BRILLIANT GREEN AGAR MODIFIED

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Selective medium for the isolation and differentiation of *Salmonella* spp. other than *Salmonella* Typhi.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	5.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Disodium hydrogen phosphate	1.0 g
Sodium dihydrogen phosphate	0.6 g
Lactose	10.0 g
Sucrose	10.0 g
Agar	13.0 g
Phenol red	90.0 mg
Brilliant green	4.7 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Originally described by Kristensen *et al.*[1], Brilliant Green Agar was modified by Kampelmacher[2,3] at National Institute of Public Health of Utrecht, to obtain a highly selective plating medium for the isolation of salmonellae from pig faces and minced meat.
Brilliant Green Agar Modified has been recommended for the isolation of *Salmonella*, other than *S.*Typhi, from water and associated materials[4], poultry and poultry products[5]. It is included in ISO 6579 as one of the second selective media for detection of *Salmonella* in foods.[6]
The presence of brilliant green may inhibit the growth of non-pathogenic enteric bacteria and *Shigella* spp., by selectively favouring *Salmonella*, except S. Typhi and S. Paratyphi.[7] For this reason, Brilliant Green Agar should be used in parallel with other enteric plating media such as XLD Agar.
Beef extract, peptone and yeast extract provide nitrogen, carbon, vitamins and minerals for microbial growth; phosphates act as buffer system; lactose and sucrose are fermentable carbohydrates; phenol red serves as an acid-base indicator giving a yellow colour to lactose and/or sucrose fermenting bacteria while lactose non-fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. This medium also contains brilliant green, which inhibits the growth of the majority of Gram-positive and Gram-negative bacteria, including *S.*Typhi and *Shigella* species.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 52.7 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation. Cool to 47-50°C and distribute into sterile Petri dishes. Do not sterilize by autoclaving.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	red-orange, fine, homogeneous, free-flowing powder
Prepared plates appearance	red-orange, limpid
Final pH at 20-25 °C	6.9 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Brilliant Green Agar Modified	Dehydrated medium	4012562	500 g (9.5 L)
Brilliant Green Agar Modified	Ready-to-use medium	541256	2 x 10 plates ø 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Food and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

The detection of *Salmonella* in foodstuffs requires four successive stages:[6]

1- Pre-enrichment in Buffered Peptone Water inoculated with the test portion, then incubated between 34°C and 38 °C for 18 h.
2- Enrichment in selective broth. Rappaport-Vassiliadis Soy Broth (RVS broth) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn broth) are inoculated with the culture obtained in Buffered Peptone Water. The RVS broth or the MSRV agar is incubated at 41.5 °C for 24 h and the MKTTn broth at 37°C for 24 h.
3- Plating out on selective solid media. From the cultures obtained in the enrichment selective broth, the following two selective solid media are inoculated:
   - Xylose Lysine Deoxycholate agar (XLD agar);
   - any other solid selective medium complementary to XLD agar (for examples, Brilliant Green Agar Modified).

The XLD agar and Brilliant Green Agar Modified plates are incubated at 37 °C and examined after 24 h.
4- Confirmation. Colonies of presumptive *Salmonella* are sub-cultured and their identity is confirmed by means of appropriate biochemical and serological tests.

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10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Salmonella spp other than *S.*Typhi and *S.*Paratyphi A form red-pink-white opaque colonies, surrounded by a diffused red halo.
E. coli/Klebsiella/Enterobacter grow less luxuriantly and form yellow-green colonies, surrounded by a halo of the same colour.
Proteus does not swarm if dry plates are inoculated and produces yellow-pink mucoid colonies (sucrose fermentation, variable).
Shigella, is completely inhibited by brilliant green.

11 - USER QUALITY CONTROL

All manufactured lots of the products 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
*S.*Enteritidis ATCC 13076	35-37°C/18-24 H/A	good growth, red colonies with red halo
*S.*Typhimurium ATCC 14028	35-37°C/18-24 H/A	good growth, red colonies with red halo
E.coli ATCC 25922	35-37°C/18-24 H/A	scanty growth, yellow colonies
E.faecalis ATCC 19433	35-37°C/18-24 H/A	growth partially inhibited

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

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative sample of all lots of dehydrated and ready to use Brilliant Green Agar Modified are 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 following target strains: *S.*Typhimurium ATCC 14028, S.Enteritidis ATCC 13076. After incubation at 35-37°C for 18-24 hours the target strains exhibit red colonies with red halo.
The selectivity is assessed with 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: *E.coli* ATCC 25922, *P.vulgaris* ATCC 9484, *S.aureus* ATCC 25923, *E.faecalis* ATCC 19433. The growth of *S.aureus* is totally inhibited while the growth of other non-target strains is partially inhibited after incubation at 37°C for 18-24 hours.

13 – LIMITATIONS OF THE METHOD

- Colonies of *Salmonella* spp. vary from red-pink-white depending on length of incubation and strain type; however, any of these colours indicate non lactose fermenter strain.[7]
- S.Typhi, S.Paratyphi and *Shigella* do not grow adequately on this medium.[7]
- Slow lactose fermenters, *Proteus*, *Citrobacter* and *Pseudomonas* may grow on Brilliant Green Agar with red colonies mimicking enteric pathogens.[7] It is advised to screen the colonies by flooding the plate with one drop of MUCAP Test reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C8 esterase enzyme, typical of *Salmonella* spp.[8]
- Since the medium is highly selective it is recommended the simultaneous inoculation of less selective media such as MacConkey Agar, and XLD Agar along with an enrichment broth.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This culture medium 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, 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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder 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 Sheets of the products are available on the website 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

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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/flasks) and the applied storage conditions (temperature and packaging). According to MacFaddin[7] the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.

16 - REFERENCES

1. Kristensen M, Lester V, Jurgens A. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br J Exp Pathol 1925; 5:291
2. Edel W, Kampelmacher EH. Comparative studies on Salmonella isolation in eight European Laboratories. Bull Wld Hlth Org 1968; 39, 487-491.
3. Edel W. Kampelmacher EH. Salmonella isolation in nine European laboratories using a standardized technique. Bull Wld Hlth Org 1969; 41, 297 - 306.
4. H. M. S. O. Methods for the isolation and identification of salmonellae (other than Salmonella typhi) from water and associated materials. 1982
5. British Poultry Meat Society. A manual of recommended methods for the microbiological. 1982
6. ISO 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection. J Clin Microbiol 1992; 30:525-526.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/06
Revision 3	Inclusion of ready-to-use plates: modification of chapters 6, 12, 14, 15	2022/12

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

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