**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.

## 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 media :** 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 media :**
500 g bottle ..... BK019HA

## 11 BIBLIOGRAPHY


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NF EN ISO 10272-2. Juillet 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Campylobacter spp. - Partie 2 : technique par comptage des colonies.

Pharmacopée Européenne. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.


## 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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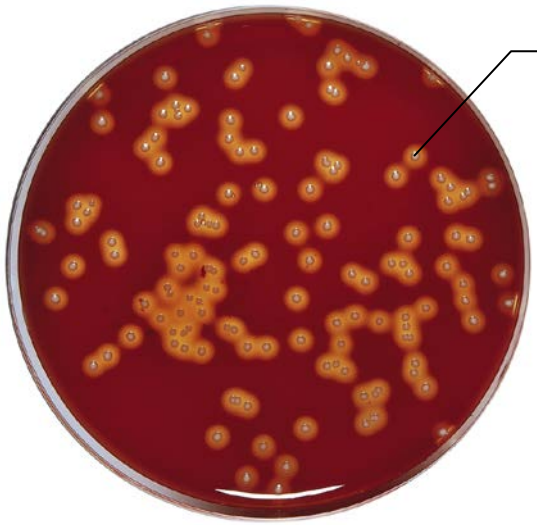
## Columbia Agar (base)

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)