Kanamycin Esculin Azide Agar (KAA Agar)
Art. No. 01-263

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 25ºC
* 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 121ºC 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 37ºC for 24 hours. Colonies that appear surrounded by a black halo are considered as group D streptococci, 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 45ºC 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 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 (Art. No. 02-263), using double strength broth if necessary.

References

Storage
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC 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). Spiral Plate Method (ISO/TS 11133-1/2)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Growth</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>Inhibited</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>Inhibited</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>Productivity &gt; 0.70</td>
<td>Brown to black colonies (Esulin +)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 19433</td>
<td>Productivity &gt; 0.70</td>
<td>Brown to black colonies (Esulin +)</td>
</tr>
</tbody>
</table>

Enterococcus faecalis ATCC 29212
Uninoculated plate (Control)
Enterococcus faecalis ATCC 19433