APT AGAR
APT BROTH
Dehydrated culture media

1 - INTENDED USE
For the cultivation and enumeration of heterofermentative lactobacilli.

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

APT Agar

Tryptone	12.50 g
Yeast Extract	7.50 g
Glucose	10.00 g
Sodium citrate	5.00 g
Sodium chloride	5.00 g
Dipotassium hydrogen phosphate	5.00 g
Manganous chloride	0.14 g
Magnesium sulphate	0.80 g
Ferrous sulphate	0.04 g
Sorbitan monoleate	0.20 g
Agar	15.00 g
Thiamine HCl	0.10 mg

APT Broth

Tryptone	12.50 g
Yeast Extract	7.50 g
Glucose	10.00 g
Sodium citrate	5.00 g
Sodium chloride	5.00 g
Dipotassium hydrogen phosphate	5.00 g
Manganous chloride	0.14 g
Magnesium sulphate	0.80 g
Ferrous sulphate	0.04 g
Sorbitan monoleate	0.20 g
Thiamine HCl	0.10 mg

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
APT (All Purpose Tween) Agar was devised by Evans and Niven[1] and developed by Shipp[2] for the non-selective enumeration of heterofermentative lactobacilli capable of elaborating H_2O_2 during growth under aerobic condition. Such reaction on cooked meats results in a green discolouration.[3] APT Agar with added 20 g/L sucrose and bromocresol purple and with added 5 g/L glucose is effective in enumerating spoilage lactic acid producers in meat products and in seafoods respectively.[4] APT Agar can also be used to propagate pediococci.[4] APT Agar and APT Broth may be used for maintaining stock cultures and for culturing of *Weissella (Lactobacillus) viridescens* ATCC™ 12706 used in the assay of thiamine.[5]
Tryptone provides nitrogen, carbon, minerals and amino acids for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, glucose is a source of carbon and energy, sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. The presence of manganese and citrate exhibits a synergic effect increasing the intensity of the growth of lactobacilli, whose lag phase is shortened by sorbitan monoleate.[6]

4- DIRECTIONS FOR MEDIUM PREPARATION
APT Agar
Suspend 61.2 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and boil for 1 minute to completely dissolve the medium. Sterilise by autoclaving at 121°C for 15 minutes. Do not overheat.
APT Broth
Suspend 46.2 g in 1000 mL of cold purified water; heat slightly to dissolve the medium with frequent agitation. Distribute and sterilise by autoclaving at 121°C for 15 minutes. Do not overheat.
Double layer plates preparation[3]
Bottom layer: APT Agar (15 mL)
Top layer: APT Agar + MnO_2 (10 mL) prepared as follows.
To each 100 mL amount of APT Agar autoclaved and cooled to 45-47°C add 10 mL of a suspension of 20 g of MnO_2 in 200 mL of APT Broth, dispensed in 10 mL amounts and sterilized by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS
APT Agar and APT Broth

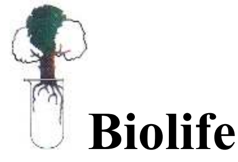
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	brown, limpid
Final pH at 20-25°C	6.7 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
APT Agar	Dehydrated medium	4010852	500 g (8,1 L)
APT Broth	Dehydrated medium	4010902	500 g (10.8 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops, sterile needles, swabs and pipettes, incubator and laboratory equipment as required, ancillary culture media and reagents.

8 - SPECIMENS
Food samples, cured meat products, tinned foods, fruit juices. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.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9 - Test procedure

Prepare the sample dilutions in 0.1% peptone water diluent. Inoculate by surface spread or poured plate methods, by single- or double-layers techniques.

1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten medium pre-cooled to 44-46°C. Prepare the other plates under the same conditions using decimal dilutions of the test sample.
2. Alternatively, sample dilutions are surface plated to obtain discrete colonies.
3. Incubate the plates under aerobic conditions at 25 °C to 32°C for 72 hours to five days.

The inoculation method, temperature and incubation time must be chosen according to the type of sample to be examined and the research objectives. Consult the appropriate references for the recommended procedures for testing and interpretation.[3,4]

10 - Reading and interpretation

H_2O_2 forms soluble compounds with suspended MnO_2. Colonies surrounded by a clear zone are regarded as potentially H_2O_2 producing lactic acid bacteria.

As these media are non-selective and permit the growth of contaminants, the presumptive diagnosis of the presence of lactobacilli should be confirmed by microscopic and biochemical examinations.

An artificial pollution test to confirm the diagnosis of bacterial greening of canned meats can be performed. Transfer a few colonies from the APT Agar plates to APT Broth tubes and incubate at 32°C for 24 hrs. Prepare a Petri dish with filter paper imbued with sterile water and put a slice of the test material under aseptic conditions. Inoculate the surface with a loopful of broth culture in APT Broth; incubate at 32°C for 24 hours and observe whether the meat has greened. If it occurs and if an un-inoculated control specimen is found to be unchanged, the diagnosis is confirmed. The presence of greening due to exceeding nitrites is to be distinguished from the bacterial greening by carrying out identification tests and assays of nitrites with the standard reagents.

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 of APT Agar supplemented with MnO_2.

Control strains	Incubation T°/ t / ATM	Expected results
L. brevis ATCC 14869	30°C / 5 days / A	good growth, colonies with transparent halo
L. sakie ATCC 15521	30°C / 5 days / A	good growth, colonies without transparent halo

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 APT Agar supplemented with MnO_2 and APT Broth supplemented with 15 g/L of agar and with MnO_2, are tested for productivity by comparing the results with a previously approved Reference Batch (RB).

The productivity is tested by a quantitative method with double layer technique with the following strains: *L. brevis* ATCC 14869, *L. sakei* ATCC 15521, *L. plantarum* ATCC 8014. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 5 days. The colonies are enumerated on both batches and the productivity ratio (Pr UFC_{TB}/UFC_{RB})) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications.

12 - Limitations of the method

- APT Agar and APT Broth a non-selective media and coliforms and many other commensals grow well.[5]
- Avoid excessive heating as thiamine is a heat-labile factor.[5]
- Studies of MacLeaod and Snell showed that Mn^{++} inhibits growth of *L. arabinosus* and *L. pentosus*.[7]

13 - Precautions and warnings

- These products are for microbiological control use and for professional use only; they are 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- These culture media contain 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.
- 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 media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture media 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 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.

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14 - 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 for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging). According to Baird RM et al. prepared plates can be stored for at least 7 days at 4 ± 2°C.[3]

15 – REFERENCES

1. Evans JB, Niven C. Nutrition of the heterofermentative Lactobacilli that cause greening of cured meat products. J Bacteriol 1951 Nov;62(5):599-603
2. Shipp HL. The green discolouration of cooked cured meats of bacterial origin. Technical circular n° 266, British Food Manufacturing Industries Research Association, Leatherhead, U.K.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 195-196.
4. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
5. Deibel RH, Evans JB, Niven CF. Microbiological assay for he thiamine using lactobacillus viridescens. J Bacteriol 1951; 62:818-821
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Sharf JM. Recommended Methods for the Microbiological Examination of Foods. 2nd ed. APHA, Washington, D.C. 1966
8. MacLeod RA, Snell EE. Some mineral requirements of lactic acid bacteria. J Biol Chem 1947; 170:351

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	2022/12

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

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