REF 04930

chromID® VRE Agar

IVD

Selective chromogenic medium for the detection and differentiation of *Enterococcus faecium* and *E. faecalis* showing acquired vancomycin resistance (VRE).

SUMMARY AND EXPLANATION

chromID® VRE Agar is a selective chromogenic medium for the detection of *E. faecium* and *E. faecalis* showing acquired vancomycin resistance (VRE), in at risk patients (1).
chromID® VRE agar enables the differentation of *Enterococcus faecium* and *E. faecalis*.
The *E. faecium* and *E. faecalis* with acquired vancomycin resistance (mainly genotypes vanA and vanB) are multi-resistant bacteria which can be responsible for health care associated infections (2). The detection of this resistance is particularly important for the prevention and epidermiological surveillance of these infections and also to prevent emergence of vancomycin resistant *Staphylococcus aureus* (VRSA), by transmission of the vanA gene (3, 4).
The medium is not a substitute for the conventional methods of antimicrobial susceptibility test methods.

PRINCIPLE

chromID® VRE Agar consists of a rich nutritive base include a variety of peptones. It also contains two chromogenic substrates and a mixture of antibiotices including vancomycin which enable:

- the specific and selective growth of VRE.
- the direct detection and differentiation of *E. faecium* and *E. faecalis* through characteristic colour of colonies:
  - *E. faecium*: violet colour for b-galactosidase-producing strains,
  - *E. faecalis* : blue-green colour for a-gulcosidase-producing strains.

The selective mixture inhibits:

- the enterococci strains that do not express acquired vancomycin resistance,
- the enterococci species that express natural vancomycin resistance (vanC genotype: *E. gallinarum* and *E. casseliflavus*),
- most Gram negative and Gram positive bacteria, yeasts and moulds.

PRESENTATION

	Ready-to-use medium
REF 04930	Pack of 1x10 plates (90 mm)
	*VRE

* printed on each plate.

COMPOSITION

Theoretical formula

This medium can be adjusted and/or supplemented according to the performance criteria required:

Casein and meat peptone (bovine and porcine)	18 g
Heart peptone (bovine or porcine)	3 g
Corn starch	1 g
Sodium chloride	6 g
Agar	15 g
Mixture of chromogenic substrates	0.12 g
Antibiotic mixture	52.3 mg
Purified water	1 L

pH 7.2 ± 0.2

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Material:

- Bacteriology incubator
- Vancomycin discs (30mg)
- Brain Heart Infusion Broth (Ref. 42081) (9 mL tube)

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This product contains materials of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure.
- Culture media should not be used as manufacturing material or components.
- Do not use past the expiration date.
- Do not use if the packaging is damaged.
- Do not use contaminated plates, or plates that exude moisture.
- Interpretation of the test results should be made taking into consideration the patient's history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed.
- Use of the medium may be difficult for people who have problems recognising colours.
- Only use one specimen per plate.
- Reading and interpretation should be performed using isolated colonies.
- Some precipitates and/or light halo may be observed in the agar but these do not affect the performance of the product.

STORAGE CONDITIONS

Protect from Light
Store product in original packaging at 2-8°C until the expiry date.

SPECIMENS

Different types of specimens may be used: stools, anal swabs.
They should be inoculated directly onto the agar or after enrichment in a broth (see instructions for use) for better detection of genotype vanB enterococci.
Good laboratory practices for collection and transport should be respected.
Note:

- It is recommended to use swabs (preferably flocked) with liquid transport medium to optimise recovery of VRE.
- VRE may be inhibited if the patient has used drug substances containing antispetic agents such as sodium hydroxide or chlorhexidine gluconate.

INSTRUCTIONS FOR USE

The medium must not be exposed to light other then during the inoculation and reading steps.

1. **Allow plates to come to room temperature in the dark.**
2. Inoculate the sample:
   - either directly onto chromID® VRE Agar, or
   - after enrichment (18-24 hours at 35±2°C) in Brain Heart Infusion Broth (Ref. 42081) containing 3.3mg/L of vancomycin (concentration obtained by addition of a 30mg vancomycin disc).
3. Incubate the plates inverted at 35±2°C in aerobic conditions, in the dark. The cultures are examined after 24 hours of incubation.

   If a negative result is obtained (no growth or colour), the medium must be incubated for a further 24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and the colour of the isolated colonies.
Typical colonies of *E. faecium* and *E. faecalis* with acquired vancomycin resistance (VRE) are:

- - a violet colour: *E. faecium* species
- - a blue-green colour: *E. faecalis* species.

Confirm that colour with a characteristic colour are Gram positive cocci.

QUALITY CONTROL

Protocol:

The capacity of the medium can be tested using the following strains:

- *Enterococcus faecalis* ATCC 51299
- *Enterococcus faecium* ATCC 700221
- *Enterococcus faecalis* ATCC 29212
- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923
- *Candida tropicalis* ATCC 9968
- *Pediococcus pentasaceus* ATCC BAA-2529
- *Enterococcus faecium* 3-299 SCV
- *Klebsiella oxytoca* 9009075
- *Enterococcus gallinarum* 05 02 053

Range of expected results:

Strain	Results at 35±2°C after 24±4 hours
Enterococcus faecalis ATCC 51299	Growth, blue-green colonies
Enterococcus faecium ATCC 700221	Growth, violet colonies
Enterococcus faecium 3-299 SCV	Growth, violet colonies

Strain	Results at 35±2°C after 48±2-6 hours
Enterococcus faecalis ATCC 51299	Growth, blue-green colonies
Enterococcus faecalis ATCC 29212	Inhibition
Escherichia coli ATCC 25922	Inhibition
Staphylococcus aureus ATCC 25923	Inhibition
Candida tropicalis ATCC 9968	Inhibition
Pediococcus pentasaceus ATCC BAA-2529	Inhibition
Enterococcus faecium 3-299 SCV	Growth, violet colonies
Klebsiella oxytoca 9009075	Inhibition
Enterococcus gallinarum 05 02 053	Inhibition

Note:

It is the responsibility of the user to perform Quality Control taking into consideration the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature…).

LIMITATIONS OF THE METHOD

- Very few *E. gallinarum* and *E. hirae* strains with acquired vancomycin resistance develop on chromID® VRE Agar and produce typical violet colonies.
- Some microorganisms other than enterococci (including yeasts, Gram negative bacilli, Pediococcus) may develop on the medium and produce typical colonies but which generally differ morphologically.
- Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains of *E. faecium* or *E. faecalis* with acquired vancomycin resistance (VRE) and which have specific requirements (substrate, temperature, incubation conditions etc.) may not develop or may not product typical colonies.
- After 48 hours of incubation, if direct inoculation has been performed with an inoculum that is particularly strong, typical vancomycin-susceptible *E. faecium* and *E. faecalis* colonies my be observed at the point of inoculation.

PERFORMANCE

Performance was evaluated at 35±2°C using 23 bacterial (*Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Pediococcus*, *Staphylococcus* and yeast (*Candida*)) strains.

Nutrient capacity:
Three (3) out of four (4) strains of *Enterococcus faecium* were isolated after 24 hours incubation.
All *Enterococcus faecalis* strains were isolated after 24 hours incubation.

Selectivity:
All bacterial organisms were inhibited after 72 hours incubation.

These results were obtained under controlled laboratory conditions using non-clinical strains. Interpretation of this performance data should include consideration for the limitations previously stated.

WASTE DISPOSAL

Unused product may be considered as non-hazardous waste and disposed of accordingly.
Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious materials.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazard and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. Muto, C.A., Jernigan, J.A., Ostrowsky, B.E., et al., Guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*, Infect. Control Hosp. Epidermiol., 2003, Vol. 24, p. 362-386.
2. Yesim Cetinkaya, Pamela Falk and C. Glen Mayhall, Vancomycin-Resistant Enterococci, Clinical Microbiology Reviews, Oct 2000, p. 686-707.
3. Tacconelli, E., New strategies to identify patients harbouring antibiotic-resistant bacteria at hospital admission, Clin. Microbiol. Infect., 2006, Vol. 12, p. 102-109.
4. Tenover, F.C., Weigel, L.M., Appelbaum, P.C. et al., Vancomycin-Resistant *Staphylococcus aureus* isolate from a Patient in Pennsylvania, Antimicrobial Agents and Chemotherapy, Jan 2004, Vol. 48, No.1, p. 275-280.

INDEX OF SYMBOLS

Symbol	Meaning
REF or **REF**	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Protect from light
	Consult Instructions for Use
	Contains sufficient for <n> tests

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