Columbia/Agar

Base agar	**356-4674 / 356-4678**
Base agar + horse blood	**356-3804**
Base agar + sheep blood + N.A.C.	**356-3954**

DEFINITION
A rich medium highly suitable for the culture of fastidious bacteria, especially with the addition of blood. It can then be used for detecting the hemolytic response of certain bacteria.

STANDARDS
FOOD MICROBIOLOGY
NF EN ISO 10272-1 (April 2006): Food microbiology - Horizontal method for detection and enumeration of *Campylobacter spp*.
Part 1: Detection method.

PRINCIPLE
The nutrient substances provided by the special blend of peptones favors the growth of most bacteria. The addition of nalidixic acid (N.A.C.) renders the medium selective.

PRESENTATION
- **Base**
  Dehydrated
  500 g **code 356-4674**
  5 kg **code 356-4678**

- **Base + horse blood**
  90 mm x 20 plates **code 356-3804**

- **Base + sheep blood + N.A.C**
  90 mm x 20 plates **code 356-3954**

STORAGE
- Pre-poured: + 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

Special blend of peptones	23 g
Starch	1 g
Sodium chloride	5 g
Agar	10 g
Distilled water	1,000 ml

Final pH (25°C) = 7.3 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Test tubes (16 x 160 mm) with autoclave proof stopper
- Sterile Petri dishes (Ø= 90 mm)
- Sterile pipettes (1 ml, etc)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- Durham bell jar (for anaerobiotic culture)
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use
Dissolve 39 g of powder in 1 liter of distilled water. Bring to the boil until completely dissolved. Dispense 15 ml per tube or 100 ml per bottle and sterilize in autoclave at 121°C ± 1°C for 15 minutes.

Reconstitution ratio: 39 g/l
500 g of powder makes 12.8 liters of medium.

PROTOCOL
• Columbia agar
Manifestation of anaerobic character in the detection of *Clostridium thermophiles*: melt the medium in a boiling water-bath and pour into Petri dishes.
Streak inoculate the surface of 4 dishes using a culture in liquid medium. Place 2 dishes in aerobiotic culture at 55°C ± 1°C for 1 to 4 days.
Place the other 2 dishes in anaerobiotic culture at 55°C ± 1°C for 1 to 4 days.

• Columbia base agar + fresh blood
This preparation promotes the culture of Gram-positive cocci, notably streptococci, including *S. pneumoniae*.
This medium permits detection of hemolytic response (presence or absence of hemolysis).
Add 5 to 10% of sterile blood to the sterile base agar, melted then cooled to 44°C - 47°C.
Swirl carefully, avoiding the creation of air bubbles in the agar.
Pour into Petri dishes. The agar is rendered selective for Gram-positive bacteria by adding the inhibitory N.A.C mixture of antibiotics (15 mg/l Nalidixic acid + 10 g/l Colistin), nearly all Gram-negative bacteria and Bacilli thus being inhibited.

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Columbia/Agar

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24 h culture at 37°C with 5% of sterile blood
Streptococcus pyogenes ATCC 19615	Good growth β-hemolysis
Streptococcus groupe C CIP A7	Good growth β-hemolysis
Enterococcus faecalis var zymogenes ATCC 29212	Good growth no hemolysis
Streptococcus pneumoniae ATCC 6303	Good growth, Greenish α-hemolysis
Neisseria meningitidis (+CO_2) ATCC 13090	Good growth No hemolysis

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

Columbia / Fastidious bacteria / Blood / NAC / Haemolytic response / Medium.

BIBLIOGRAPHY

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