TECHNICAL DATA SHEET

COLUMBIA AGAR (BASE)

CULTURE & ISOLATION OF FASTIDIOUS MICROORGANISMS

1 INTENDED USE

Columbia Agar is a highly nutritive medium used for the growth and isolation of a large variety of microorganisms, particularly very fastidious bacteria: streptococci and pneumococci in animal samples. When blood, selective agents or growth accelerators are added, it becomes possible to prepare a wide variety of media adapted to specific uses.

The typical composition corresponds to that defined in European (EP), United States (UP) and Japanese (JP) Pharmacopeias.

2 HISTORY

Developed by Ellner in 1966, Columbia Agar enables luxuriant colonies, perfectly defined hemolytic zones and well characterized colonies and pigmentation to be obtained.

3 PRINCIPLES

Peptones included in the composition of the medium favor the excellent growth of colonies.

Yeast extract is a source of vitamin B complex.

Starch is a detoxifying agent and also an energy source.

Defibrinated sheep blood, which can be added to the medium, favors the detection of hemolytic reactions and supplies X factor (heme) required for the growth of a large number of bacteria, but lacks V factor (nicotinamide adenine dinucleotide) due to the presence of an NADase, which destroys any NAD present. *Haemophilus influenzae*, which requires both X and V factors, does not grow on agar containing ordinary blood.

The following media can be prepared with Columbia base :

- **Blood agar:** By adding 5 or 10% sterile sheep blood after autoclaving and cooling, the medium is suitable for the growth of *Streptococcus*, *Pneumococcus*, *Staphylococcus*, *Listeria* and *Erysipelothrix*. It can be made selective by adding colistin and nalidixic acid to preclude the development of Gram-negative bacteria and *Bacillus*.
- **Chocolate agar:** By adding 10% sheep or horse blood to sterile Colombia Agar and heating to 70°C for 5 minutes until a chocolate color develops, an excellent medium is obtained for growth of *Haemophilus, Neisseria, Taylorella or Campylobacter*.
- **Base media without enrichment:** Columbia Agar can be used to grow *Brucella abortus*, *Yersinia pestis* and *Clostridium perfringens*, as well as all enterobacteria.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of base media:

- Polypeptone ..... 23,0 g
- Starch ..... 1,0 g
- Sodium chloride ..... 5,0 g
- Bacteriological agar ..... 13,5 g

pH of ready-to-use media at 25 °C: 7,3 ± 0,2.

5 PREPARATION

- Suspend 42.5 g of dehydrated medium (BK019) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47°C.
- Aseptically add 5 to 7 mL of sterile, defibrinated sheep blood per vial.
- Mix well.
- Pour into sterile Petri dishes and let solidify on a cool surface.
- Dry the plates with the covers partially removed.

✓ **Reconstitution :**
42,5 g/L

✓ **Sterilization :**
15 min at 121°C

NOTE:
For other applications, use the corresponding protocol.

6 INSTRUCTIONS FOR USE

- Inoculate in order to obtain isolated colonies.
- Incubate at 37 °C for 24 to 48 hours in optimal conditions for the culture of the inoculated germs.

7 RESULTS

Observe the bacterial growth.

Beta hemolysis

Streptococci belonging to Lancefield group A appear as small, grey colonies, translucid or opaque, surrounded by a zone of beta hemolysis. Other bacteria may present the same type of hemolysis: *Listeria*, hemolytic *Staphylococci*, *Escherichia coli* and *Pseudomonas*.
Staphylococci appear as opaque, yellow-gold or white colonies, with or without type β hemolysis zones.
Listeria present small zones of beta hemolysis.
Bacillus cereus form a clear zone surrounding the colonies.

See ANNEX 1 : PHOTO SUPPORT.

Alpha hemolysis

Pneumococci appear as flat, shiny, grey and occasionally mucoid colonies surrounded by a zone of narrow, greenish hemolysis referred to as alpha hemolysis.

CAMP Factor

Group B *Streptococci* produce an extracellular, thermoresistant substance (CAMP Factor) which provokes a triangle of total hemolysis in a zone of incomplete staphylococcal hemolysis, at the junction of the two cultures.

8 QUALITY CONTROL

Dehydrated media: beige powder, free-flowing and homogeneous.
Prepared media: (with 5% defibrinated sheep blood) : opaque, red agar.

Typical culture response after 48 hours of incubation at 37 °C, with 5% sheep blood (qualitative method of inoculation):

Microorganisms		Growth	Type of hemolysis
Streptococcus pyogenes	ATCC® 19615	Good, score 2	beta
Streptococcus pneumoniae	ATCC 6303	Good, score 2	alpha
Listeria monocytogenes	ATCC 19115	Good, score 2	beta
Staphylococcus aureus	WDCM 00034	Good, score 2	-
Escherichia coli	WDCM 00013	Good, score 2	-

9 STORAGE / SHELF LIFE

Dehydrated base medium: 2-30 °C.
The expiration date is indicated on the label.

Prepared based media in vials (*): 180 days at 2-8 °C.
Prepared base media with sheep blood (*): 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated medium:
500 g bottle..... BK019HA

11 BIBLIOGRAPHY

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The Japanese Pharmacopoeia. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count and II. Microbiological examination of non-sterile products: Test for specified products.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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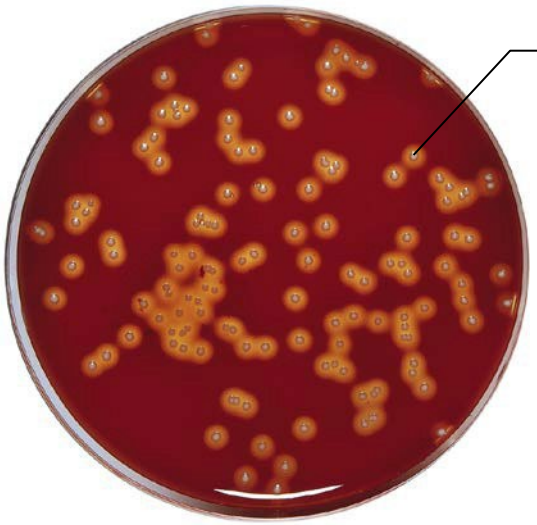
Columbia Agar (base)

A highly nutritive media allowing the culture and isolation of a large variety of microorganisms.

Results :

Agar with 10% sterile sheep blood added.
Incubation 48 hours at 37 °C.



Group D *Streptococci*

Characteristic colonies surrounded by a zone of clear hemolysis (β-hemolysis)