Reference: 451830ZA **Technical Data Sheet**

Product: # Kanamycin Esculin Azide Agar

Specification

Solid medium for confirmative detection and isolation of Lancefield's group D streptococci in food samples, according to Mossel et al.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottle 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	8-25 ºC

Composition

Composition (g/l):

Tryptone	20.00
Yeast extract	5.00
Sodium chloride	5.00
Sodium citrate	1.00
Esculin	1.00
Ammonium ferric citrate	0.50
Sodium azide	0.15
Kanamicin	0.02
Agar	15.00

Description /Technique

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:
Melt the medium contained in the bottles in a water bath (100ºC) or in a microwave oven, avoiding overhating before pouring into Petri dishes when cooled to room temperature.
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 37°C for 24 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.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

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Quality control

Physical/Chemical control

Color : Olive brown pH: 7.0 ± 0.2 at 25ºC

Microbiological control

Melt the medium and inoculate $10^3$10^4 CFU (Productivity test qualitative)/ 10^4-10^6 CFU (Selectivity)

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 36 ± 2 ºC, reading at 44±4 h

Microorganism	Growth
Enterococcus faecalis ATCC® 29212, WDCM 00087	Good. Brown to black colonies. Positive esculin.
Enterococcus faecalis ATCC® 19433, WDCM 00009	Good. Brown to black colonies. Positive esculin.
Escherichia coli ATCC® 25922, WDCM 00013	Inhibited
Stph. aureus ATCC® 25923, WDCM 00034	Inhibited - poor

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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