

## Hektoen Agar – DM134

### Introduction

MAST® Hektoen Agar is a selective medium for the isolation and differentiation of *Salmonella* spp. and *Shigella* spp.. 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

Hektoen Agar dehydrated culture medium powder is intended to produce a selective medium. When prepared in accordance with the instructions for use, it produces a medium capable of supporting the growth of many enteric pathogens, including *Salmonella* spp. and *Shigella* spp.

Hektoen 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 DM134\*

Material:	Concentration in medium:
Peptone mixture	25.0g/L
Lactose	10.0g/L
Sucrose	12.0g/L
Salicin	1.0g/L
Sodium chloride	2.0g/L
Sodium thiosulphate	1.0g/L
Ferric ammonium citrate	2.0g/L
Trisodium citrate	1.25g/L
Bile salts	1.5g/L
Acid fuchsin	0.025g/L
Bromo-thymol blue	0.05g/L
Agar A	14.0g/L

\*Formulation may change to meet performance criteria.

The formulation is illustrative of the DM134 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, CO<sub>2</sub> 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. Hektoen 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® Hektoen 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.  
**DO NOT AUTOCLAVE.**

4. Cool the solution to 50 to 55°C and hold at this temperature in a water bath until ready to pour culture media plates.
5. Prepare the required number of empty plates and mix the solution well. Following aseptic technique, pour culture plates to a volume of 15 to 20ml per plate and allow to set.
6. Prepared culture plates 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

After incubation *Shigella* spp. produce green moist colonies. *Salmonella* spp are blue-green colonies, with or without a black centre. Coliforms are salmon-pink to orange colonies.

## 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	Result
<i>Escherichia coli</i> ATCC® 25922	Significant or complete inhibition
<i>Enterococcus faecalis</i> ATCC® 29212	Significant or complete inhibition
<i>Salmonella typhimurium</i> ATCC® 14028	Growth (blue-green colonies, with or without black centre)

## References

Bibliography is available on request.