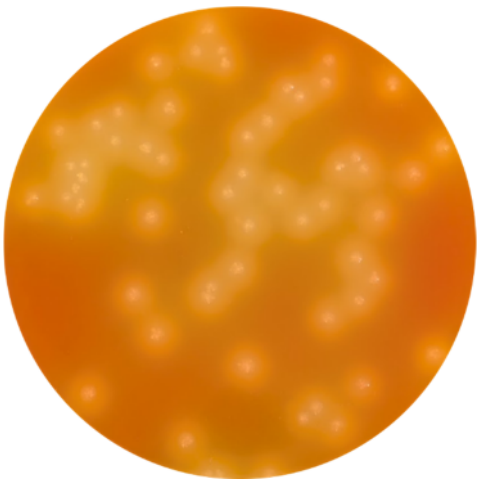
LACTOSE EGG-YOLK NEOMYCIN AGAR (LENA) BASE
LACTOSE EGG YOLK NEOMYCIN AGAR (LENA)
NEOMYCIN ANTIMICROBIC SUPPLEMENT

Dehydrated and ready-to-use culture medium and selective supplement



Lactose Egg-Yolk Neomycin Agar: *Clostridium perfringens*

1 - INTENDED USE

For the detection of *Clostridium perfringens* in foods, according to ISO 15213-3:2024.

2 – COMPOSITION*

LACTOSE EGG-YOLK NEOMYCIN AGAR (LENA) BASE - DEHYDRATED MEDIUM TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10 g
Meat extract	10 g
Sodium chloride	5 g
Lactose	10 g
Phenol red	0.1 g
Agar	14 g

NEOMYCIN ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 mL OF MEDIUM)

Neomycin sulphate	125 mg

LACTOSE EGG-YOLK NEOMYCIN AGAR (LENA) - READY TO USE PLATES

Peptone	10 g
Meat extract	10 g
Sodium chloride	5 g
Lactose	10 g
Phenol red	0.1 g
Agar	14 g
Egg yolk emulsion 20%	50 mL
Neomycin sulphate	250 mg
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1] The detection of *C. perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks and for this purpose various culture media have been proposed since the 1950s.
The Lactose Egg-Yolk Neomycin Agar medium, alongside TSC agar (REF 402158), is suitable for the detection of *C. perfringens* according to ISO 15213-3.[2]
The mixture of peptones and meat extract provides carbon, nitrogen and trace elements necessary for bacterial growth, sodium chloride ensures osmotic balance, lactose is the fermentable substrate and phenol red is the pH indicator to highlight the reaction.
Egg Yolk Emulsion 20% (REF 42111205) is the lecithinase indicator and neomycin sulphate (REF 4240034) is an antibiotic that inhibits protein synthesis of contaminants, contributing to the selective isolation of *C. perfringens*.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 24.55 g in 500 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes and cool to 47-50°C. Add 25 mL of Egg-Yolk Emulsion 20% (REF 42111205) and the contents of one vial of Neomycin Antimicrobic Supplement (REF 4240034) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

LENA

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	red, clear
Prepared plates appearance	red-orange, opalescent
Final pH at 20-25 °C	7.3 ± 0.2

Neomycin Antimicrobic Supplement

Freeze-dried supplement appearance	short, white pastille
Reconstituted supplement appearance	colourless, clear

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lactose Egg-Yolk Neomycin Agar (LENA) Base	Dehydrated medium	4015732	500 g (10.18 L)
Neomycin Antimicrobic Supplement	Freeze-dried supplement	4240034	10 vials, each for 500 mL of medium
Egg Yolk Emulsion 20%	Liquid supplement	42111205	100 mL
Lactose Egg-Yolk Neomycin Agar (LENA)	Ready-to-use plates	541573	2 x 10 plates ø 90 mm

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7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, controlled atmosphere generators and jars, ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption and for animal feeding, environmental samples in the area of food and feed production and handling, samples from the primary production stage. Refer to applicable International Standards for the collection, transport, storage and preparation of samples and operate in accordance with good laboratory practice. [2]

9 - TEST PROCEDURE

Detection of *C. perfringens* with Lactose Egg-Yolk Neomycin Agar (ISO/TS 15213-3) [2]

1. Prepare the initial suspension in the case the product of concern is not liquid. Add 1 mL of the liquid sample or 1 mL of the initial suspension (0,1 g product) to 9 mL of Rapid Perfringens Medium-RPM (REF 401984) and incubate for 18 ± 2h at 46°C.
2. Equilibrate plates to room temperature, if stored at low temperatures, and dry the surface if necessary.
3. From the selective enrichment in RPM, inoculate 10 µL on a TSC Agar plate (REF 402158) and 10 µL on a LENA plate.
4. Incubate anaerobically the TSC Agar at 37°C for 24 h ± 2 h and the LENA at 46°C 24 h ± 2 h.
5. Take 5 typical colonies from both plates and inoculate them on a non-selective medium (e.g. Columbia Blood Agar REF 541136 or a highly nutritive non-selective medium such as BHI Agar) and incubate anaerobically for 20 h ± 2 h at 37°C.
6. Proceed with confirmation tests: acid phosphatase test (REF 192010) or SIM Agar test (REF402037).

10 - READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On LENA, *C. perfringens* produces yellow colonies as a result of lactose fermentation, with an opaque lecithinase halo.
On TSC Agar, *C. perfringens* usually produces black or grey-yellow-brown colonies as a result of sulphite reduction to sulphide.
C. perfringens colonies are positive to acid phosphatase test, or are positive for sulfite production, negative for indole production and mobility on SIM Agar.

11 - USER QUALITY CONTROL

All manufactured lots of the products 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
C. perfringens ATCC 13124	46°C/ 22-26 H / AN	growth, yellow colonies with opaque halo
E. coli ATCC 25922	46°C/ 22-26 H / AN	totally inhibited
B. subtilis ATCC 6633	46°C/ 22-26 H / AN	possible growth, yellow colonies without halo

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated LENA Base supplemented with Neomycin Antimicrobic Supplement and Egg-Yolk Emulsion 20%, and ready-to-use plates of LENA, are tested for productivity, selectivity and specificity by comparing the results with previously approved Reference Batches.
Productivity is tested by a quantitative technique with the target strains *C. perfringens* ATCC 13124 and *C. perfringens* ATCC 12916, with anaerobic incubation at 46°C for 24 hours. The target strains show good growth with yellow colonies and an opaque halo.
Selectivity is tested by using the modified Miles-Misra method with *E. coli* ATCC 25922. The strain is totally inhibited.
Specificity is tested by the semi-quantitative ecometric technique with *B. subtilis* ATCC 6633. The strain can develop yellow colonies without an opaque halo.

13 - LIMITATIONS OF THE METHOD

The medium is designed to provide an aid in the detection of *Clostridium perfringens*, alongside the TSC Agar medium.

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplements and the ready-to-use plates are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Neomycin Antimicrobic Supplement is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- 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, avoid 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.
- Be careful when opening the metal ring of vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.

- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets 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

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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).

Freeze-dried supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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/bottles) and the applied storage conditions (temperature and packaging). According to ISO 15213-3[2], the LENA medium plates prepared in Laboratory can be stored at 2-8 °C for up to 4 weeks and the medium base can be stored at 2-8 °C for up to 4 weeks in closed containers or tubes; prior to use, the stored medium is melted completely and cooled down to 44 °C to 47 °C before use.

16 - REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. ISO/TS 15213-3:2024 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Clostridium* spp. – Part 3: detection of *Clostridium perfringens*.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First edition	2025/03

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