Product :
REINFORCED CLOSTRIDIAL AGAR

Also known as
RCA

Specification
Solid medium for the cultivation and enumeration of clostridia and other anaerobic bacteria.

Formula * in g/L
Casein peptone..... 10,00
Yeast extract..... 3,00
Meat extract..... 10,00
Dextrose..... 5,00
Sodium chloride..... 5,00
Sodium acetate..... 3,00
Soluble starch..... 1,00
Cysteine..... 0,50
Agar..... 15,00

Final pH 6,8 ±0,2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 52,5 g of powder in 1 L of distilled water and bring to the boil constanty stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description
Reinforced Clostridial Agar was originally described by Hirsch and Grinstead to enhance the growth of small inoculums and achieve a higher clostridial count. Later, Barnes and Ingram used the medium to develop vegetative cells in assays of *Clostridium perfringens*. Barnes also used this medium to count clostridia in food; moreover other authors used this medium in enumeration assays of *C. thermoscharolyticum* in sugar, the study of intestinal flora, and for bacterial counts in human or animal faeces, etc. Tartera *et al.* (1999) modified it by the addition of antibiotics for the isolation and counting of phages infecting Bacteroides. Later this medium was adopted in the 10705-4:2001 ISO Standard.
For enumeration by the MPN method, the liquid version is preferred.

Technique
Material to be examined is ground in a grinder or Stomacher®, and a dilution bank is prepared. From each of the dilutions, take an aliquot and add to plates or tubes, pour the molten medium at 50°C over the sample. Let it solidify. Incubate for a time and temperature suitable to the Microorganism. An anaerobic environment can be achieved in tubes by covering with oil immediately after the Reinforced Clostridial Medium is solidified. If plates are used, they must be incubated in an anaerobic atmosphere.
Muñoa and Parés added a filter sterilized solution of Nalidixic acid 0,02 g/L, Polymyxin 0,025 g/L, Kanamycin sulfate 0,05 g/L, Sodium iodoacetate 0,025 g/L and triphenyl-tetrazolium HCl 0,025 g/L to obtain a selective and differential medium for bifidobacteria in water and wastewater.

Quality control
Incubation temperature: 30 - 35 ºC **Incubation time:** 44 ± 4h
Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) according to ISO 11133:2014/Amd 1:2018. Anaerobic conditions. Double layer.

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 27853	Inhibited	-
Clostridium perfringens ATCC® 13124	Good	Gas(+)
Clostridium perfringens ATCC® 10543	Good	Gas(+)
Clostridium sporogenes ATCC® 19404	Good	Gas (D)

References

· ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. Boca Raton Fla. USA.
· HIRSCH, A. & E. GRINSTEAD (1954) Methods for the Growth and Enumeration of Anaerobic Sporeformers from Cheese, with Observations on the Effect of Nisin.
· INGRAM, M. & E.M BARNES (1956) A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Practice 5, 4:145.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MUÑOA, F.J., R. PARÉS (1988) Selective medium for isolation and enumeration of Bifidobacterium spp. Appl. Environm. Microgiol 54:1715-1718.
· TARTERA, C., R. ARAUJO, T. MICHEL & J. JOFRE (1992) Culture and decontamination methods affecting enumeration of phages infecting Bacteroides fragilis in sewage. Appl. Environm. Microbiol. 58:8:2670-2673.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).