DIFFERENTIAL CLOSTRIDIAL AGAR

Dehydrated culture medium

1 - INTENDED USE

Basic medium to which iron (III) ammonium citrate and sodium sulphite solutions are added, for enumeration of sulphite-reducing clostridia spores in dried foodstuffs.

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

Pancreatic digest of casein	5.0 g
Enzymatic digest of meat	5.0 g
Meat extract	8.0 g
Yeast extract	1.0 g
Starch	1.0 g
Glucose	1.0 g
L-cysteine HCl	0.5 g
Resazurin	2.0 mg
Agar	20.0 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Differential Clostridial Agar has been formulated by Weenk et al.[1] for the enumeration of spores of mesophilic clostridia in dried foods. They established that the sulphite activity and the ferrous ion should be rigorously standardised and the basal medium should be free of added acetate and lactate.
Differential Clostridial Agar is included by APHA[2] in the list of commonly used media for the isolation and enumeration of clostridia in foodstuffs.
The medium is very rich and permits the growth of most clostridia, and many other anaerobes and facultative anaerobes. Pancreatic digest of casein, enzymatic digest of meat and meat extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group and glucose is a source of carbon and energy. L-cysteine is a reducing agent and favours the growth of anaerobes. Starch helps to detoxify metabolic by-products. Sodium sulphite and ferric citrate are added to the medium and act as indicators: sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black medium. The redox indicator resazurin is used to monitor anaerobiosis. Agar is the solidifying agent.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45-48°C and add 5 mL of Solution A and 1 mL of Solution B.
Solution A (freshly prepared): 1 g of ferric (III) ammonium citrate in 5 mL of purified water, sterilized in the autoclave at 121°C for 15 minutes or sterilised by filtration.
Solution B: 2.5 g of sodium sulfite anhydrous in 10 mL of purified water, sterilised by filtration. May be stored for no more than one month.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid or slightly opalescent
Final pH at 20-25 °C	7.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Differential Clostridial Agar	Dehydrated medium	4013122	500 g (12 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, test tubes, Petri dishes, controlled atmosphere generators and jars, Erlenmeyer flasks, ferric (III) ammonium citrate, sodium sulphite, ancillary culture media and reagents.

8 - SPECIMENS

Foodstuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

9 - TEST PROCEDURE,

Prepare samples or heated samples and dilutions and inoculate duplicate Petri dishes with 1 mL of each of the appropriate dilutions of the food material.
Pour 15 mL of melted medium and mix well the inoculum with the medium.
Let the medium solidify and overlay the plates with a layer of the same medium.
Incubate 2-5 days at 30- 35 °C under anaerobic conditions.
Weenk *et al*. developed an alternate procedure by using spiral plating with Differential Clostridial Agar and a DCA overlay.[1,2]

10-READING AND INTERPRETATION

Clostridia reduce sulphite to sulphide and the iron sulphide produced causes the culture medium to turn black.
Count the black colonies on the plates containing between 15 and 150 characteristic colonies.

10 - 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.

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CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	30°C / 72h / AN	growth with black colonies
C. sporogenes ATCC 19404	30°C / 72h / AN	growth with black colonies

AN: anaerobic 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 Differential Clostridial Agar supplemented with sodium sulphite and ferric ammonium citrate solutions (Test Batch TB) is assessed for productivity and specificity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a pour plate quantitative method with the target strains *C. perfringens* ATCC 13124, *C. bifermentans* NCTC 506, *C. sporogenes* ATCC 1904. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 72 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr:CFU_{TB}/CFU_{RB}$) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical (black colonies with black halo) the results are considered acceptable and conform to the specifications. Specificity is tested by pour plate quantitative method with the non-target strain *B. subtilis* ATCC 6633. After incubation at 30°C for 72 hours the non-target strain exhibits a good growth with colourless colonies.

13 - LIMITATIONS OF THE METHOD

- Some bacilli might mimic clostridia under the conditions of the procedure.[1]
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product is for Laboratory use 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.
- 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 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).

16 - REFERENCES

1. Weenk GH, Van den Brink JA, Struijk CB, Mossel DA. Modified methods for the enumeration of spores of mesophilic Clostridium species in dried foods. Int J Food Microbiol 27(2-3):185-200.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

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	2023/02

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

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