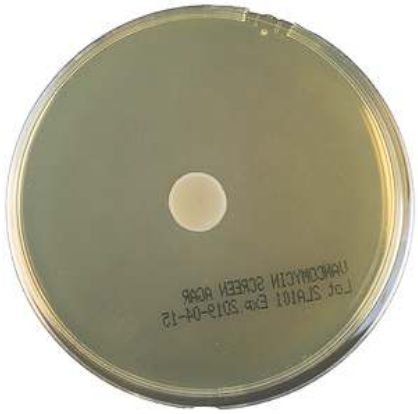
INSTRUCTIONS FOR USE

VANCOMYCIN SCREEN AGAR

Ready-to-use plates



Vancomycin Screen Agar:
Vancomycin resistant *Enterococcus faecalis*

1 - INTENDED USE

In vitro diagnostic device. Screen agar for the detection of presumptive resistance of enterococci to vancomycin and presumptive reduced susceptibility of *Staphylococcus aureus* to vancomycin.

2 - COMPOSITION -TYPICAL FORMULA *

Brain heart infusion and peptones	27.5 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g
Agar	15.0 g
Vancomycin	0.006 g
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

Enterococci are common cause of urinary tract infections, intra-abdominal and intra-pelvic abscesses or post-surgery wound infections and blood-stream infections. The emergence and spread of resistant enterococci, particularly vancomycin resistant enterococci (VRE), represents a major public health problem. Glycopeptide resistance is mediated by different Vancomycin resistance (*Van*) gene operons: at least nine acquired resistance types and an additional type (*Van*C) that is intrinsic to *E.casseliflavus* and *E.gallinarum*.[1]
Staphylococcus aureus is a virulent microorganism responsible for many serious infections among the general population. The first clinical isolate of *S.aureus* with reduced susceptibility to vancomycin was identified by Hiramatsu *et al*. in 1997[2], and these strains have now been reported worldwide.
The Clinical and Laboratory Standards Institute (CLSI), recommends Brain Hearth Infusion Agar supplemented with 6 mg/L of vancomycin as a screening test for the presumptive detection of pure cultures of vancomycin resistant enterococci and of *S.aureus* strains with a reduced susceptibility to vancomycin.[3]
Vancomycin Screen Agar contains brain heart infusion and peptones as sources of nitrogen, carbon, vitamins and minerals for microbial growth; glucose provides an energy source, sodium chloride maintains osmotic balance, disodium hydrogen phosphate is included as a buffer system; vancomycin at the concentration of 6 mg/L is used to detect the resistance to vancomycin.

4 - PHYSICAL CHARACTERISTICS

Prepared plates appearance	pale yellow, limpid
Final pH at 20-25°C	7.4 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Vancomycin Screen Agar	Ready-to-use plates	549520	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator, laboratory equipment as required, sterile loops, swabs, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Vancomycin Screen Agar is inoculated with pure cultures of clinical isolates, presumptively identified as enterococci or *S.aureus*. It is not intended as primary isolation medium for clinical specimens.

8- TEST PROCEDURE

Allow plates to come to room temperature before inoculation.
Select several well isolated colonies from a non-selective agar plate and prepare a suspension in Tryptic Soy Broth equivalent to 0.5 McFarland standard.
Preferably, using a micropipette, spot 1-10 µL drop (enterococci) or 10 µL drop (*S.aureus*) onto Vancomycin Screen Agar plate. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10-15 mm in diameter or streak a portion of the plate.
A blood agar plate should be also inoculated as a growth control, to check strain viability and purity.
Invert the plate and incubate at 35 ± 2°C, aerobically for a full 24 hours.

9- READING AND INTERPRETATION

Examine the incubated plates carefully with transmitted light.
Observe control plate for growth and purity that indicates viable test organisms in the inoculum.
Examine carefully the Vancomycin Screen Agar plate for growth.
Enterococci with a presumptive resistance to vancomycin: presence of > 1 colony.

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S.aureus with a presumptive reduced susceptibility to vancomycin: presence of > 1 colony or light film of growth.

Additional testing and reporting (enterococci)

Perform vancomycin MIC on *Enterococcus* spp. that grow on Vancomycin Screen Agar and test for motility and pigment production to distinguish species with acquired resistance (eg. *van*A and *van*B) from those with intrinsic intermediate-level resistance to vancomycin (eg. *van*C), such as *E.gallinarum* and *E.casseliflavus* which often grow on the medium. In contrast to other enterococci, *E.gallinarum* and *E.casseliflavus* with vancomycin MICs of 8-16 µg/mL (intermediate) differ from vancomycin-resistant enterococci for infection prevention purposes.

Additional testing and reporting (*S.aureus*)

Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S.aureus* that grows on Vancomycin Screen Agar.

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.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis (VR) ATCC 51299	33-37°C / 24 h / A	growth
E. faecalis ATCC 29212	33-37°C / 24 h / A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Vancomycin Screen Agar is tested for performance characteristics with one vancomycin susceptible strain (*E.faecalis* ATCC 29212) and one vancomycin resistant strain (*E.faecalis* ATCC 51299).

After incubation at 33-37°C for 24 hours in ambient air, *E.faecalis* ATCC 51299 shows growth, while *E.faecalis* ATCC 29212 is completely inhibited.

12 - LIMITATIONS OF THE METHOD

- Testing on Vancomycin Screen Agar doesn't reliably detect all vancomycin-intermediate *S.aureus* strains: some strains for which the vancomycin MICs are 4µg/mL will fail to grow.[3]
- Perform vancomycin MICs with a reliable method on *Enterococcus* spp. and *S.aureus* that grow on Vancomycin Screen Agar for resistance confirmation and for determining the precise concentration of vancomycin to which the isolate is resistant.
- *E.gallinarum* and *E.casseliflavus,* with an intrinsic intermediate level resistance to vancomycin, often grow on the medium.[3]
- There are insufficient data to recommend the use of this medium for the detection of vancomycin resistant strains other than enterococci and *S.aureus*.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- 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.
- 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.
- 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

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).

15 - REFERENCES

1. Orsi GB, Ciorba V. Vancomycin resistant enterococci healthcare associated infections. Ann Ig 2013; 25: 485-492

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2. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J. Ant Chem 1997;40(1):135–6
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 30th ed. CLSI supplement M100-S30. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/11
Revision 2	Removal of obsolete classification	2023/03

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

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