REF **414685**

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chromID® CARBA SMART Agar (CARB/OXA)

IVD

MULTIMEDIA

Selective chromogenic media for the screening of Carbapenemase-Producing *Enterobacteriaceae* (CPE).

SUMMARY AND EXPLANATION

chromID® CARBA SMART Agar consists of two chromogenic culture media dispensed into one Petri dish containing separate compartments (CARB/OXA).

chromID CARBA SMART Agar is used for the screening of Carbapenemase-Producing *Enterobacteriaceae* (CPE), in patients who are chronic carriers or in patients at risk of carriage (1, 2, 3, 4, 5, 6, 7).
This agar does not replace conventional susceptibility test methods.
CPE are particularly multi-resistant bacteria that are capable of causing nosocomial infections and hospital epidemics (8, 9, 10, 11). The detection of CPE carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of chromID CARBA SMART agar contributes to the active surveillance of CPE.

PRINCIPLE

chromID CARBA SMART Agar (patents pending) consists of a nutrient-rich base combining different peptones. It contains:

- a mixture of antibiotics which enables the selective growth of:
  - mainly KPC and metallo-carbapenemase-type CPE, for the CARB medium.
  - OXA-48-type CPE for the OXA medium.
- three chromogenic substrates which enable the identification of the most frequently isolated CPE:
  - *Escherichia coli*: spontaneous coloration (pink to burgundy) of strains producing ß-glucuronidase (ß-GUR) and/or ß-galactosidase (ß-GAL) (12).
  - *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC): spontaneous bluish-green to bluish-grey or purple coloration of strains producing ß-glucosidase (ß-GLU).

CONTENT OF THE KIT

	Ready-to-use media:
REF **414685**	Pack of 20 plates (90 mm)
	CARB/OXA *

* printed on each plate

- **CARB** identifies the compartment on the plate containing chromID CARBA agar
- **OXA** identifies the compartment on the plate containing chromID OXA-48 agar

COMPOSITION

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

CARB medium

Casein peptone (bovine)	5 g
Soy peptone	5 g
Meat peptone (bovine or porcine)	8 g
Carbohydrates	1 g
L-Tryptophan	0.9 g
Phosphate buffer	1 g
Chromogenic mixture	1.4 g
Nurient mixture	2.8 g
Selective CARB mixture	0.3 g
Agar	18 g
Purified water	1 l

pH 7.4

OXA medium

Casein peptone (bovine)	5 g
Soy peptone	5 g
Meat peptone (bovine or porcine)	8 g
Carbohydrates	1 g
L-Tryptophan	0.9 g
Phosphate buffer	1 g
Chromogenic mixture	1.4 g
Nutritive mixture	2.8 g
Selective OXA mixture	0.88 g
Agar	18 g
Purified water	1 l

pH 7.4

MATERIALS REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.

POSSIBLE ADDITIONAL REAGENTS

- Etest® strips
- ATCC® Quality Control strains (see list of LyfoCults® Plus strains)

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 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.
- 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 recognizing colors.
- Only use one specimen per plate.
- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
- Interpretation of test results should be made taking into consideration the patient's history, 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 in the cellophane sachet for 2 weeks at 2-8°C **in the dark**.

SPECIMENS

Different types of specimens may be used: stools and rectal swabs. They are inoculated directly on the agar without enrichment.
Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

INSTRUCTIONS FOR USE

1. **Allow plates to come to room temperature.**
2. Inoculate the specimen directly onto the chromID® CARBA SMART agar.

   It is recommended to inoculate the CARB compartment first, followed by the OXA compartment, using the same swab.
3. Incubate the inverted plates at 35 ± 2°C in aerobic conditions. The cultures are generally examined after 18 to 24 hours of incubation.

The user is responsible for choosing the appropriate temperature for the intended use, in accordance with current standards.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and the appearance of the colonies.

CPE produce the following characteristic colors:

- **Pink to burgundy** colonies or translucent colonies with a pink to burgundy center: ***E. coli*** species.
- Bluish-green to bluish-grey or purple colonies: **KESC group**.
  Identification of the microorganism must be followed by additional tests.

- Presence of typical colonies in the CARB compartment: suspicion of CPE.
- Presence of typical colonies in the OXA compartment: suspicion of OXA-48-type CPE.

Note:
The antimicrobial susceptibility test with a VITEK® 2 AST card or a rapid ATB™ E 4 strip must be performed using a subculture obtained on a conventional medium.

Carbapenemase production must be confirmed using an appropriate method.

QUALITY CONTROL

Protocol:

The nutrient capacity and the selectivity of the medium can be tested using the following strains.
Prepare a suspension calibrated to 0.5 McF, and then dilute in sterile saline solution in order to obtain an inoculum, after isolation on the agar plate:

- of 10^4 CFU for CARB and 10^6 CFU for OXA:
  Klebsiella pneumoniae ATCC® BAA-1705™
- of 10^6 CFU for CARB and OXA:
  Klebsiella pneumoniae ATCC 700603
- of 10^4 CFU for OXA:
  Escherichia coli ATCC BAA-2523

Range of expected results:

Strain	Results at 35 ±2°C	
	CARB	OXA
Klebsiella pneumoniae ATCC BAA-1705 (KPC)	Green colonies within 24 hours	No growth within 24 hours
Escherichia coli ATCC BAA-2523 (OXA-48)	Not applicable	Pink to burgundy colonies within 24 hours
Klebsiella pneumoniae ATCC 700603	No growth within 24 hours	No growth within 24 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 incubation time, etc.).

LIMITATIONS OF THE METHOD

- Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains which have specific requirements (substrate, temperature, incubation conditions, etc.) may not grow.
- Some multi-resistant microorganisms other than CPE may develop on the CARB medium producing colonies with a characteristic color: some vancomycin-resistant enterococcus strains (particularly *E. faecium* Van A: small blue-turquoise colonies) or *Enterobacteriaceae* resistant to carbapenems by impermeability.
- Some *Enterobacteriaceae* strains with weak carbapenemase-producing activity, may not develop on the media.

PERFORMANCE

Performance was evaluated at a national reference center for the detection of antimicrobial resistance using human clinical specimens (stools and rectal swabs) and characterized strains.

The agars (chromID® OXA-48 and chromID CARBA) were simultaneously inoculated with the specimens or calibrated bacterial suspensions. The readings were performed after 18-24 hours of incubation at 35 ± 2°C in aerobic conditions.

Sensitivity

Sensitivity was evaluated using 54 OXA-48 Carbapenemase-Producing *Enterobacteriaceae* or OXA-48-like variants (Ambler class D) and 20 non-OXA-48 Carbapenemase-Producing strains (Ambler class A and class B) inoculated on a plate with a 10^3 CFU inoculum.

Combined sensitivity of the 2 media = 95.9% **[88.6-99.2]**
or detection of 71 out of 74 CPE strains

Specificity for strains

Specificity was evaluated:
- using 20 carbapenem-sensitive *Enterobacteriaceae* strains that are ESBL producers or AmpC hyperproducers
- using 20 *Enterobacteriaceae* strains that are intermediate or resistant to carbapenems by impermeability.

These strains were inoculated using a strong inoculum (10^7 CFU per plate).

chromID CARBA SMART	chromID OXA-48	chromID CARBA	**Combined specificity**
20 carbapenem-sensitive strains	100% [83.2-100.0] (20/20)	100% [83.2-100.0] (20/20)	**100% [83.2-100.0]** (20/20)
20 strains intermediate or resistant to carbapenems by impermeability	100% [83.2-100.0] (20/20)	35% [18.1-56.7 (7/20)	**35% [18.1-56.7]** (7/20)

Specificity for specimens

149 CPE-negative clinical specimens (rectal swabs and stools) were inoculated on CARB and OXA agars. The following results were obtained after direct reading of the media. Identical results were obtained after Gram staining.

chromID CARBA SMART	chromID OXA-48	chromID CARBA	**Combined specificity**
149 clinical specimens	100% [97.6-100] (149/149)	96.6% [92.3-98.9] (144/149)*	**96.6% [92.3-98.9] (144/149)***

* 2 Gram-negative oxidase-positive bacillus strains and 3 *K. pneumoniae* strains, showing carbapenem resistance by impermeability, grew on the medium and were a characteristic blue color.

Results of previous evaluations performed on the chromID CARBA medium

Performance was evaluated at 2 sites (United Kingdom and Greece) according to the same protocol, using human clinical specimens (stools and rectal swabs) from patients at risk or chronic carriers being screened for carbapenemase-producing *Enterobacteriaceae*.

The specimens were directly inoculated on the agars. The readings were performed after 18-24 hours of incubation at 35 ± 2°C in aerobic conditions.

The 2 evaluations (United Kingdom and Greece) were performed using 806 specimens (88 stools and 718 rectal swabs). CARB Agar was compared to a Mac Conkey agar + Imipenem (1 mg/L).

All of the colonies were confirmed: Gram stain, identification to species level, carbapenemase production.

151 specimens were found to be positive by at least one of the methods used (culture medium with confirmation of colonies by PCR and modified Hodge test).

Sensitivity (95% Confidence Interval)

chromID CARBA	Mac Conkey + Imipenem
97.4% [93.4-99.3]	82.1% [75.1-87.9]

Specificity (95% Confidence Interval)

Without Gram stain		With Gram stain	
chromID CARBA	Mac Conkey + Imipenem	chromID CARBA	Mac Conkey + Imipenem
90.7% [88.2-92.8]	46.6% [42.7-50.5]	99.7% [98.9-100.0]	83.8% [80.8-86.6]

At one of the sites (Greece), the media were compared to the CDC method (13) using 177 specimens (rectal swabs) including 86 positive specimens.

Sensitivity (95% Confidence Interval)

chromID CARBA	Mac Conkey + Imipenem	CDC
96.5% [90.1-99.3]	89.5% [81.1-95.1]	98.8% [93.7-100.0]

Specificity (95% Confidence Interval)

Without Gram stain			With Gram stain		
chromID CARBA	Mac Conkey + Imipenem	CDC	chromID CARBA	Mac Conkey + Imipenem	CDC
91.2% [83.4-96.1]	31.9% [22.5-42.5]	80.2% [70.6-87.8]	100.0% [96.0-100.0]	70.3% [59.8-79.5]	80.2% [70.6-87.8]

WASTE DISPOSAL

Unused reagents may be considered as non-hazardous waste and disposed of accordingly. Dispose of used or unused reagents as well as any other contaminated disposable material 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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14. Guidance for control of Carbapenem-resistant *Enterobacteriaceae* (CRE) – 2012 CRE Toolkit.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue 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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