Instructions for Use

REF - PP0060 **Brilliant Green Modified Agar**

Pre-Poured Media

1. Intended Use

For in vitro diagnostic use. PP0060 Brilliant Green Modified Agar is used for the selective isolation of *Salmonella* species, other than *S. typhi,* from clinical specimens, food and dairy products.

2. Composition*

Ingredient	g/L
Beef extract	5.0
Peptone	10.0
Yeast extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant Green	0.0047
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Brilliant Green Agar (BGA) was first cited by Kristensen *et al.* [1] and later modified to improve selectivity [2]. The medium is highly selective and may not be suitable for the isolation of *Salmonella* spp. from samples where numbers of salmonellae are low. If this is suspected to be the case, or if the sample is suspected to contain *Salmonella typhi*, other selective media such as XLD (E&O PP0320), Hektoen Enteric Agar (E&O PP1520) or Deoxycholate Citrate Agar (E&O PP0370) may be inoculated with the specimen in parallel. The United States Pharmacopeia [3] indicates that the medium may be used to confirm the absence of salmonellae in nutritional and dietary supplements. This product may also be used as a secondary plating medium for subculture from selective enrichment media [4, 5] during food and environmental testing.

4. Principle

Beef extract, peptone and yeast extract supply the required nitrogen and other essential nutrients. pH is maintained by the phosphate buffer system. Lactose and sucrose are fermentable carbohydrates. Phenol red is a pH indicator and turns the medium yellow when acid is produced by fermentation. Brilliant green inhibits Gram-positive bacteria and Gram-negative bacilli other than *Salmonella* species.

5. Physical Characteristics

	Medium
Appearance and Colour	Orange firm gel
pH	6.9 ± 0.2

6. Materials Provided

PP0060 Brilliant Green Modified Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0060-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP0060 Brilliant Green Modified Agar is suitable for the testing of the following specimens:

- Faecal specimens and other clinical samples
- Food and Environmental: products for human consumption, animal feed and environmental samples

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Use standard, local, or national procedures to obtain isolated colonies.

Allow the plates to come to room temperature.

For faecal specimens, inoculate plates directly with the faecal sample and spread over the plate to obtain single colonies using a sterile loop or automated plate streaker. Incubate plated samples aerobically at 37 ± 1°C for 18-24 hours.

For food and environmental samples, if PP0060 Brilliant Green Modified Agar has been selected as the secondary plating medium. Pre-enrich the sample in Buffered Peptone Water (E&O BM0082), followed by selective pre-enrichment in Rappaport-Vassiliadis Broth (E&O BM0320) & Muller-Kauffmann tetrathionate-novobiocin broth (E&O BM0947) then inoculate PP0060 Brilliant Green Modified Agar. Incubate plated samples aerobically at 37 ± 1°C for 18-24 hours.

Non-typhi *Salmonella* spp. will produce presumptive pink/red colonies typically with a red halo. Colony identity may be confirmed using biochemical, serological, molecular, or spectrographic techniques.

Refer to standard, local, or national guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
S. typhimurium (NCTC 12023)	37 ± 1°C for 18-24 hours	Growth: Pink colonies with red halo
E. coli (NCTC 12241)	37 ± 1°C for 18-24 hours	Inhibited
E. faecalis (NCTC 12697)	37 ± 1°C for 18-24 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify PP0060 Brilliant Green Modified Agar performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated aerobically at 37 ± 1°C for 18-24 hours. All samples showed expected recovery and morphology of the required target organism: *Salmonella typhimurium* (NCTC 12023), and no recovery of the non-target organisms: *Escherichia coli* (NCTC 12241) and *Enterococcus faecalis* (NCTC 12697). Therefore, it can be concluded that PP0060 Brilliant Green Modified Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- This medium is not recommended for the isolation of *S. typhi* or *Shigella* spp.
- Due to natural variation, some strains may grow poorly on this medium.
- The plated medium may change colour from tan to red on incubation at 37°C but should revert on cooling.
- This medium is highly selective and may not be suited to the isolation of salmonellae from samples containing low numbers of target species. Such samples should be pre-enriched in a suitable enrichment medium before subculture to PP0060.
- Taylor [6] showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar (BGA) as red colonies.
- If required, confirm identity using biochemical, serological, molecular or spectroscopic techniques.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0060 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date, cracked or a precipitate is present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Kristensen, M., Lester, V. and Jurgens, A. (1925) On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Brittish Journal for Experimental Pathology, 6(6), pp.291-299.
2. Kauffmann, F. (1935) Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren Für Salmonellabacillen. Zeitschrift für Hygiene und Infektionskrankheiten, 117, pp.26-32.
3. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37.

Rockville, MD: United States Pharmacopeial Convention.
4. Association of Official Analytical Chemists (AOAC) 1978. Bacteriological Analytical Manual, 5th edn., Washington D.C.
Pharmacopoeia of culture media for food microbiology. (1987). Int. J. Food Microbiol. 513: 245-247
5. International Organization for Standardization (2017) 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Horizontal method for the detection of Salmonella spp. Geneva, ISO.
6. Taylor, W. (1965) Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. American Journal for Clinical Pathology, 44(4), 471-475.

Version History*

001 14/08/23 - New Document Created

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

EC REP

E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com

Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place