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Brilliant Green Agar Base, Modified (DM044)

Intended Use
Brilliant Green Agar Modified (DM044) is used for selective isolation of Salmonellae other than Salmonella typhi from faeces, foods, dairy products.

Product Summary and Explanation
Kampelmacher[1] proposed the formula for a selective medium to isolate *Salmonella* from pig feces and minced meat. Brilliant Green Agar Modified is more selective than Desoxycholate Citrate Agar and other brilliant green media, and inhibits the growth of *Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* spp. which may resemble *Salmonella*. *Salmonella enterica* grows well on Brilliant Green Agar Modified compared to Desoxycholate Citrate Agar.[2] Brilliant Green Agar Modified is recommended for the isolation of *Salmonella* other than *Salmonella Typhi*, from water and associated materials[3] and meat and meat products.[4] It is recommended by the British Poultry Meat Society[5] for the examination of poultry and poultry products.
Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of Salmonella disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[10]
Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. [7] and further modified by Kauffmann (8). Brilliant Green Agar is also recommended by APHA [9,10] FDA[11] and described in EP, BP and IP.[12-14] This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli* , *Pseudomonas species* , *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar.

Principles of the Procedure
The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which and is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further be supplemented with sulphacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	10.00
Yeast extract	3.00
Lactose	10.00
Sucrose	10.00
Sodium chloride	5.00
Phenol red	0.08
Brilliant green	0.0125
Agar	20
Final pH 6.9± 0.2 at 25°C	

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Formula may be adjusted and/or supplemented as required to meet performance specifications

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 29 grams of the medium in 500 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle. AVOID OVERHEATING.
4. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (MS042).
5. Mix well before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Greenish brown clear to slightly opalescent gel forms in Petri plates
Reaction of 2.9% Solution	pH : 6.9 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% agar gel.

Expected Cultural Response: Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (MS042).

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of Colony
1.	*Salmonella typhimurium ATCC 14028*	50 -100	Good-luxuriant	25 -100	>=50 %	pinkish white
2.	*Salmonella abony NCTC 6017*	50 -100	Good-luxuriant	25 -100	>=50 %	pinkish white
3.	*Salmonella enteritidis ATCC 13076*	50-100	Luxuriant	25 -100	>=50 %	pinkish white
4.	*Salmonella typhi ATCC 6539*	50-100	Fair-good	15 -40	30 -40 %	reddish pink
5.	*Escherichia coli ATCC 25922*	50-100	None-poor	0 -10	0 -10 %	yellowish green
6.	*Escherichia coli ATCC 8739*	50 -100	None-poor	0 -10	0 -10 %	yellowish green
7.	*Escherichia coli NCTC 9002*	50 -100	None-poor	0 -10	0 -10 %	yellowish green
8.	*Staphylococcus aureus ATCC 25923*	>=10^3	Inhibited	0	0%	--
9.	*Staphylococcus aureus ATCC 6538*	>=10^3	Inhibited	0	0%	--

The organisms listed are the minimum that should be used for quality control testing.

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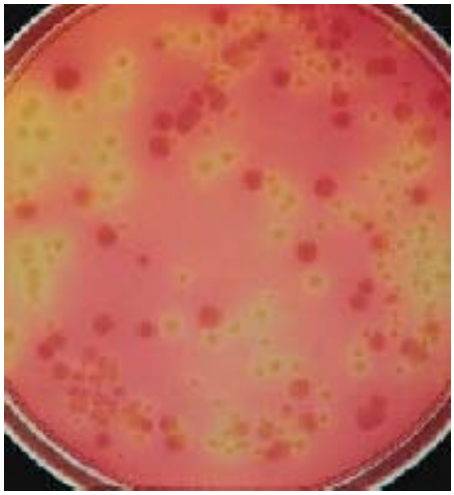

Brilliant Green Agar Modified (DM044)
Salmonella Typhimurium ATCC 14028 : pinkish white colonies
Escherichia coli ATCC 25922 : yellowish green colonies

Test Procedure

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 mL of Buffered Peptone Water (DM049).
2. Macerate for a sufficient time to give 15,000-20,000 revolutions.
3. Aseptically transfer the contents of the blender jar to a 500 mL flask. Incubate at 37 ± 0.1°C for 16-20 hours.
4. Transfer 10 mL samples to 100 mL Muller Kauffmann Tetrathionate Broth (DM1105).
6. Incubate the Muller Kauffmann Tetrathionate Broth (DM1105) at 42-43°C.

Sewage Polluted Natural Water

This procedure is applicable to the isolation of *Salmonella* spp. other than *S.* Typhi.

1. Inoculate 25 mL aliquots of the sample into 25 mL of double strength Buffered Peptone Water (DM049) and incubate at 37°C for 18 hours.
2. Transfer 1 mL samples into 10 mL of Muller Kauffmann Tetrathionate Broth (DM1105).
3. Incubate at 43°C for 48 hours.

Results

1. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus, Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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Limitations of the Procedure

1. Organisms other than *Salmonella* spp., such as *Morganella morgani* and some *Enterobacteriaceae,* may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and sero-agglutination, should be carried out on all presumptive *Salmonella* spp.

Packaging

Product Name : Brilliant Green Agar Base, Modified
Product Code : DM044
Available Pack sizes : 100gm / 500gm

Reference

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11. Bacteriological Analytical Manual, 5th Ed, 1978, AOAC, Washington D.C.
12. The European Pharmacopoeia, 2008, Council or Europe, Strasbourg.
13. The British Pharmacopoeia, 2007 vol. II, London.
14. Indian Pharmacopoeia, 2007, Ministry of Health and Family Welfare, Govt., of India,

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