chromID™ CARBA agar (CARB)
Selective chromogenic medium for the screening of Carbapenemase-Producing Enterobacteriaceae (CPE).

SUMMARY AND EXPLANATION

chromID™ CARBA agar is a selective chromogenic medium for the screening of Carbapenemase-Producing Enterobacteriaceae (CPE), particularly KPC and NDM-1, in patients who are chronic carriers or in patients at risk (1, 2, 7, 8, 9, 10). This medium does not replace conventional susceptibility test methods.

CPE are particularly multi-resistant bacteria that are capable of causing nosocomial infections and hospital epidemics (3, 4). 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 agar contributes to the active surveillance of CPE.

PRINCIPLE

chromID™ CARBA agar (patent pending) consists of a rich nutritive base combining different peptones. It contains:
- a mixture of antibiotics which enable the selective growth of CPE;
- 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) (5).

CONTENT OF THE KIT

Ready-to-use medium:
- Pack of 20 plates (90 mm)

* printed on each plate

COMPOSITION

Theoretical formula:
This medium can be adjusted and/or supplemented according to the performance criteria required:
- 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
- Nutrient mixture …………………………………………….2.8 g
- Selective mixture …………………………………………….0.3 g
- Agar …………………………………………………………….18 g
- Purified water …………………………………………………….1 l

pH 7.4

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagent:
- Oxidase reagent (Ref. 55 635)

Material:
- Bacteriology incubator
- Non-impregnated disks Ø 6mm (Ref. 54 991)

POSSIBLE ADDITIONAL REAGENTS
- Etest® strips
- ATCC® LyfoCults® PLUS quality control strain

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® 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 tests results should be made taking into consideration colonial and microscopic morphology and, if necessary, the results of any other tests performed.

STORAGE CONDITIONS

- The plates can be stored 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 agar.

3. Incubate the inverted plates at 35 ± 2°C in aerobic conditions. The cultures are generally examined after 18-24 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 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** colonies: KESC group.

Identification of the micro-organism must be followed by additional tests.

**Note:** Due to the presence of tryptophan in the medium, beige colonies with a brown halo or a brown coloration of the bacterial growth may develop (Proteeae tribe). In this case, identification must be continued using a subculture on a conventional medium if the VITEK® 2 GN card is used.

In any case, carbapenemase production must be confirmed.

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 an agar plate:

- of 10⁴ CFU:
  - with *Klebsiella pneumoniae* ATCC® BAA-1705™
- of 10⁵ CFU:
  - with *Klebsiella pneumoniae* ATCC® 700603™

**Range of expected results:**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Results at 33-37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
</tr>
<tr>
<td>ATCC® BAA-1705™</td>
<td>Growth within 24 hours</td>
</tr>
<tr>
<td>ATCC® 700603™</td>
<td>No growth within 24 hours</td>
</tr>
</tbody>
</table>

**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

- Some multi-resistant micro-organisms other than CPE may develop on the medium producing colonies with a typical color: some vancomycin-resistant enterococcus strains (particularly *E. faecium* Van A: small blue-turquoise colonies) or *Enterobacteriaceae* resistant to carbapenem due to impermeability.
- Some *Pseudomonas* strains may show a brown pigmentation. The oxidase test enables them to be differentiated from *Proteeae*.
- The ability to detect carbapenemase-producing *Proteeae* has not been determined for this medium.
- Some *Enterobacteriaceae* strains with weak carbapenemase-producing activity, such as OXA-48, VIM or IMP, may not develop on the medium.
- Growth depends on the requirements of each individual micro-organism. It is therefore possible that certain strains which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.
PERFORMANCE

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 and 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 two evaluations (United Kingdom and Greece) were performed using 806 specimens (88 stools and 718 rectal swabs). chromID™ CARBA bioMérieux was compared to 1 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)

<table>
<thead>
<tr>
<th></th>
<th>chromID CARBA</th>
<th>Mac Conkey + Imipenem</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>97.4% [93.4-99.3]</td>
<td>82.1% [75.1-87.9]</td>
</tr>
</tbody>
</table>

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 (6) using 177 specimens (rectal swabs) including 86 positive specimens.

Sensitivity (95% Confidence Interval)

<table>
<thead>
<tr>
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<th>chromID CARBA</th>
<th>Mac Conkey + Imipenem</th>
<th>CDC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>96.5% [90.1-99.3]</td>
<td>89.5% [81.1-95.1]</td>
<td>98.8% [93.7-100.0]</td>
</tr>
</tbody>
</table>

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 all used 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


6. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing Klebsiella spp. and E. coli from rectal swabs - www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoli.pdf


8. BEREKSI N., GIRAUD D., JOYEUX F. and al.– Evaluation of a chromogenic medium, chromID CARBA, for the detection of carbapenemase-producing Enterobacteriaceae. – Poster 1718 – London 2012 – 22nd ECCMID.


INDEX OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>M</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>℃</td>
<td>Temperature limitation</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>Σ</td>
<td>Contains sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td>⚠</td>
<td>Protect from light</td>
</tr>
</tbody>
</table>

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