Product :
KANAMYCIN ESCULIN AZIDE AGAR (KAA AGAR)

Specification
Solid medium for confirmative detection and isolation 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
Agar	15,00

Final pH 7,0 ±0,2 at 25 ºC

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

Directions
Suspend 48 g of powder in 1 L of distilled water and let it soak. Heat to boiling point and distribute into suitable containers. Sterilize in the autoclave at 121°C for 15 minutes.

Description
KAA confirmative Agar is a medium that several organisations and institutes recommend for detecting, enumerate and isolate Lancefield's group D streptococci in samples of food and beverages e.g.: bottled water, fresh/refrigerated/frozen/minced meat, fish, molluscs, soft drinks, pastries and spices. Kanamycin and sodium azide are the selective inhibitory compounds.

Technique
From samples considered positive, aliquots of 0,1 mL are inoculated onto the surface of the plates of KAA, spreading with a Drigalsky loop. Incubate the plates, in an inverted position, at 36°C for 24-48 hours. Colonies that appear surrounded by a black halo are considered as group D streptoccoci, and are isolated to confirm them biochemically and morphologically with the following tests: microscopical examination; catalase assay (that should be negative) in an azide-less medium; growth at 45°C and resistance to a high saline concentration (6,5% of NaCl in BHI Broth).
Finally, they have to grow in Bile Esculin Agar with an appearance similar to the colonies on the KAA Confirmative Agar. 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 is recommended with KAA Presumptive Broth, using double strength broth if necessary.

Quality control
Incubation temperature: 36°C ±2.0 **Incubation time:** 44 ± 4h

Inoculum: 10^{3}-10^{4} CFU (Productivity test qualitative)/ 10^{4}-10^{6} CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Inhibited	-
Staphylococcus aureus ATCC® 25923	Inhibited	-
Enterococcus faecalis ATCC® 29212	Good	Brown to black colonies (Esculin +)
Streptococcus bovis ATCC® 33317	Good	Brown to black colonies (Esculin +)
Enterococcus faecalis ATCC® 51229	Good	Brown to black colonies (Esculin +)

References

· CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD. (2003) Handbook of Culture Media for Food Microbiology. Elsevier Sci. B.V. Amsterdam. The Netherlands.
· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
· GUINEA, J., SANCHO, J. & PARES, R. (1979) Análisis Microbiológico de Aguas. Ed. Omega. Barcelona.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and 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. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

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