on each pack label.

- Store packs in a dry environment.
- Store packs at room temperature (10°C to 25°C).
- Avoid sources of moisture such as autoclaves, CO₂ incubators and water-baths.
- Limit the time a pack remains open whilst in use.
- This product is hygroscopic, avoid prolonged exposure to ambient moisture.
- For opened packs of dehydrated culture media ensure lid is firmly closed after every use.
- Before use ensure the appearance of the media conforms to the expected colour and texture i.e. free flowing, no excessive lumps. Media that is discoloured or lumpy should be further examined for performance against the recommended QC organism panel.

Warnings and precautions

1. Wilkins Chalgren Agar is for *in vitro* use only, and must be used by trained professional laboratory staff.
2. All microbiological cultures and equipment used to transfer and manipulate them should be treated as infectious. Autoclave sterilise all biohazard



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- waste before disposal in accordance with local regulations.
- On receipt, store MAST[®] dehydrated culture media at the recommended storage temperature and conditions stated on the pack.
 - Do not store near sources of moisture or within high humidity environments.
 - Do not use if media powder is discoloured and/or lumpy, examine against recommended QC organism panel before continuing use. Discolouration could be a sign of degradation and must be examined further.
 - When handling the device ensure that local and regulatory health and safety advice is followed.
 - When handling the sterilised solution, beware of the temperature, use thermal resistant gloves where appropriate.
 - When preparing culture media after sterilisation, ensure that this is performed in an aseptic manner.

MAST[®] dehydrated culture media are supplied in a sealed primary container, which helps to prevent moisture ingress from the environment. The nature and frequency of use of the device is conducive to an end-user re-entering the container. When the product is not in use, the primary container should remain sealed.

Materials Provided

Mast[®] dehydrated culture media is supplied in a powder form contained within a re-usable primary container for end-user reconstitution.

Materials required but not provided

Standard microbiological supplies and equipment such as petri dishes, bottles, tubes, laminar flow cabinet, water bath, autoclave, balance, weigh boats, spatulas, thermometer, timer, additives such as defibrinated blood, deionised water, or suitable control strains of microorganisms.

Procedure

- Refer to pack label for quantities and volumes required. Prepare MAST[®] Wilkins Chalgren Agar by suspending the powder in distilled or deionised water.
- Sterilise the solution in an autoclave at 121°C (15 p.s.i) for 15 minutes.
- Cool the solution to 50 to 55°C and hold at this temperature in a water bath until ready to pour culture media plates.
- If required add 5 to 7% sterile defibrinated sheep blood to enhance the growth of fastidious anaerobic species.
- Prepare test plates for agar dilution susceptibility testing by addition of appropriate antibiotic

- solutions.
- Pour culture plates (20ml per 100mm diameter plate or alternative volumes according to the methodology followed) and allow to set.
 - Prepared culture plates should be used immediately.

Refer to local Health and Safety handling procedures for infectious waste disposal guidelines.

Technical Guidance

Observe the powder before use. If the powder is discoloured or lumpy, this could be a sign of degradation and must be further examined.

Interpretation of results

Following incubation record the growth end point and determine the Minimum Inhibitory Concentration (MIC) of the test organism. Interpret results as sensitive, intermediate or resistant according to the criteria laid down in the method of use.

Limitations of use

MAST[®] media are not intended to be used as the sole, and primary isolation medium in instances where a failure to detect a pathogenic infection would result in death, serious illness or possible transmission of infectious disease.

Quality Control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate expected performance. Do not use the product if the result with the control organism is incorrect. The list below illustrates a range of performance control strains which the end user can easily obtain.

Table 2. Suggested organisms for QC

Test Organism	Result
<i>Bacteroides fragilis</i> ATCC [®] 25285	Growth and correct susceptibility pattern
<i>Bacteroides thetaiotaomicron</i> ATCC [®] 29741	Growth and correct susceptibility pattern

References

Bibliography is available on request.

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Type Culture Collection, Manassas, Virginia, USA
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