

Triple Sugar Iron (T.S.I.) Agar – DM224

Introduction

MAST® Triple Sugar Iron Agar is used for the differentiation of Enterobacterales based on hydrogen sulphide production and fermentation of lactose, sucrose and dextrose. 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 clinical laboratory purposes.

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

Intended Purpose

Triple Sugar Iron Agar is a medium for the differentiation of Enterobacterales according to their ability to ferment lactose, sucrose and glucose, and to produce hydrogen sulphide.

Triple Sugar Iron Agar is intended to be used in conjunction with other *in vitro* tests to aid the differentiation of Enterobacterales. 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. Slopes are inoculated with the target organism or specimen. Slopes 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 slope 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 DM224*

Material:	Concentration in medium:
Peptone mixture	18.0g/L
Yeast extract	3.0g/L
Meat extract	4.0g/L
Lactose	10.0g/L
Sucrose	10.0g/L
Dextrose	1.0g/L
Sodium chloride	5.0g/L
Ferric ammonium citrate	0.3g/L
Sodium thiosulphate	0.3g/L
Phenol red	0.025g/L
Agar	14.0g/L

*Formulation may change to meet performance criteria

The formulation is illustrative of the DM224 product range. The product does not contain any material from animal origin and 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, 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. Triple Sugar Iron 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 waste before disposal in accordance with local regulations.

3. On receipt, store MAST® dehydrated culture media at the recommended storage temperature and conditions stated on the pack.
4. Do not store near sources of moisture or within high humidity environments.
5. 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.
6. When handling the device ensure that local and regulatory health and safety advice is followed.
7. When handling the sterilised solution, beware of the temperature, use thermal resistant gloves where appropriate.
8. 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

1. Refer to pack label for quantities and volumes required. Prepare MAST® Triple Sugar Iron Agar by suspending the powder in distilled or deionised water.
2. Allow to stand for 15 minutes.
3. Bring to the boil until completely dissolved.
4. Mix well and distribute into suitable containers.
5. Sterilise the solution in an autoclave at 121°C (15 p.s.i) for 15 minutes.
6. Allow to set in a slanted position to form a long slope and a 3.5cm butt.
7. Prepared culture slopes may be used immediately or stored at 2 to 8°C for up to one week before use.

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 read tubes for acid production of slope/butt, gas and hydrogen sulphide reactions. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented. Cracks or bubbles in the medium indicate gas production. A black precipitate in the butt indicates hydrogen sulphide production.

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 Organisms	Slope	Butt	Gas	H ₂ S
<i>Escherichia coli</i> ATCC® 25922	A(K)	A	+(-)	-
<i>Shigella sonnei</i> ATCC® 25931	K	A	-	-
<i>Proteus mirabilis</i> ATCC® 29906	K(A)	A	+	+++
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	A	++	-

A= Acid, K= Alkaline, () = indicates occasional reactions.

References

Bibliography available on request.