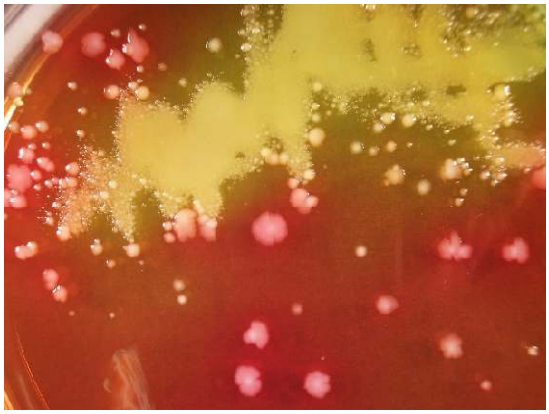
BRILLIANT GREEN AGAR

Dehydrated culture medium



Brilliant Green Agar: *Salmonella* sp. colonies (red) and *E.coli* colonies (yellow)

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)

Peptocomplex	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Agar	20.0 g
Phenol red	80.0 mg
Brilliant green	12.5 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 subsequently modified by Kauffmann[2] to obtain a highly selective plating medium for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products.
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.[3] For this reason, Brilliant Green Agar should be used in parallel with other enteric plating media such as MacConkey Agar, and XLD Agar.
Peptocomplex and yeast extract provide nitrogen, carbon, vitamins and minerals for microbial growth; sodium chloride maintains the osmotic equilibrium; 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 *Salmonella* Typhi and *Shigella* species.
A modification of Brilliant Green Agar which reduces growth of contaminants such as *Citrobacter* spp, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas* and *Proteus mirabilis* has been described by Watson[4] and Walker[5] by the addition of sulfacetamide sodium salt (1.0 mg/mL) and mandelic acid sodium salt (0.25 mg/mL).

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 58 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-pink, fine, homogeneous, free-flowing powder
Prepared plates appearance	reddish-brown, limpid
Final pH at 20-25 °C	6.9 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Brilliant Green Agar	Dehydrated medium	4012552	500 g (8.6 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 for the complete identification of the colonies.

8 – SPECIMENS

Refer to applicable International Standards and regulations for the collection of food and water samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

Heavily inoculate a Brilliant Green Agar plate directly with the specimen and/or with the enriched cultures in a pre-enrichment medium such as Buffered Peptone Water, and in selective enrichment broths such as Selenite Broth, Selenite Cystine Broth, Tetrathionate Broth.
Incubate the Brilliant Green Agar plate at 35-37°C and examine for suspected colonies after 18 to 24 hours and after 42 to 48 hours.

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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

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
*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, a representative sample of all lots of dehydrated Brilliant Green 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 following target strains: *S.*Typhimurium ATCC 14028, S.Enteritidis ATCC 13076, *S.arizonae*, ATCC 13314. After incubation at 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: *P.vulgaris* ATCC 9484, *E.coli* ATCC 25922, *E.faecalis* ATCC 29212. The growth of *E.coli* is partially inhibited while the growth of *E.faecalis* and *P.vulgaris* is totally 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.[3]
- S.Typhi, S.Paratyphi and *Shigella* do not grow adequately on this medium.[3]
- Slow lactose fermenters, *Proteus, Citrobacter* and *Pseudomonas* may grow on Brilliant Green Agar with red colonies mimicking enteric pathogens.[3] 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.[6]
- 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]
- The medium is normally reddish brown in colour; after incubation turns bright red but returns to normal colour at room temperature.[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 product is for microbiological control only 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 the 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.
- 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 plates 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.
- 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.
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[3] 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. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg.Infektionskr. 1935; 117: 26.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Watson C, Walker AP. A modification of brilliant green agar for improved isolation of Salmonella J Appl Bacteriol. 1978 Oct;45(2):195-204.
5. Walker AP. A note of the inhibition of Pseudomonas aeruginosa by a modification of brilliant green agar for improved salmonella isolation J Appl Bacteriol. 1981 Dec;51(3):405-8.
6. 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	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	2022/06

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it