Columbia Agar Base EP/USP/ISO

Cat. 1104

For the isolation and cultivation of fastidious microorganisms and the determination of hemolytic reactions.

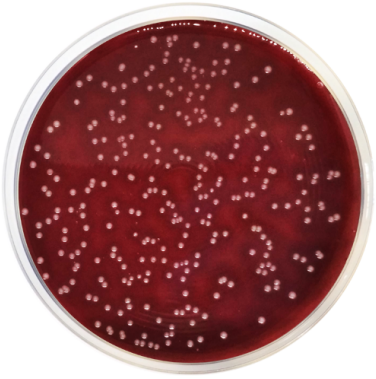
Practical information

Aplications	Categories
Growth	Campylobacter
Selective isolation	Fastidious microorganisms
Detection	Hemolytic reactions

Industry: Pharmaceutical/Veterinary / Clinical / Food / Quality Control

Regulations: USP / ISO 10272 / ISO 11133 / European Pharmacopoeia

CE IVD



Principles and uses

Columbia Agar Base is a highly nutritive general purpose medium for the cultivation of fastidious organisms, especially when used as a base for Blood Chocolate Agar. It can also be used as a selective isolation medium by adding antimicrobial agents. It is suitable for the isolation and cultivation of a wide variety of microorganisms with difficult growth characteristics. By adding blood, it can be used to determine hemolytic reactions. The majority of the common pathogenic bacteria, however, grow well without the addition of blood.

Pancreatic digest of casein, meat peptic digest and Heart pancreatic digest provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract and maize starch are a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The blood is another source providing growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary according to the type of blood or base medium used. For example, defibrinated sheep blood gives best results for Group A streptococci.

To make the medium more selective, the following supplements may be added if desired: CNA Staph/Streph Supplement (Cat. 6016) for staphylococci, streptococci and pneumococci isolation. Brucella Supplement (Cat. 6017) for Brucella isolation.

The European Pharmacopoeia, USP, recommends the Agar Columbia in the Paragraph 2.6.13 “Microbiological examination of non-Sterile products: test for specified microorganisms” for the testing of Clostridia in products.

ISO 10272 indicates that Columbia Agar Base with blood can be used to allow the development of well-isolated colonies, to use these colonies in confirmation tests.

With the addition of 5-10% sterile defibrinated blood and, especially with Polyenrichment Supplement (Cat. 6011), Polyenrichment CC Supplement (Cat. 6071) and VCN Supplement (Cat. 6013), Columbia Agar Base becomes an excellent chocolate agar that can be used to isolate pathogenic Neisseria, gonococci and meningococci, as good as or better than Thayer-Martin Medium. Depending on the selection of inhibitors, alternative supplements to VCN may be VCNT (Cat. 6026) or VCAT (Cat. 6014).

Formula in g/L

Bacteriological agar	13,5	Maize starch	1
Pancreatic digest of casein	10	Sodium chloride	5
Yeast extract	5	Meat peptic digest	5
Heart pancreatic digest	3		

Preparation

Suspend 42,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. The medium is generally enriched with sterile defibrinated blood (sheep, rabbit or horse), serum or some other material. Cool to 44-47 ºC and aseptically add 5-10 % sterile defibrinated blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood.

Instructions for use

» For clinical diagnosis:
- Used with 5-10% blood.
- Type of sample: Blood.
- Inoculate and incubate at 35±2 ºC for 18-72 hours.
- Reading and interpretation of results.

Types of Hemolysis:
1. Alpha-hemolysis: greenish discoloration of medium.
2. Beta-hemolysis: clear zone around the colony.
3. Gamma-hemolysis: no change.

» For other uses not covered by the CE marking:

Test of specified microorganisms (Clostridia) according to European Pharmacopoeia:
- Used without blood.
- Inoculate and incubate under anaerobic conditions the Reinforced Clostridial Medium (Cat. 1007) at a temperature of 30-35 ºC for 48 hours.
- Make subcultures on Columbia Agar (Cat. 1104).
- Add 20 mg / liter of gentamicin if necessary.
- Inoculate and incubate under anaerobic conditions at 30-35 ºC for 48 hours.
- Colonies grown in anaerobiosis that are negative catalases indicate presence of clostridia. This result must be confirmed by biochemical tests.

Detection and enumeration of Campylobacter spp. according to ISO 10272:
- Used with 5% blood.
- From characteristic colonies of Campylobacter Agar CCDA (Cat. 1129 + Cat. 6053), inoculate and incubate on Columbia Agar (Cat. 1104) at 41,5 ºC for 24-48 hours in microaerobic atmosphere.
- Perform confirmation tests.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Cherry red opaque	7,3±0,2

Microbiological test

According to European Pharmacopoeia; Clostridium sporogenes:
Incubation conditions: (30-35 ºC, anaerobic conditions / 48-72 h).
Inoculation conditions: (<=100 CFU).

According to ISO 10272; Campylobacter jejuni, Campylobacter coli:
Incubation conditions: Productivity qualitative (41,5±1 ºC, microaerobic atmosphere / 24-48 h).
Inoculation conditions: (10^3-10^4 CFU).
Reference media: Media bathch Blood Agar already validated.

Rest of strains; Neisseria meningitidis, Streptococcus pyogenes, Staphylococccus aureus, Streptoccocus pneumoniae:
Incubation conditions: (35±2 ºC, 5-10% CO2 atmosphere / 48 h).

Microorganisms	Specification	Characteristic reaction
Clostridium sporogenes ATCC 11437	Good growth	
Neisseria meningitidis ATCC 13090	Good growth	
Streptococcus pyogenes ATCC 19615	Good growth	Beta hemolysis
Staphylococcus aureus ATCC 25923	Good growth	Beta/Gamma hemolysis
Campylobacter jejuni ATCC 29428	Good growth	
Campylobacter jejuni ATCC 33291	Good growth	
Campylobacter coli ATCC 43478	Good growth	
Streptococcus pneumoniae ATCC 6305	Good growth	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Ellner, Stossel, Drakeford and Vasi. AM J. Clin. Path. 45:502-504. 1966.
European Pharmacopoeia.
ISO 10272-1: Microbiology of the food chain Horizontal method for detection and enumeration of Campylobacter spp. Part 1: Detection method
ISO 10272-2: Microbiology of the food chain Horizontal method for detection and enumeration of Campylobacter spp. Part 2: Colony-count technique