Chapman Stone Agar

Cat. 1017

Selective and differential medium for the isolation of pathogenic staphylococci in foods

Practical information

Aplications	Categories
Differentiation	Staphylococcus

Industry: Food

Principles and uses

Champan Stone Agar is used for the isolation of pathogenic Staphylococci in foods. It is similar to Staphylococcus N° 110 Agar (Cat. 1032), but contains ammonium sulfate to detect the gelatinase activity (Stone's reaction), and sodium chloride concentration is reduced to 5,5%.

The main modification of this medium is the inclusión of ammonium sulfate that allows the direct observation of gelatin hydrolysis instead of adding reagents to the plate medium. Due to the presence of ammonium sulfate in the medium itself, there is no need to flood the plate with the ammonium solution to detect the gelatin liquefaction (Stone's method). Ammonium sulfate precipitates unhydrolysed gelatin, so a transparent halo will appear around the gelatinase (+) colonies.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. D-Mannitol is the fermentable carbohydrate providing carbon and energy. Sodium chloride, in high concentrations, inhibits most bacteria except Staphylococci. Gelatin is a protein derived by the hydrolysis of collagen, found abundantly in bones, skin, tendons, cartilage and animal tissue. It is used in culture media to determine gelatinolysis by bacteria. The gelatinoses produced by the microorganisms hydrolyze the gelatin liquefying a solid medium or preventing the gelation of a medium containing gelatin. Bacteriological agar is the solidifying agent.

The staphylococcal colonies are yellow, yellow-gold or orange, ferment mannitol, coagulase-positive, produce beta-hemolysis in media such as Blood Agar (Cat. 1108) and are gelatinase-positive (positive Stone´s reaction). Any pigmented colony (yellow or soft orange) that is surrounded by a clear zone is probably a pathogenic Staphylococcus. Pale colonies, practically lacking in color or not producing pigment, should not be considered as positives, even if they are surrounded by a clear zone (halo), and are presumptively identified as S. epidermidis colonies.

It is recommended to pick the colony and inoculate it in 0,1-0,2 ml of Brain Heart Infusion Broth (Cat. 1400) and perform the coagulase test. At the same time, add a drop of Bromocresol purple to the colony site in order to determine mannitol fermentation: a yellow color formation is a positive reaction. The zones of clear halos around the colonies indicate degradation by the enzyme gelatinase (gelatin hydrolysis).

Formula in g/L

Ammonium sulfate	75	Bacteriological agar	15
Casein peptone	10	Dipotassium phosphate	5
D-mannitol	10	Gelatin	30
Sodium chloride	55	Yeast extract	2,5

Preparation

Suspend 202,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 10 minutes. Cool to 50 ºC, mix well and dispense into plates.

Instructions for use

Streak plate method:
- In a Petri dish, add 12-15 ml of molten agar and let it solidify.
- Inoculate 10 µl of the initial suspension and/or diluted sample.
- Extend the inoculum with a sterile loop on the agar surface.
- Incubate the plates in an inverted position at a temperature of 30±2 ºC for 18-48 hours.
- Examine for growth and the presence or absence of clear zones (halos) around colonies.

-To determine mannitol fermentation, add a few drops of bromcresol purple. Any change in color of the indicator, compared with the uninoculated medium, indicates fermentation of mannitol.

Expected results:
- Mannitol fermentation: Positive = change in color of the indicator to yellow. Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as Staphylococcus.
- Gelatinase activity: Positive Stone reaction = formation of clear zones (halos) around the colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Light beige	Amber, slightly opalescent	7,0±0,2

Microbiological test

Incubation conditions: (30±2 ºC / 18-48 h).

Microrganisms	Specification	Characteristic reaction
Staphylococcus epidermidis ATCC 12228	Good growth	Mannitol (-), Halo (+)
Escherichia coli ATCC 25922	Inhibited growth	Mannitol (-), Halo (-)
Staphylococcus aureus ATCC 25923	Good growth	Mannitol (+), Halo (+)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Chapman J. Bact. 1945. 50: 201 Recommended Methods for the Microbiological Examination of Foods APHA. Inc. New York 1958. Standards Methods for Examination of Dairy Products, 1st Ed. APHA. Inc. New York, 1960.