ChromArt

E.C.O.A.GAR
(ENTEROCOCCUS CHROMOGENIC OTTAVIANI & AGOSTI AGAR)

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

E.C.O.A.gar:
colonies of Enterococcus sp. (blue)
and contaminants

1 - INTENDED USE

For the isolation, enumeration and presumptive confirmation of enterococci in water, milk, food.

2 - COMPOSITION

E.C.O.A.GAR, DEHYDRATED MEDIUM (401430)
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Peptones	**28.0 g**
Sodium chloride	5.0 g
Glucose	1.0 g
Emulsifying agents	5.7 g
Phosphate buffer	5.0 g
Agar	15.0 g
Chromogenic substrates	180.0 mg
Selective compounds	26.0 mg

KANAMYCIN SELECTIVE SUPPLEMENT (4240055C)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Kanamycin sulphate	10 mg

E.C.O.A.GAR, READY-TO-USE PLATES (491430) *

E.C.O.A.gar, dehydrated medium	60 g
Kanamycin sulphate	20 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

E.C.O.A.gar is a selective chromogenic medium for the isolation, enumeration, and presumptive confirmation of enterococci in water, milk, food, and other materials of sanitary interest. It is prepared according to the formulation developed by F. Ottaviani and M. Agosti.[1] Selectivity of the medium is achieved with a mixture of antimicrobial compounds included in the base medium and with the addition, after sterilization, of kanamycin sulphate.
The medium contains no sodium azide, is not classified as hazardous, and therefore requires no special precautions for use and disposal.
The differential characteristics of the medium are due to a mixture of chromogenic compounds for the determination of specific enterococcal enzymes. Compared with conventional KAA and KF media, E.C.O.A.gar exhibits higher specificity and sensitivity for the detection of *Enterococcus* strains lacking specific enzyme complexes (e.g., for the for hydrolysis of esculin) and that do not form typical colonies: specifically *Enterococcus avium* strain FAIR-E101, *Enterococcus faecium* strains FAIR-E 102, E130, E 131, and E-338, *Enterococcus hirae* strain FAIR-E 174, and *Enterococcus malodoratus* strains FAIR-E168 and E169, all from the BCCM/LMG Bacteria Collection of the University of Ghent (B)[1].

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 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 contents of one vial of Kanamycin Selective Supplement (REF 4240055) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale violet, clear
Freeze-dried selective supplement	low, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
E.C.O.A.gar	Dehydrated medium	4014302	500 g (8.3 L)
Kanamycin Selective Supplement	Freeze-dried supplement	4240055	10 vials, each for 500 mL of medium base
E.C.O.A.gar	Ready to use plates	491430	3 x 10 plates ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Water, milk, food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

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9 - TEST PROCEDURE

The medium can be used in accordance with standard laboratory procedures, inoculated by spread-plate or poured plate or membrane filtration techniques and incubated at 37°C for 24 hours.
The E.C.O.A.gar medium provides the laboratory with the following advantages:

- higher enterococcal recovery than traditional KAA and KF media due to the higher differential power;
- greater safety for operators, limited environmental impact, and easier management of laboratory waste due to the absence of sodium azide.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth, recording any specific morphological and colour characteristics of the colonies.
Enterococci grow blue-green colonies.
Rare colonies of non-*Enterococcus* strains, resistant to antimicrobials in the culture medium, grow with colourless, violet-grey or magenta-red colonies or with natural pigmentation.

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
E. faecalis	ATCC	29212	37°C / 24H / A	good growth, blue colonies
E. faecium	ATCC	19434	37°C / 24H / A	good growth, blue colonies
E. coli	ATCC	25922	37°C / 24H / A	inhibited
S. aureus	ATCC	25923	37°C / 24H / A	inhibited

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use E.C.O.A.gar are tested for productivity and selectivity with incubation at 37°C for 24 hours, by comparing the results with a previously approved Reference Batch.
The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *E. faecalis* ATCC 29212, *E. faecalis* ATCC 33186, *E. faecium* ATCC 19434, *E. hirae* ATCC 8043. After incubation at 37°C for 24 hours the target strains exhibit good growth with blue colonies.
The selectivity is assessed 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 following non-target strains: *S. mitis* ATCC 9811, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. mirabilis* ATCC 10005, *P. aeruginosa* ATCC 27853. The growth of the non-target strains is totally inhibited after incubation at 37°C for 18-24 hours

13 - LIMITATIONS OF THE METHOD

- 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, the supplement 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 supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Kanamycin Selective Supplement is classified as hazardous. 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 supplement vial 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 sterilized 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.

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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 +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 2-8°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/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 - REFERENCE

1. Ottaviani F, Agosti M. (2000) Personal communication

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 1	Updated layout and content	2022/08

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