chromID™ Salmonella Agar (SM2)

Chromogenic medium for the selective isolation and differentiation of the genus Salmonella

SUMMARY AND EXPLANATION

chromID™ Salmonella agar is a selective isolation and differentiation medium for the detection of Salmonella in human specimens (stools, rectal specimens) and food products according to standards NF EN ISO 6579 (1) and NF V 08-052 (2).

The detection principle for chromID™ Salmonella agar is different to that of SM® ID agar (Ref. 43 291). In addition, it enables the detection of lactose (+) Salmonella, with higher specificity of coloration.

PRINCIPLE

chromID™ Salmonella agar consists of a nutritive base combining different peptones and 3 chromogenic substrates which enable:

- the growth of all Salmonella.
- the detection of activities of specific enzymes.
The differentiation of Salmonella, including lactose (+) Salmonella, is based on the following principle: spontaneous pale pink to mauve coloration of strains producing esterase.

Other bacterial strains produce colonies that are different colors.

The selective mixture inhibits most Gram (+) bacteria and yeasts.

CONTENT OF THE KIT

<table>
<thead>
<tr>
<th>Ready-to-use medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Réf 43 621 Pack of 2 x 10 plates (90 mm)</td>
</tr>
<tr>
<td>Réf 43 629 Pack of 10 x 10 plates (90 mm)</td>
</tr>
</tbody>
</table>

* Printed on each plate

POSSIBLE ADDITIONAL REAGENTS

- Selenite F broth (Ref. 42 099).
- Rappaport broth (Ref. 42 091).
- Rappaport-Vassiliadis broth (Ref. 42 043).
- Selenite Cystine broth (Ref. 42 052).
- Rappaport-Vassiliadis Soy broth (Ref. 42 110).
- Muller-Kauffmann broth with tetrathionate and novobiocin (Ref. 42 114).
- VIDAS® ICS (Ref. 30 435)

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use and microbiological control 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® Approved Guideline – Current Revision". For further 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 past the expiry date.
- Do not use reagents 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, macro 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 recognizing colors.
- The performance data were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- Store the plates in their box at 2°C-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.
- Protect from light.

COMPOSITION

Theoretical formula.

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

<table>
<thead>
<tr>
<th>Peptides (porcine or bovine)</th>
<th>6.25 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>0.16 g</td>
</tr>
<tr>
<td>Bile salts (bovine or ovine)</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>9.63 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>9 g</td>
</tr>
<tr>
<td>Selective mixture</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Agar</td>
<td>14 g</td>
</tr>
<tr>
<td>Purified water</td>
<td>1 l</td>
</tr>
</tbody>
</table>

pH 7.3

MATERIAL REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.
SPECIMENS
For use in medical bacteriology
The medium is inoculated directly using stools (liquid stools or a suspension of stools in sterile physiological saline), rectal specimens or an enrichment broth. Good laboratory practices for collection and transport should be respected.

For use in food bacteriology
Follow the recommendations in the current standards to perform specimen collection and preparation.

INSTRUCTIONS FOR USE
For use in medical bacteriology:
The detection of Salmonella using chromID™ Salmonella agar may be performed according to the usual fecal culture protocol:
1. Allow plates to come to room temperature.
2. Inoculate the chromID™ Salmonella agar directly from the specimens or after enrichment using Rappaport or Selenite F broth.
3. Incubate with the cover bottom side at 37°C in aerobic conditions. The user is responsible for choosing the appropriate temperature for the intended use, in accordance with current standards. The cultures are examined after 18-24 hours of incubation. In certain cases it may be necessary to prolong incubation.

For use in food bacteriology:
This medium is suited to the simplified application of the standardized protocol NF EN ISO 6579 (1) and NF-V 08-32 (2) for the detection of Salmonella in food products. According to the NF EN ISO 6579 standard (1), isolation on chromID™ Salmonella agar should be performed in parallel with XLD agar after pre-enrichment in buffered peptone water, followed by enrichment in Rappaport-Vassiliadis Soy broth and Muller-Kauffmann broth with tetrathionate and novobiocin.
1. Allow plates to come to room temperature.
2. Inoculate each of the 2 enrichment broths on 2 separate plates.
3. Incubate with the cover bottom side at 35 or 37°C in aerobic conditions.
   The cultures are generally examined after 18-24 hours of incubation.

chromID™ Salmonella agar is also compatible for use with the VIDAS Immuno-Concentration Salmonella assay (VIDAS® ICS).

READING AND INTERPRETATION
• After incubation, observe the bacterial growth.
• Record the presence of characteristic colonies of Salmonella: pale pink to mauve colonies
• Identification of the microorganism(s) isolated must be followed by biochemical and/or immunological tests (3).

QUALITY CONTROL
Protocol:
The nutrient capacity of the medium can be tested using the following strain:
- Salmonella Typhimurium ATCC®14028

Range of expected results:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Results at 33-37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Typhimurium ATCC®14028</td>
<td>Growth within 24 hours Pink to mauve colonies</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...).

LIMITATIONS OF THE METHOD
• White precipitates may be observed when certain microorganisms grow. These precipitates do not have the characteristic appearance of Salmonella colonies.
• Some Salmonella serovars (in particular Salmonella Dublin, Salmonella Abortusovis, Salmonella Gallinarum) may produce a weak coloration or be slow to produce a coloration.
• Certain Gram (-) bacilli other than Salmonella may product characteristic colonies. Complete identification must therefore be performed using additional tests.
• Certain stool specimens may contain free esterases which are likely to produce a pink to mauve coloration of the medium at the inoculation point.
• Depending on the sensitivity of strains to selective agents, certain Gram (+) bacteria or yeasts may grow on this medium.
• Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains of Salmonella, which have specific requirements, may not develop.
• Depending on the specimens analyzed, it is recommended to use chromID™ Salmonella agar in conjunction with additional media intended for fecal culture (e.g. Campylosel, Yersinia, Clostridium difficile agars, etc.).
• In food microbiology, it is recommended to use chromID™ Salmonella agar in conjunction with media recommended by the reference standard.
• chromID™ Salmonella agar has been evaluated on the main food matrices and on a large number of bacterial strains. Given the wide variety of food products, manufacturing processes and microbial flora, it may be necessary to check that chromID™ Salmonella agar is properly suited to the specific nature of the products tested.
PERFORMANCE

Validation using specimens

chromID™ Salmonella agar was compared with SM® ID agar and another chromogenic medium. 260 human specimens (stools and rectal specimens) were tested, including:
- 231 specimens which were naturally contaminated or not contaminated with salmonella.
- 29 negative stool specimens which were artificially contaminated with salmonella.

The stool specimens were inoculated directly on the media and after enrichment in Selenite F broth. Rectal specimens were inoculated only after enrichment in Selenite F broth. Performance was evaluated after 24 hours of incubation at 37°C. Nutrient capacity and sensitivity of Salmonella detection

Forty of the 260 specimens studied produced positive salmonella cultures on at least one of the 3 media (identification confirmed).

<table>
<thead>
<tr>
<th>chromID™ Salmonella detection</th>
<th>SM® ID</th>
<th>Other medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity of Salmonella detection</td>
<td>39/40 (98%)</td>
<td>36/40 (90%)</td>
</tr>
</tbody>
</table>

Specificity of coloration

Among the characteristic colonies observed on each of the media, the proportion of colonies confirmed as Salmonella is the following:

<table>
<thead>
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<th>SM® ID</th>
<th>Other medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity of coloration</td>
<td>39/42 (93%)</td>
<td>36/50 (72%)</td>
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</tbody>
</table>

Detection of lactose (+) Salmonella:

chromID™ Salmonella agar was compared to SM® ID agar.
Nine pure lactose (+) Salmonella cultures were achieved. Seven strains produced characteristic colonies on chromID™ Salmonella agar, whereas no colonies were characteristic on SM® ID agar.

WASTE DISPOSAL

Dispose of used or 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

1. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella. – NF EN ISO 6579, December 2002, ISSN 0335-3931.
5. DELORME S., SENTA-LOYS A., SAUVONNET V. et al. – SM ID 2, a new chromogenic medium for isolation, detection and presumptive identification of Salmonella: comparison with SM ID, Hektoen and four commercially available chromogenic media. – Poster 933, Glasgow 2003, 13th 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></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Use by</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td></td>
<td>Consult Instructions for Use</td>
</tr>
<tr>
<td></td>
<td>Contains sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td></td>
<td>Protect from light</td>
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</table>

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