PRODUCT SPECIFICATION SHEET

Chapman Stone Agar (DM058)

Intended Use
Chapman Stone Agar is recommended for selective isolation of food poisoning *Staphylococci*

Product Summary and Explanation
Chapman Stone Medium is prepared according to the formula described by Chapman.[1] It is similar to Staphylococcus Medium 110, previously described by Chapman,[2] except that the sodium chloride concentration is reduced to 5.5% and ammonium sulfate is included in the formulation. The inclusion of ammonium sulfate in the medium negates the need to add a reagent after growth has been obtained in order to detect gelatinase activity by Stone's method. Chapman Stone Medium is especially recommended for suspected food poisoning studies involving *Staphylococcus*.[4] It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation and the presence or absence of gelatin liquefaction. Chapman Stone Agar is a selective media used for the isolation of food poisoning staphylococci. Foods commonly contaminated with *S. aureus* included synthetic creams, custards and high-salted food. *Staphylococcus aureus* is one of the pathogens most frequently isolated from clinical specimens. In fact, *S. aureus* is currently the most common cause of nosocomial infections [3] Treatment of infection caused by *S. aureus* has become more problematic since the development of multiple drug resistant strains. To identify *S. aureus* from contaminated samples more easily and reliably, selective media have been developed. It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation and the presence or absence of gelatin liquefaction.

Principles of the Procedure
Casein enzymic hydrolysate, yeast extract provide nitrogen, carbon, sulphur, vitamin B and trace elements. Sodium chloride acts as a selective agent, which inhibits most of the bacterial species. Mannitol is the fermentable carbohydrate and its fermentation can be detected by adding a few drops of bromocresol purple resulting in production of yellow colour. Gelatin hydrolysis is observed as clear zones around colonies. Due to the presence of ammonium sulphate in the medium itself there is no need to flood the plate with ammonium sulphate solution for detection of gelatin liquefaction by the isolates, which is known as Stones method.[5] Dipotassium phosphate provides buffering capability. Material under test is inoculated on the surface and incubated at 30°C for 48 hours to produce separated colonies. After incubation, cream to golden yellow colonies surrounded by clear zones are presumptively identified as *S. aureus*. White or non-pigmented colonies, with or without a clear zone, are presumptively identified as *S. epidermidis*. Coagulase activity should be performed to confirm the findings. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by gram stain and the catalase test.[6]

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Yeast extract	2.50
Gelatin	30.00
D-Mannitol	10.00
Sodium chloride	55.00
Ammonium sulphate	75.00
Dipotassium phosphate	5.00
Agar	15.00
Final pH (at 25°C) 7.0 ± 0.2	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 20.25 grams of the medium in 100 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.
4. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow coarse free flowing powder
Prepared Medium	Light amber coloured, opalescent gel forms in Petri plates.
Reaction of 20.25% solution	pH 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel and 3.0% gelatin gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 25-30°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Gelatinase production
1.	*Escherichia coli* ATCC 25922	>=10^3	Inhibited	0%	----	----
2.	*Staphylococcus aureus* ATCC 25923	50-100	Luxuriant	>=50%	Positive reaction, production of yellow colour on addition of Bromo cresol purple	Positive reaction, clearing or halo
3.	*Staphylococcus epidermidis* ATCC 12228	50-100	Luxuriant	>=50%	Negative reaction, no production of yellow colour on addition of Bromo cresol purple	Positive reaction, clearing or halo

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Streak a sample of the specimen onto the surface of the agar. Make several stabs into the medium along the streak.
2. Incubate, aerobically, at 32°C for up to 48 hours.
3. Examine for growth and the presence or absence of clear zones around colonies.
4. To determine mannitol fermentation, add a few drops of bromcresol purple to areas on the medium from which colonies have been removed. Any change in color of the indicator, compared with that of the uninoculated medium, indicates fermentation of mannitol.

Results

Mannitol fermentation: Positive -change in color of the indicator to yellow. Gelatinase activity: Positive Stone reaction = formation of clear zones around the colonies. Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as *Staphylococcus aureus*. White or nonpigmented colonies, with or without a clear zone, are probably *S. epidermidis*.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if 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 Procedure

1. Confirm the presumptive identification of pathogenic staphylococci with additional tests, such as coagulase activity.
2. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by Gram stain and the catalase test.

Packaging

Product Name :Chapman Stone Agar
Product Code : DM058
Available Pack sizes : 100gm / 500gm

References

1. Chapman. 1948. Food Res. *13*:100.
2. Chapman. 1946. J. Bacteriol. *51*:409.
3. Chapman G. H., 1949, J. Bacteriol., 58:823
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, Md.
5. Stone, 1935, Proc. Soc. Exp. Biol. N.Y., 33:185.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore

Further Information

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