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Kanamycin Esculin Azide Broth (KAA Broth)

Art. No. 02-263



WARNING

H: 3.1.0/4; H302-4.1/3; H412
P: P273; P264+P270; P301+P312; P330+P361+P501a

Specification

Liquid medium for the presumptive detection of Lancefield's group D streptococci in food samples, according to Mossel *et al.*

Formula* in g/L

Tryptone.....	20,00
Yeast extract.....	5,00
Sodium chloride.....	5,00
Disodium citrate.....	1,00
Esculin.....	1,00
Ferric-ammonium citrate.....	0,50
Sodium azide.....	0,15
Kanamycin sulfate.....	0,02
Final pH 7,0 ± 0,2 at 25°C	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 33 g of powder in 1 L of distilled water. Distribute in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

KAA Presumptive Broth is a medium that several International Organizations recommend to detect, enumerate and isolate Lancefield's group D streptococci in samples of food and beverages like: bottled water, fresh, refrigerated, or frozen minced meat, fish, molluscs, soft drinks, pastries, spices and other products. Kanamycin and sodium azide are the selective inhibitory compounds.

Technique

Prepare tubes with 9 mL of broth, and Petri plates with the agar. Make a 10-fold serial dilution bank from the sample in duplicate, and inoculate 1 mL amounts in the tubes. Incubate at 37°C for 24 hours.

The presumptive presence of streptococci is indicated by the development of a blackish-brown colour and the loss of fluorescence using a Wood's lamp. These tubes are considered as positive, and then, from these, inoculate 0,1 mL aliquots over the surface of the KAA plates spreading with a Drigalsky loop. Incubate the plates, in an inverted position, at 37°C for 24 hours. Colonies surrounded by a black halo are considered as group D streptococci, and are isolated to confirm their identity biochemically and morphologically with the following tests: microscopic examination, catalase assay (that should be negative) in an azide free medium, growth at 45°C and resistance to a high saline concentration (6,5% of NaCl in BHI Broth (Art. No. 02-599). Finally, they have to grow in Bile Esculin Agar (Art. No. 01-265) with a similar appearance to that of the colonies on the KAA. Nonetheless, there are some exceptions to this rule, i.e. *Streptococcus equinus* and *S. bovis* do not grow in the hypersaline broth, and therefore,

definitive identification has to be performed by serological methods.

This methodology does not allow the enumeration of bacteria from the original sample, and as this is a necessary, the Most Probable Number (MPN) technique using the presumptive broth, is recommended, using double strength broth if necessary.

For bottled water, soft drinks and molluscs, CeNAN (*Centro Nacional de Alimentación y Nutrición*, in Spain) suggest the following technique:

Prepare broth tubes at normal concentration and at double strength. Using a sterile pipette, inoculate five broth tubes of double strength with 10 mL of sample. Inoculate five tubes of normal concentration with 1 mL of sample and five tubes of normal concentration with 0,1 mL of sample. Homogenize them well and incubate at 37°C for 48 hours. Tubes that show a blackish-brown colour after the incubation period, are considered positive. Carry out the counting using the MPN tables.

References

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- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- MOSSEL, D.A.A., P.G.M. BUKER, J. ELDERING (1978) Streptokokken der Lancefield Gruppe D in Lebensmitteln und Trinkwasser. Arch F. Lebensmittelhyg. 29:121-127.
- PASCUAL ANDERSON, M^aR^a (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
- VANDERZANT & SPLITTSTOESSER (1992) Compendium of Methods for the Microbiological Examination of Food. 3rd ed. APHA. Washington.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Quality control

Incubation temperature: 35°C ± 2.0

Incubation time: 24 - 48 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity) (ISO/TS 11133-1/2)



WARNING

H: 3.1/O/4; H302-4.1/C/3; H412
P: P273-P264-P270-P301+P312-P330-P501a

Microorganism	Growth	Remarks
<i>Escherichia coli</i> ATCC 25922	Inhibited	-
<i>Staphylococcus aureus</i> ATCC 25923	Inhibited	-
<i>Enterococcus faecalis</i> ATCC 29212	Good	Black medium
<i>Enterococcus faecalis</i> ATCC 19433	Good	Black medium



Left: Uninoculated Tube
Right: *Enterococcus faecalis* ATCC 29212