RAPID PERFRINGENS MEDIUM (RPM) BASE
INSTANT WHOLE MILK POWDER
RPM SELECTIVE SUPPLEMENT

Dehydrated culture medium and supplements

1 - INTENDED USE
For the enrichment of *Clostridium perfringens* in food according to ISO 15213-3 and its presumptive identification.

2 – COMPOSITION*
RAPID PERFRINGENS MEDIUM (RPM) BASE - DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	11.0 g
Enzymatic digest of casein	15.0 g
Peptone	10.0 g
Glucose	10.5 g
Sodium thioglycollate	0.5 g
Sodium chloride	5.5 g
L-cystine	0.5 g
Resazurin	0.001 g
Dipotassium hydrogenphosphate	10.0 g
Iron (II) sulphate·$7H_2O$	1.0 g
Gelatin	120.0 g
Agar	1.4 g

INSTANT WHOLE MILK POWDER (PACKAGE CONTENTS)

Instant whole milk powder	300 g

RPM SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Neomycin sulphate	37.5 mg
Polymyxin B	6.25 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
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.
In 1978, the first study was published that demonstrated the excellent performance of Rapid Clostridium Medium compared to SPS medium. 774 samples naturally contaminated with *C. perfringens* were tested: 546 (71%) were positive on RPM and only 168 (22%) on SPS agar.[2]
Rapid Perfringens Medium (RPM) was developed by Erickson and Deibel[2] for the detection and estimation of low numbers of *C. perfringens* in foodstuffs by the MPN method in test tubes and is recommended by ISO 15213-3:2024[3] as a selective enrichment medium for *C. perfringens* prior to inoculate on TSC Agar and LENA. The medium allows presumptive identification of *C. perfringes* due to the characteristic coagulation reaction and gas production (stormy fermentation).
The Rapid Perfringens Medium consists of two separately prepared solutions, which are combined after sterilisation. The complete medium contains all the necessary elements to promote the growth of clostridia such as peptones, sodium chloride, glucose; L-cystine and sodium thioglycolate are reducing agents that promote anaerobiosis. Whole milk allow the fermentation reaction to be visualised. Selectivity is ensured by the antibiotics polymyxin B sulphate and neomycin sulphate, combined with an incubation temperature of 46° C.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION
Prepare the two solutions separately.
RPM solution
Suspend 46.35 g in 250 mL of cold purified water. Dissolve the powder, heating if necessary and sterilise at 121°C for 5 minutes. Cool to 44-47°C.
Instant Whole Milk Powder solution
Suspend 25 g in 250 mL of cold purified water. Mix well and sterilise at 121°C for 5 minutes. Cool to 44-47°C and add the contents of one vial of RPM Selective Supplement (REF 4240051) reconstituted with 5 mL of sterile purified water.

Combine the two solutions, mix well and dispense into sterile tubes (9 mL) or flasks (90 mL) If not used within 8 hours, just before use, heat in boiling water or steam for 15 minutes, then cool rapidly to incubation temperature.

5 - PHYSICAL CHARACTERISTICS
RPM

RPM dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Instant Whole Milk Powder appearance	creamy white, very fine, free flowing powder
Appearance of the complete solution	milky, opaque solution
Final pH at 20-25 °C	6.8 ± 0.2

RPM SELECTIVE SUPPLEMENT

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

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6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Rapid Perfringens Medium (RPM) Base	Dehydrated medium	4019842	500 g (5.3 L)
Instant Whole Milk Powder	Raw material/supplement	4120502	300 g (6 L)
RPM Selective Supplement	Freeze-dried supplement	4240051	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, 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* according to ISO/TS 15213-3[3]

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 .Alternatively, 10 mL of the liquid sample or of the initial suspension (1 g product) is added to 90 mL of Rapid Perfringens Medium RPM
2. Incubate for 18 ± 2 h at 46°C.
3. If stored at low temperatures, equilibrate plates for the subculture to room temperature, and dry the surface if necessary.
4. From the selective enrichment in RPM, inoculate 10 µL on a TSC Agar plate (REF 402158) and 10 µL on a LENA plate (REF 401573).
5. Incubate anaerobically the TSC Agar plates at 37°C for 24 h ± 2 h and the LENA plates at 46°C 24 h ± 2 h.
6. 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.
7. Proceed with confirmation tests: acid phosphatase test (REF 192010) or SIM Agar test (REF 402037).

Enumeration of *C. perfringens* by MPN method[2]

1. Prepare the initial suspension in the case the product concern is not liquid. Add 1 mL of the liquid sample or 1 mL of the initial suspension (0.1 g product) and 1 mL of decimal dilutions to 9 mL of Rapid Perfringens Medium-RPM, according to a MPN scheme with three tubes for each dilution.
2. Incubate at 46°C for 18-24 hours.

10 - READING AND INTERPRETATION

ISO/TS 15213-3 method

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

MPN method

The tubes are considered positive if coagulation and gas formation (stormy fermentation) are observed.

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/ 16-20 H	growth with visible fermentation
E. coli ATCC 25922	46°C/ 16-20 H	totally inhibited

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 RPM Base supplemented with RPM Selective Supplement, is tested for productivity and selectivity by comparing the results with previously approved Reference Batches.
The medium is tested by inoculating the tubes with *C. perfringens* ATCC 13124 and *C. perfringens* ATCC 12916 by dilution to extinction method. After incubation at 46°C for 16-20 hours *C. perfringens* shows visible fermentation.
To assess selectivity, the medium is inoculated with *E. coli* ATCC 25922: after incubation at 46°C for 20 hours no appreciable change is visible of the medium and, after subculture of 10 µL onto Mac Conkey Agar, there is no growth.

13 - LIMITATIONS OF THE METHOD

- The medium allows only presumptive identification of *C. perfringens* and therefore appropriate confirmatory tests must be carried out.
- If the medium is not used within 8 hours, it needs to be warmed beforehand to remove oxygen and promote its liquefaction, so that both solid and liquid samples can be inoculated.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements 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. RPM Selective 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, 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.
- Be careful when opening the metal ring of vials to avoid injury.
- The selective supplement is sterilized by membrane filtration.
- 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 supplements 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

Dehydrated medium base and powder supplement

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, they may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottles in humid places. After use, the containers must be tightly closed. Discard the products if the containers and/or the caps are damaged, or if the containers are 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 the sterilised basal medium can be stored for a maximum of 4 weeks at 2-8 °C in closed containers or test tubes; before use the medium must be completely redissolved and cooled to 44 °C - 47 °C. The sterilised Instant Whole Milk solution can be stored at 2-8°C for up to 4 weeks in closed containers or tubes.[3]

16 - REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. J.E. Erickson, R.H. Deibel. New medium for rapid screening and enumeration of *Clostridium perfringens* in foods. Applied and environmental microbiology, American Society for Microbiology, Oct. 1978, p567-571
3. 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	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

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.