



## GASSNER MEDIUM

### Dehydrated culture medium

**1 - INTENDED USE**

For the detection and isolation of pathogenic *Enterobacteriaceae* in foodstuffs and other materials.

**2 - COMPOSITION - TYPICAL FORMULA \***

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	14.00 g
Sodium chloride	5.00 g
Lactose	43.000 g
Aniline Blue	0.62 g
Metachrome yellow	1.25 g
Agar	12.00 g

\*The formula may be adjusted and/or supplemented to meet the required performances criteria.

**3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE**

Gassner Medium is based on the formulation proposed by Gassner<sup>1</sup> for the detection and isolation of pathogenic *Enterobacteriaceae* in foodstuffs and other materials. This medium is also known as Water-blue Metachrome-Yellow Lactose Agar.

Peptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Methachrome yellow inhibits the accompanying Gram-positive microbial flora. Lactose, at high concentration, is the fermentable carbohydrate and a source of carbon and energy. The colour of the medium is green and when lactose is fermented by coliforms, an acidic environment is created that induces a colour change of the pH indicator aniline blue from green to deep blue; at alkaline pH levels the yellow colour of methachrome yellow becomes increasingly evident. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Agar is the solidifying agent.

**4 - DIRECTIONS FOR MEDIUM PREPARATION**

Suspend 76 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes.

**5 - PHYSICAL CHARACTERISTICS**

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue-green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

**6 - MATERIALS PROVIDED - PACKAGING**

Product	Type	REF	Pack
Gassner Medium	Dehydrated medium	4015012	500 g (6.57 L)

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

**8 - SPECIMENS**

Foodstuffs and other materials of sanitary interest. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

**9 - TEST PROCEDURE**

The method followed will depend upon the sample under test.

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C for 18-24 hours.

**10 - READING AND INTERPRETATION**

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies

Coliforms grow on the medium with blue colonies surrounded by a deep-blue halo.

Lactose non-fermenting *Enterobacteriaceae* grow with yellow or pale green colonies surrounded by a halo of the same colour.

**11 - USER QUALITY CONTROL**

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 25922	35-37°/ 18-24 H-A	good growth, blue colonies with blue halo
<i>S. Typhimurium</i> ATCC 14028	35-37°/ 18-24 H-A	good growth, yellow colonies with yellow halo
<i>E. faecalis</i> ATCC 19433	35-37°/ 18-24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection





### 12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Gassner Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

The productivity characteristics are tested by semi-quantitative ecometric technique with the lactose positive target strains *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736 and with the lactose-negative strains *S. Typhimurium* ATCC 14028, *P. mirabilis* ATCC 10005, *S. flexneri* ATCC 12022. After incubation at 37°C for 24 hours the target strains exhibit good growth with typical colonies: coliforms grow with blue colonies surrounded by a blue halo, *S. Typhimurium* and *P. mirabilis* grow with yellow colonies with yellow halo, while *S. flexneri* exhibits colourless colonies.

The selectivity is assessed by modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433. The growth of *S. aureus* is partially inhibited while *E. faecalis* is totally inhibited after incubation at 37°C for 24 hours

### 13 – LIMITATIONS OF THE METHOD

- The isolated colonies on the plates should be identified with suitable tests.

### 14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

### 15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

### 16 – REFERENCES

- Gassner G. Ein neuer Dreifarbenährboden zur Typhus-Ruhr-Diagnose. *Centralbl f Bakt I Orig* 1918; 80:219-222.

### TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

### REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

