Modified Giolitti and Cantoni Broth (ISO)

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(NCM0184)

Intended Use

Modified Giolitti and Cantoni Broth (ISO) is used for the enumeration and detection of coagulase-positive staphylococci from food and animal feeding stuffs using the Most Probable Number (MPN) technique as described in ISO 6888-3:2003. Modified Giolitti and Cantoni Broth (ISO) is not intended for use in the diagnosis of disease or other conditions in humans.

Description

An enumeration and detection medium of coagulase-positive Staphylococci from food and animal feeding stuffs using the Most Probable Number (MPN) technique, as described in ISO 6888-3:2003. Originally described by Giolitti and Cantoni as a medium for the enrichment of staphylococci from foodstuffs, Mossel later applied the medium to use with samples from dried milk and infant food. Optimized for use in samples where staphylococci may be stressed and/or in low numbers, growth of the target organisms is promoted by sodium pyruvate, Glycine and the high concentration of mannitol. Selectivity is achieved via lithium chloride, which inhibits Gram-negative bacilli, and potassium tellurite, which inhibits Gram-positive organisms other than staphylococci. Further selectivity is achieved by use of anaerobiosis either by pouring a plug of agar/paraffin or by incubation in a jar or incubator under anaerobic conditions. Anaerobiosis particularly inhibits the growth of *Micrococcus* spp. The presence of coagulase-positive staphylococci is indicated by the reduction of tellurite, resulting in a blackening of the broth or a black precipitate. Coagulase-positive staphylococci are principally *Staphylococcus aureus* but may also include the species *Staphylococcus intermedius* and *Staphylococcus hyicus*. According to ISO 6888-3:2003, subculture is performed separately on Baird Parker Agar or Rabbit Plasma Fibrinogen Agar (RPFA). This medium conforms to the performance and formulation requirements of ISO 6888-3:2003.

Typical Formulation

Enzymatic digest of casein	10.0 g/L
Meat extract	5.0 g/L
Yeast extract	5.0 g/L
Lithium chloride	5.0 g/L
Mannitol	20.0 g/L
Sodium chloride	5.0 g/L
Glycine	1.2 g/L
Sodium pyruvate	3.0 g/L

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

NCM4012	Potassium Tellurite 1%
NCM4081	Tween 80

Precaution

Refer to SDS

Preparation

Single Strength:
1. Dissolve 54.2 grams of the medium in one liter of purified water.
2. Swirl to mix and add 1 gram of Polyoxyethylene sorbitan mono-oleate (Tween 80), NCM4081.
3. Heat with frequent agitation to completely dissolve.
4. Dispense the medium in appropriate tubes and autoclave at 121°C for 15 minutes then allow to cool to 44-47°C.
5. Prior to use add NCM4012 Potassium Tellurite 1% to give a final concentration of 0.1 g/L, e.g. add 0.1mL NCM4012 to 9mL of single strength base. DO NOT REHEAT MEDIA CONTAINING POTASSIUM TELLURITE.

Double Strength:
6. Dissolve 108.4 grams of the medium in one liter of purified water.
7. Swirl to mix and add 2 grams of Polyoxyethylene sorbitan mono-oleate (Tween 80) NCM4081.
8. Heat with frequent agitation to completely dissolve.
9. Dispense the medium in appropriate tubes and autoclave at 121°C for 15 minutes then allow to cool to 44-47°
10. Prior to use add NCM4012, Potassium Tellurite 1% to give a final concentration of 0.1g/L, e.g. add 0.2mL NCM4012 to 9mL of double strength base. DO NOT REHEAT MEDIA CONTAINING POTASSIUM TELLURITE.

If product is to be used on day of preparation, allow to cool to 44-47ºC and use immediately after supplementation with potassium tellurite. If the medium is not used as above then the base medium must be re-heated to 100° C for 15 minutes to expel any dissolved oxygen and cooled to 44-47ºC. DO NOT REHEAT MEDIA CONTAINING POTASSIUM TELLURITE.

Test Procedure

For the enumeration and detection of coagulase-positive Staphylococci- Refer to ISO 6888-3:2003

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is clear, pale yellow (will be darker if prepared at double-strength)

Expected Cultural Response: Cultural response at 37°C ± 1°C after 24 ± 2 hours to 48 ± 2 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results		
		Reaction in Modified Giolitti and Cantoni	Recovery on Baird Parker or RPFA	Recovery on TSA
Staphylococcus aureus WDCM 00034 + *Escherichia coli* WDCM 00013	10-100 + $>10^4$	Blackening or black precipitate	>10 CFU* + Total inhibition	N/A
Staphylococcus aureus WDCM 00032 + *Escherichia coli* WDCM 00013	10-100 + $>10^4$	Blackening or black precipitate	>10 CFU* + Total inhibition	N/A
Escherichia coli WDCM 00012	$>10^4$	No reaction	N/A	Total inhibition
Escherichia coli WDCM 00013	$>10^4$	No reaction	N/A	Total inhibition

* With characteristic colonies according to each medium.
The organisms listed are the minimum that should be used for Quality Control testing.

Results

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Blackening in the broth or the presence of a black precipitate may or may not be detected in the tubes due to reduction of tellurite.

Baird Parker Agar with Egg Yolk Tellurite

Presumptive Coagulase-positive Staphylococci produce black, shiny, convex colonies demonstrating lecithinase activity (an opaque zone around the colony) and lipase activity (a zone of clearing encircling the opaque zone).

Rabbit plasma fibrinogen (RPFA)

Presumptive Coagulase-positive Staphylococci appears as black colonies surrounded by a zone of precipitation (demonstrating coagulase activity).

Expiration

The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Blackening or a black precipitate may or may not be detected, subculture whether blackening has developed or not.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 6888-3:2003 Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)- Part 3: Detection & MPN technique for low numbers.
2. ISO 11133:2014+A1:2018 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.