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 differentiation of *Enterococcus faecium* and *E. faecalis.*
The *E. faecium* and *E. faecalis* with acquired vancomycin resistance (mainly genotypes *van*A and *van*B) 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 epidemiological surveillance of these infections and also to prevent the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA), by transmission of the *vanA* gene (3, 4).

This medium is not a substitute for the conventional antimicrobial susceptibility test methods.

PRINCIPLE

chromID™ VRE agar (patents pending) consists of a rich nutritive base including a variety of peptones. It also contains two chromogenic substrates and a mixture of antibiotics including vancomycin (8 mg/l) which enable:

- the specific and selective growth of VRE.
- the direct detection and the differentiation of *E. faecium* and *E. faecalis* through the characteristic color of colonies.
  - *E. faecium*: violet color for β-galactosidase-producing strains,
  - *E. faecalis*: blue-green color for α-glucosidase-producing strains.

The selective mixture inhibits:

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

CONTENT OF THE KIT

	Ready-to-use media:
REF 43 004	Pack of 20 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	15.0 g
Mixture of chromogenic substrates	0.12 g
Selective mixture	52.3 mg
Purified water	1 l

pH 7.2

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.
- Vancomycin discs (30 µg).
- Brain-Heart broth (Ref. 42 081) (9 ml tube).

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products 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 as infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers from Occupationally Acquired Infections*; *Approved Guideline* – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest Edition " or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates or plates that exude moisture.
- 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 halos may be observed in the agar but these do not affect the performance of the product.
- Interpretation of the test results should be made taking into consideration colonial and microscopic morphology and if necessary, the results of any other tests performed.

STORAGE CONDITIONS

- **Store the plates in their box at 2-8°C until the expiry date.**
- If not in the box, plates can be stored for 2 weeks at 2-8°C in the cellophane sachet **in the dark.**

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 *van*B enterococci.
Note:

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

Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

INSTRUCTIONS FOR USE

The medium must not be exposed to light other than 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 37°C) in Brain-Heart Infusion broth (Ref. 42 081) containing 3.3 mg/l of vancomycin (concentration obtained by addition of a 30 µg vancomycin disc).
3. Incubate the plates inverted at 37°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 color), the medium must be incubated for a further 24 hours.

READING AND INTERPRETATION

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

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

Confirm that colonies with a characteristic color are Gram-positive cocci.

QUALITY CONTROL

Protocol:

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

- *Enterococcus faecium* ATCC® 700221.
- *Enterococcus faecalis* ATCC® 51299.
- *Enterococcus faecalis* ATCC® 29212.

Range of expected results:

Strain	Results at 33-37°C
Enterococcus faecium (*van*A) ATCC® 700221	Growth and violet color within 24 hours
*Enterococcus faecalis (van*B) ATCC® 51299	Growth and blue-green color within 24 hours
Enterococcus faecalis ATCC® 29212	No growth after 48 hours

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 and time, etc.).

LIMITATIONS OF THE METHOD

- Very few *E. gallinarum* and *E. hirae* strains with acquired vancomycin resistance develop on chromID VRE and produce typical violet colonies.
- Some micro-organisms 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 micro-organism. 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 produce 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 may be observed at the point of inoculation.

PERFORMANCE

chromID™ VRE medium (ref. 43004) was compared to chromID™ VRE medium (ref. 43002) after 18-24 hours (D1) and 48 hours (D2).
Two studies were performed in Canada and Germany, using 540 human specimens (stools and anal swabs). The specimens were inoculated directly onto the agar or following enrichment in Brain-Heart broth with a vancomycin disk for 18-24 hours, and then incubated at 37°C. 62 specimens were found to be positive (*E. faecium* *van*A and *van*B) on at least one of the 2 media.
Note: all of the VRE strains isolated belonged to the species *E. faecium*. An in-house study performed using collection strains showed that the sensitivity of detection for *E. faecalis* is equivalent to that of *E. faecium.*

Results obtained without enrichment

Sensitivity of detection for VRE (95% confidence interval)

	chromID™ VRE (ref. 43004)	chromID™ VRE (ref. 43002)
D1	*64.1%* [47.2-78.8] ***van*A: 10/12** ***van*B: 15/27**	*59%* [42.1-74.4] ***van*A: 10/12** ***van*B: 13/27**
D2	*97.4%* [86.5-99.9] ***van*A: 11/12** ***van*B: 27/27**	*97.4%* [86.5-99.9] ***van*A: 11/12** ***van*B: 27/27**

Specificity (95% confidence interval)

	chromID™ VRE (ref. 43004)	chromID™ VRE (ref. 43002)
D1	*98.8%* [97.4-99.6] **495/501**	*98.4%* [96.9-99.3] **493/501**
D2	*95,6%* [93.4-97.2] **479/501**	*94.4%* [92-96.3] **473/501**

Results obtained after selective enrichment (see instructions for use)

Sensitivity of detection for VRE (95% confidence interval)

	chromID™ VRE (ref. 43004)	chromID™ VRE (ref. 43002)
D1	*69%* [55.5-80.5] ***van*A: 11/12** ***van*B: 29/46**	*65.5%* [51.9-77.5] ***van*A: 11/12** ***van*B: 27/46**
D2	*94.8%* [85.6-98.9] ***van*A: 12/12** ***van*B: 43/46**	*91.4%* [80-97.1] ***van*A: 11/12** ***van*B: 42/46**

Specificity (95% confidence interval)

	chromID™ VRE (ref. 43004)	chromID™ VRE (ref. 43002)
D1	*98.1%* [96.5-99,1] **473/482**	*96.9%* [94.9-98.3] **467/482**
D2	*97.9%** [96.2-99] **472/482**	*94.8%** [92.4-96.6] **457/482**

* the specificity of chromID VRE (Ref. 43004) is significantly higher than the specificity of chromID (Ref. 43002).

Negative predictive values (VPN) according to the method used

	chromID™ VRE (ref. 43004)		chromID™ VRE (ref. 43002)	
	Direct inoculation	After enrichment in Brain-Heart broth	Direct inoculation	After enrichment in Brain-Heart broth
D1	*92.7%* [90.1-94.8]	*95.5%* [93.3-97.2]	*92.3%* [89.7-94.5]	*95.1%* [92.8-96.8]
D2	*95%* [92.7-96.8]	*98.5%* [97-99.4]	*94.9%* [92.6-96.7]	*98.1%* [96.3-99.1]

WASTE DISPOSAL

Dispose of used and unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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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, N°1, p. 275-280.
5. NAAS T., FORTINEAU N., SNANOUDJ R. *et al.* – First nosocomial outbreak of Vancomycin-Resistant *Enterococcus faecium* expressing a VanD-like phenotype associated with a *van*A genotype. *J. Clin. Microbiol*. – Aug. 2005, Vol. 43, N°8, p. 3642-3649

INDEX OF SYMBOLS

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

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BIOMÉRIEUX

bioMérieux SA
RCS LYON 673 620 399

69280 Marcy-l'Etoile / France
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

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