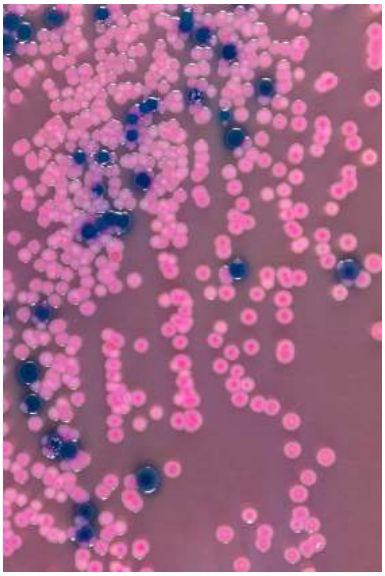
ChromArt

CHROMOGENIC YERSINIA AGAR BASE
CHROMOGENIC YERSINIA SUPPLEMENT

Dehydrated culture medium and selective supplement



Chromogenic Yersinia Agar:
Yersinia enterocolitica with "bull's eye" colonies;
Serratia marcescens: blue colonies.

1 - INTENDED USE

For the determination of the presence or absence of *Yersinia enterocolitica* in foods.

2 – COMPOSITION*

CHROMOGENIC YERSINIA AGAR BASE
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptones	22.0 g
Cellobiose	20.0 g
Sodium pyruvate	2.0 g
Sodium chloride	5.0 g
Sodium deoxycholate	0.5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Irgasan	0.004 g
5-Bromo-4-chloro-3-indolyl β-D-glucopyranoside (X-Glupy)	0.2 g
Growth factors	0.15 g
Agar	12.0 g

CHROMOGENIC YERSINIA SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Antibiotic mix	0.0875 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

Chromogenic Yersinia Agar is a modification of Cefsulodin-Irgasan-Novobiocin (CIN) Agar originally developed in 1979 by Schiemann,[1] devised to differentiate, with a chromogenic reaction, the colonies of some enterobacteria from those of *Yersinia enterocolitica*, thus increasing the specificity of the method.
Chromogenic Yersinia Agar, complete with the antibiotic mix of the selective supplement, may be used as the second plating medium in the procedure recommended by ISO 10273[2] for the determination of the presence or absence of pathogenic *Y. enterocolitica* in food chain samples.
Peptone and special growth factors provide nutrients for bacterial growth. Sodium chloride maintains the osmotic balance of the medium. Sodium pyruvate aids in the resuscitation of sub-lethally injured cells. Gram-positive and some Gram-negative bacteria (e.g. *E.coli, K. pneumoniae, P. mirabilis, P. aeruginosa*) are inhibited by the selective agents present in the medium base (sodium deoxycholate, crystal violet, irgasan) and in the lyophilized supplement. Cellobiose is present as a fermentable carbohydrate: cellobiose fermenting bacteria induce acidification of the medium with precipitation of deoxycholate and absorption of neutral red; *Y. enterocolitica* therefore cultivates with the characteristic aspect of the "bull's eye" colonies: the center of the colony deep red with a transparent margin. Organisms that do not metabolize cellobiose to acid end-products will form colourless colonies. The medium also contains X-Glupy: the colonies of β-glucosidase positive strains hydrolyze the chromogenic compound with the formation of a green-blue chromophore. *C. freundii, P. rettgeri, S. marcescens, K. oxytoca* form blue or blue-green colonies.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30.9 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add the content of one vial of Chromogenic Yersinia Supplement (REF 4240095) dissolved with 5 mL of sterile purified water using aseptic precautions. Mix well and pour into sterile Petri dishes

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-purple, slightly opalescent
Freeze-dried selective supplement	short, dense, white, pellet
Reconstituted selective supplement	colourless, limpid
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Chromogenic Yersinia Agar Base	Dehydrated medium	4080502	500 g (8.09 L)
Chromogenic Yersinia Supplement	Freeze-dried supplement	4240095	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile inoculation needles, loops, incubator and laboratory equipment as required, sterile Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

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8 - SPECIMENS

Food products and ingredients intended for human consumption and the feeding of animals; environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[2]

9 - TEST PROCEDURE

Prepare the suspension of the sample with Yersinia PSB Broth (REF 402270) to obtain a 1:10 dilution (e.g.: 25 g of sample +225 mL of liquid medium).
The ISO 10273 method prescribes the direct inoculum of the sample suspension on CIN Agar plates and on a chromogenic medium or the inoculum of the sample after enrichment.

DIRECT PLATING

Using the initial PSB suspension, divide a volume of 1 mL onto two to four CIN agar plates and a volume of 1 mL onto two to four Chromogenic Yersinia Agar plates. Spread it over the plates with a spreader. Invert the plates and place them in the incubator set at 30°C for 24 h ± 2 h.

PLATING AFTER ENRICHMENT

Transfer 10 mL of the initial suspension in PSB (REF 402270) into 90 mL of Yersinia ITC Broth (REF 402265).
Incubate the initial suspension in PSB and the selective enrichment broth ITC at 25 °C for 44 h ± 4 h.
Using a sterile pipette, transfer 0.5 mL of the PSB enrichment into 4.5 mL of KOH solution (0.5 g in 100 mL of sterile saline solution) prepared the day before use, and mix. After 20 s ± 5 s of the addition of the PSB enrichment to the KOH solution, streak by means of a loop the surface of a CIN Agar plate and of a Chromogenic Yersinia Agar plate.
Repeat the procedure for ITC enrichment.
Invert the plates and place them in the incubator set at 30°C for 24 h ± 2 h.
Inoculate CIN Agar plates and Chromogenic Yersinia Agar plates also with untreated PSB and ITC cultures.

10 - READING AND INTERPRETATION

Observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
After an incubation at 30°C for 24 h ± 2 h on Chromogenic Yersinia Agar, *Y. enterocolitica* grows with red colonies, with transparent surrounding rim. The colonies diameter changes from one strain to another but is the same within the same serotype.
K. oxytoca, C. freundii, P. rettgeri, S. marcescens can grow and form blue or green/blue colonies. *Aeromonas hydrophila* grows with pink colonies. Gram positive bacteria are totally inhibited. Perform the confirmation tests, according to ISO 10273[2], on the typical colonies.

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.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
Y. enterocolitica	ATCC	9610	30°C / 18-24H / A	good growth, colonies with red centre
S. marcescens	ATCC	8100	30°C / 18-24H / A	good growth, blue colonies
E. coli	ATCC	25922	30°C / 18-24H / A	inhibited
S. aureus	ATCC	25923	30°C / 18-24H / A	inhibited

A: aerobic 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 Chromogenic Yersinia Agar Base supplemented with Chromogenic Yersinia Supplement are tested for productivity, specificity and selectivity with incubation at 30°C for 24 hours, by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative test with the target strains *Y. enterocolitica* ATCC 23715 and ATCC 9610: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical ("bull's eye" colonies: the centre of the colony deep red with a transparent margin) the results are considered acceptable and conform to the specifications.
Specificity is tested by a semi-quantitative ecometric technique with the non-target strains *S. marcescens* ATCC 8100 and *A. hydrophila* ATCC 7965. The amount of growth and colonies characteristics are evaluated after incubation: *S. marcescens* grows with blue-green colonies while *A. hydrophila* exhibits a light growth with colourless colonies with pink centre.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. After incubation, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- In case of dense growth of background flora on the plates, the colony size of *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent.
- *Y. intermedia, Y. frederiksenii,* and *Y. kristensenii* grow equally as well as *Y. enterocolitica* and exhibit the same colony morphology.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement 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 supplement 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. Chromogenic Yersinia 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 the supplements 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 supplements 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

Dehydrated medium

Upon receipt, store at +2°C /+8°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°C /+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/flasks) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Schiemann D A. Synthesis of a selective agar medium for Yersinia enterocolitica. Can J Microbiol 1979; 25(11):1298-1304,
2. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica

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	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/06

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