DEFINITION
Medium used to identify of the respiratory mode of bacteria and for the deep isolation of anaerobes.

PRINCIPLE
The nutrient substances provided by the meat-liver base, and the glucose used as an energy source, favor the growth of most anaerobic bacteria.

PRESENTATION
- Ready-to-use
  7.5 ml x 30 tubes  code 355-4716
- Dehydrated
  500 g code 356-4564

STORAGE
- Ready-to-use: + 2°C to 8°C.
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA
Meat-liver base  30 g
Glucose  2 g
Agar  6 g
Distilled water  1,000 ml
Final pH (25°C) = 7.6 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes with autoclave-proof stoppers
- Sterile pipettes (code 355-0751) or inoculating loop
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 38 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to boiling point until completely dissolved. Dispense 7 – 8 ml per tube. Sterilize in autoclave at 121°C (± 1°C) for 20 minutes.

Reconstitution ratio : 38 g/l.
500 g of powder makes 13.1 liters of medium.

PROTOCOL
- Inoculation and incubation
At the moment of use, melt the medium in a boiling water-bath and regenerate for 20 minutes. Leave it to cool to 44°C - 47°C.

1. Detection of respiratory mode
Immerse the tip of a flame-sterilized and closed Pasteur pipette in the bacterial test culture. Drain the tip, then place the inoculum in the bottom of the tube, executing several twisting up-and-down movements.

2. Isolation of anaerobes
On completion of the preceding operation, and to use up all the inoculum, continue with a 2nd tube, then a 3rd, and so on until the 8th tube. Immediately immerse the inoculated tubes in cold water in a vertical position. Then place them in an incubator at 37°C and observe for 1 to 7 days.

READING AND INTERPRETATION
1. Identification of respiratory mode
After the period of incubation required for bacterial growth, four main types of respiratory mode can be recognized:
- strict aerobes, which develop only in the superficial zone;
- strict anaerobes, which develop only at depth;
- facultative aerobes-anaerobes, which develop along the top of the medium;
- micro-aerobes, which form a ring in the intermediate aerobiosis-anaerobiosis zone.
2. Isolation of anaerobic bacteria
After incubation, the colonies appear within the tubes of medium. Their shape and color depend on the species in question.
Only the tubes containing perfectly isolated colonies are retained. These are then subcultured in a solid or liquid medium for identification.
The tube of medium may be fractured by gases released during growth. In this case, an early reading must be taken of tubes containing the minimum number of colonies.

PRECAUTIONS
Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST
The growth performances of the media are verified with the following strains:

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>Results after 24 - 48h culture at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis ATCC 6633</td>
<td>Aerobic growth</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>Aerobic-anaerobic growth</td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 13124</td>
<td>Anaerobic growth</td>
</tr>
</tbody>
</table>

QUALITY CONTROL OF MANUFACTURER
Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS
Meat-Liver (6% semi-agar) / Bacteria / Detection / Deep isolation / Respiratory mode / Glucose / Medium.