Baird Parker Agar Base (DM034)

Intended Use
Baird Parker Agar Base (DM034) is recommended for isolation and enumeration of coagulase positive *Staphylococci* from foods and other specimen.

Product Summary and Explanation
Baird Parker Agar was developed by Baird-Parker[1,2] from the Tellurite-glycine formulation of Zebovitz et al[3] for isolation of S. aureus from foods. Baird Parker medium was reported to be the best medium for selective detection of coagulase positive and entero-toxigenic Staphylococcus.[6] Researchers have found a high correlation between the coagulase test and the presence of clear zone of lypolysis in this medium, which is because of the lecithinase of Staphylococci that breakdown, the egg yolk. On the other hand, some studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, whereas other Staphylococci cannot reduce tellurite. Baird Parker Agar was found to be less inhibitory to Staphylococcus aureus than other media at the same time being more selective. [4, 5, 6] Subsequently the use of Baird-Parker Agar was officially adopted by AOAC International [7] and is recommended in the USP for use in the performance of Microbial Limit Tests.[8] Recently, ISO committee has also recommended this medium for the isolation and enumeration of Staphylococci.[9]
The identity of Staphylococcus aureus isolated on Baird-Parker Agar must be confirmed with a coagulase reaction. Baird- Parker Agar can also be used to detect coagulase activity by adding fibrinogen plasma.[11] Smith and Baird-Parker [10] found that the addition of 50 mg/l Sulphamethazine in the medium, suppresses the growth and swarming of Proteus species.

Principles of the Procedure
In Baird Parker Agar Base beef extract, yeast extract and casein enzymic hydrolysate provides essential mineral, vitamin, nitrogenous compounds and other growth requirements. Sodium pyruvate is incorporated to protect injured cells and help recovery and growth of *S. aureus* without destroying the selectivity. Lithium chloride and potassium tellurite inhibit most of contaminating microflora except *S.aureus*. The tellurite additive is toxic to egg yolk- clearing strains other than S. aureus. The reduction of tellurite is a characteristic of coagulase-positive staphylococci, and imparts a black color to the colonies. Glycine, pyruvate enhances growth of *Staphylococcus*. With the addition of egg yolk the medium becomes yellow and opaque. Glycine neutralizes aldehyde, while egg yolk neutralizes phenolic compounds, if any, in the test samples.
The egg yolk additive, in addition to being an enrichment, also aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). Staphylococci that contain lecithinase break down the Egg Yolk and cause clear zones around the colonies. A clear zone and grey-black colonies on this medium are diagnostic for coagulase positive Staphylococci. Upon further incubation, an opaque zone is developed around colonies, which can be due to lipolytic activity. Identity of S. aureus isolated on Baird-Parker Agar must be confirmed with a coagulase reaction and deoxyribonuclease test. The sterility of product is confirmed by absence of growth of S. aureus on this medium.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of Casein	10.00
Beef extract	5.00
Yeast extract	1.00
Glycine	12.00
Sodium pyruvate	10.00
Lithium chloride	5.00
Agar	15.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions checked

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Lithium Chloride is harmful. Avoid all bodily contact and inhalation of vapours. On contact with skin wash with plenty of water immediately

Directions

1. Suspend 58 grams of the medium in 950 ml of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C and add aseptically 50 ml concentrated Egg Yolk Emulsion (MS038) and 3 ml sterile 3.5% Potassium Tellurite solution (MS025) or 50 ml Egg Yolk Tellurite Emulsion (MS061).
5. If desired add rehydrated contents of 1 vial of BP Sulpha Supplement (MS110).
6. Alternatively 1 vial of Fibrinogen Plasma Trypsin Inhibitor Supplement (MS118) may be used per 90 ml medium in place of Egg yolk Tellurite Emulsion (MS061) for identification of coagulase, positive Stapylococci.
7. Mix well and pour into sterile Petri plates.

Warning : Lithium Chloride is harmful. Avoid all bodily contact and inhalation of vapours. On contact with skin wash with plenty of water immediately.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium: Yellow coloured clear to slightly opalescent gel After addition of Egg Yolk Emulsion and Tellurite Emulsion: Yellow coloured opaque gel forms in Petri plates.
Reaction of 6.3% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after incubation at Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of colony	Lecithinase
1.	*Staphylococcus aureus ATCC 6538*	50 -100	good-luxuriant	25-100	>=50 %	grey-black shiny	Positive, opaque zone around the colony
2.	*Staphylococcus aureus ATCC 25923*	50 -100	good-luxuriant	25-100	>=50 %	grey-black shiny	Positive, opaque zone around the colony
3.	*Proteus mirabilis ATCC 25933*	50 -100	good-luxuriant	50-100	>=50 %	brown – black	Negative
4.	*Micrococcus luteus ATCC 10240*	50-100	poor- good	15 -40	30-40%	shades of brown-black (very small)	Negative
5.	*Staphylococcus epidermidis ATCC 12228*	50-100	poor- good	15 -40	30-40%	black	Negative
6.	*Bacillus subtilis ATCC 6633*	50-100	none- poor	0 -10	0 -10	dark brown matt	Negative
7.	*Escherichia coli ATCC 8739*	50-100	none- poor	0 -10	0 -10	large brown black	Negative

8.	*Escherichia coli* ATCC 25922	50-100	none- poor	0 -10	0 -10	large brown black	Negative
9.	*Escherichia coli* NCTC 9002	50-100	none- poor	0 -10	0 -10	large brown black	Negative

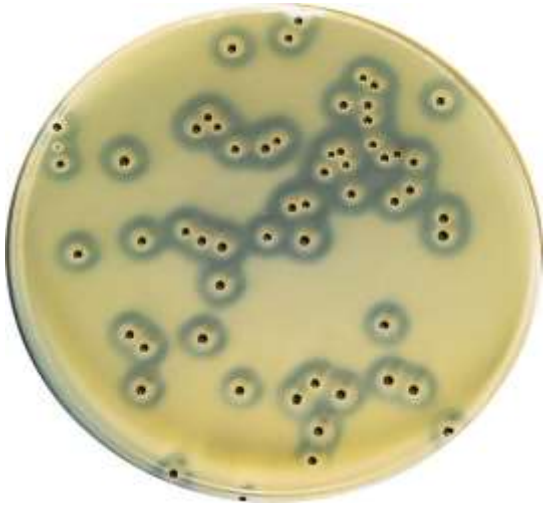
The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Food samples are macerated in suitable broth medium, diluted as desired and the dilutions spread-inoculated onto the agar surfaces, which should be dry when inoculated.
2. Alternatively, inoculate the material to be examined (0.1 ml per plate of diameter 90-100 mm),
3. Incubate plates aerobically for 24 hours at 35 - 37°C.
4. Perform the coagulase test on the colonies with the typical characteristics, which have developed during the incubation period.
5. Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones.
6. The basal medium, without the egg yolk or the tellurite, is perfectly stable.
7. To detect coagulase activity by adding Fibrinogen Plasma Trypsin Inhibitor supplement (MS118), is dissolved in 10 ml sterile distilled water added to 90 ml sterile molten media kept at 45-50°C. On this medium coagulase positive colonies appear white to grey-black surrounded by an opaque zone due to coagulase activity within 24-48 hours incubation at 35°C. Reduction in tellurite is necessary because of absence of egg yolk emulsion. This results in translucent agar and white to grey coloured colonies of Staphylococci. For quantitative results select 20-200 colonies. Count Staphylococcus aureus like colonies and test them for coagulase reaction. Report Staphylococcus aureus per gram of food.
8. Refer appropriate references for detailed instructions of specific procedures.

Results

1. Coagulase-positive typical colonies of *Staphylococcus aureus* produce grey-black, shiny, convex colonies with entire margins and clear zones, (egg yolk reaction) with or without an opaque zone.
2. Coagulase-negative staphylococci generally produce poor or no growth. If growth occurs, colonies are black; typical clear or opaque zones are rare.
3. The majority of other organisms are inhibited or grow poorly. If growth appears, colonies are dark-brown matt growth, with no clear or opaque zones.
4. Colonies of some contaminating organisms may digest the coagulase halo reaction.
5. Other bacteria may grow on this media but biochemical test will differentiate coagulase positive Staphylococci from the other organisms.



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Staphylococcus aureus ATCC 6538 showing grey-black shiny colonies with, opaque zones around the colony (Lecithinase Positive reaction).

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. Baird-Parker Agar is selective for coagulase-positive staphylococci, but other bacteria may grow.
2. Microscopic examination and biochemical tests will differentiate coagulase-positive staphylococci from other organisms.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Baird Parker Agar Base
Product Code : DM034
Available Pack sizes : 100gm / 500gm

References

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8. The United States Pharmacopoeia, 2008, USP31, The United States Pharmacopoeial Convention. Rockville, MD.
9. International Organization for Standardization (ISO), 1983, Draft ISO/DIS 6888.
10. Smith B. A. and Baird-Parker A.C., 1964, J. Appl. Bacter

Further Information
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