Formula * in g/L

Tryptone..... 15,00
Soya peptone..... 5,00
Yeast extract..... 5,00
Disodium disulfite ($Na_2S_2O_5$).....1,00
Iron ammonium citrate..... 1,00
Agar.....15,00

Final pH 7,6 ±0,2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 42 g of powder in 1 L of distilled water. Bring to the boil and distribute into suitable containers.
Sterilize in the autoclave at 121ºC for 15 minutes. If the medium is not used on the same day of preparation, the medium must be reduced before use.

Description

This modification of the Iron Sulfite Agar is formulated according to ISO 15213:2003 Standard that specifies a horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
The method can be used with foods and animal feeding stuffs and environmental samples in the food production and handling area. In the *Nordisk Metodikkommitté för Livsmedel* Standard (NMKL No. 95:1997 Sulfite-reducing Clostridia: Determination in food) this medium is used in the clostridia presumptive test, before the confirmatory (respiratory tests, spore-forming test) step. In the ISO Standard also it is also stated that this method is applicable only for clostridia and after the isolation on this medium a confirmatory study of black colonies must be performed.

Technique

Transfer aliquots from the dilution bank of the sample into sterile Petri dishes in duplicate. Into each inoculated Petri dish, add 15 mL of melted, reduced medium cooled to 44-47ºC. Carefully mix the inoculum with the medium and allow it to solidify. After the medium has solidified, overlay with another 10 mL of the same medium. The time elapsing between inoculation of Petri dishes and the addition of the melted medium should not exceed 15 minutes.
The inoculated Petri dishes are incubated in anaerobic conditions at 37 ± 1ºC for 24-48 hours. If thermophilic bacteria are suspected a second set of petri dishes must be incubated at 50 ± 1ºC for 24-48 hours.
The black colonies, surrounded or not by a black zone are considered as sulfite-reducing bacteria, presumptive clostridia. Their identity must be confirmed with suitable biochemical and serological tests.

Quality control

Incubation temperature: 37ºC ±1,0 **Incubation time:** 24-48±2 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^3-10^4 CFU (Specificity) according to ISO 11133:2014/Amd 1:2018 . Anaerobic conditions.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Fair to good	w/o blackening
Clostridium sporogenes ATCC® 11437	Fair to poor	Black colony
Clostridium perfringens ATCC® 10543	Productivity > 0.50	Black colony
Clostridium perfringens ATCC® 13124	Productivity > 0.50	Black colony

References

· ISO 15213:2003 Standard. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

Storage

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