**MEDIUM PURPOSE**
Chromogenic medium for detection of Colistin resistant Gram-negative bacteria.

Polymyxin E (colistin) and B are increasingly used as antimicrobials in the treatment of multi-drug resistant bacterial infections. Polymyxin resistance, although intrinsic in Gram-positive and some Gram-negative species (*Proteus, Morganella, Serratia*), is now a problem in a number of other pathogens (*Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica, Klebsiella pneumoniae*). \(^1\)

Resistance arises due to mutations/insertions in genes involved in LPS biosynthesis (lpx, pmrA/B, mgrB, phoP/Q) and/or the acquisition of phosphoethanolamine transferases (PEtN). Of great concern is the recently described plasmid-encoded PEtN, MCR-1, now found worldwide in a range of animal, human and environmental bacterial isolates. \(^1\)

\(^1\) Novel Chromogenic Culture Media (CHROMagar™ COL-APSE) for the Isolation and Differentiation of Colistin Resistant Gram-negative Pathogens. ECCMID 2017.

**COMPOSITION**
The product is composed of a powder base (B) and a supplement (S).

<table>
<thead>
<tr>
<th>Product</th>
<th>Base (B)</th>
<th>Supplement (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>42.5 g/L</td>
<td>2 mL/L</td>
</tr>
<tr>
<td>Composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar 15.0</td>
<td></td>
<td>Growth factors mix</td>
</tr>
<tr>
<td>Peptones 20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt 5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromogenic and selective mix 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth factors 1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ASPECT**

<table>
<thead>
<tr>
<th>Powder Form</th>
<th>Liquid Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30 °C</td>
<td>15-30 °C</td>
</tr>
</tbody>
</table>

**FINAL MEDIA pH**

7.1 +/- 0.2

**PREPARATION (Calculation for 1 L)**

**Step 1**  Preparation of Base + S

- Disperse slowly 42.5 g of powder base in 1 L of purified water.
- Add 2 mL of CHROMagar™ COL-APSE supplement S into slurry.
- Stir until the agar is well thickened.
- Heat and bring to boiling (100 °C) while swirling or stirring regularly.

**Warning 1:** If using an autoclave, do so without pressure.

**Advice 1:** For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

- Cool in a water bath to 45-50 °C, swirling or stirring gently to homogenize.

**Advice 2:** In case of product samples containing a high load of *Proteus*, Cefixime can be added after the previous step at 0.05 mg/L.

**Step 2**  Pouring

- Pour into sterile Petri dishes.
- Let it solidify and dry.

**Storage**

- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.
- Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration.
**SPECIMEN COLLECTION AND HANDLING**

CHROMagar™ COL-APSE can be used with the following specimens: perineal, rectal specimens, stools and urine.

This medium can be also used in food industry with the following specimens: livestock and poultry.

Use of transport devices approved for collection of such specimens is recommended.

**MATERIAL REQUIRED BUT NOT PROVIDED**

Standard microbiological laboratory material for culture media preparation, control, streaking, incubation and waste disposal.

**INOCULATION**

Related samples can be processed by direct streaking on the plate.

- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Incubate in aerobic conditions at 37 °C for 18-24 hours.

**INTERPRETATION**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Typical colony appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL-R E. coli</td>
<td>→ dark pink to reddish</td>
</tr>
<tr>
<td>COL-R Coliforms</td>
<td>→ metallic blue</td>
</tr>
<tr>
<td>COL-R Pseudomonas</td>
<td>→ translucent, +/- natural pigmentation cream to green</td>
</tr>
<tr>
<td>COL-R Acinetobacter</td>
<td>→ cream, opaque</td>
</tr>
<tr>
<td>Other COL-R Gram (-)</td>
<td>→ colourless, natural pigmentation</td>
</tr>
<tr>
<td>COL-S Gram (-)</td>
<td>→ inhibited</td>
</tr>
<tr>
<td>Gram (+) bacteria, yeasts</td>
<td>→ inhibited</td>
</tr>
</tbody>
</table>

**Typical colony appearance**

- E. coli
- Coliforms
- Pseudomonas
- Acinetobacter

The pictures shown are not contractual.

**PERFORMANCE**

In the following studies, 84 pure strains and 98 human stool samples were tested during 24 h at 37 °C.

<table>
<thead>
<tr>
<th>CHROMagar™ COL-APSE</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100 % *</td>
</tr>
<tr>
<td>Specificity</td>
<td>81 % *</td>
</tr>
<tr>
<td>NPV</td>
<td>100 % *</td>
</tr>
<tr>
<td>PPV</td>
<td>83 % *</td>
</tr>
</tbody>
</table>

* Data obtained from studies 1 «CHROMagar™ COL-APSE: a selective bacteria culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogen» David W. Wareham et Al. Journal of Microbiology 2017

2 «Wide dissemination of colistin-resistant Escherichia coli with the mobile resistance gene mcr in healthy residents in Vietnam» Marc Saulmont et Al. Journal of Antimicrobial Chemotherapy 2018

**LIMITATIONS AND COMPLEMENTARY TESTS**

- Species final identification may require additional testing such as biochemical tests.
- Resistance to colistin for E. coli, Klebsiella, Enterobacter and Salmonella can be confirmed by microdilution method.
- Some Hafnia can grow in mauve like E. coli but they can be identified with an additional test such as Indole test.

**QUALITY CONTROL**

Please perform Quality Control according to the use of the medium and the local QC regulations and norms.

Good preparation of the medium can be tested, isolating the following ATCC strains:

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Typical colony appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCR-1 E. coli NCTC 13846</td>
<td>→ dark pink to reddish</td>
</tr>
<tr>
<td>COL-S E. coli ATCC® 25922 (WDCM 00013)</td>
<td>→ inhibited</td>
</tr>
<tr>
<td>S. marcescens ATCC® 13880</td>
<td>→ green-blue</td>
</tr>
<tr>
<td>E. faecalis ATCC® 29212 (WDCM 00087)</td>
<td>→ inhibited</td>
</tr>
</tbody>
</table>

**WARNINGS AND PRECAUTIONS**

- For Research Use Only (RUO). Not for use in diagnostic procedures.
- This laboratory product should be used only by trained personnel (healthcare professional, etc). Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with procedures and good laboratory practices.
- Use of the medium may be difficult for people who have problems recognising colours.
- For a good microbial detection, collection and transport of specimen should be well handled and adapted to the particular specimen according to good laboratory practices.
- Culture media should not be used as manufacturing material or components.
- Do not ingest or inhale the product.
- Do not use the product after the expiry date.
- Do not use the product if it show any evidence of contamination or any sign of deterioration.
- Do not use the product if the packaging is damaged.
- Any change or modification in the procedure may affect the results.
- Unappropriate storage may affect the shelf life of the product.
- Recap the bottles/vials tightly after each preparation and keep them in a low humidity environment, protected from moisture and light.
- Reading and interpretation should be performed using isolated colonies.

Some precipitates 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.
- Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local and national regulations.
- For hazard and precaution recommendations related to some chemical components in this medium, please refer to the pictogram(s) mentioned on the labels. The Safety Data Sheet (SDS) is available on www.chromagar.com
DISPOSAL OF WASTE
After use, all plates and any other contaminated materials must be sterilized or disposed of by appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121 °C for at least 20 minutes.

LITERATURE REFERENCES
Please contact DRG for further information.

IFU/LABEL INDEX
- Catalogue reference
- Consult instructions for use
- Quantity of powder sufficient for X liters of media
- Expiry date
- Required storage temperature
- Store away from humidity
- Protect from light
- Manufacturer