Technical Information

Baird Parker Agar with Sulpha

Product Code: DM 2140

Application: - Baird Parker Agar Base with supplements is recommended for the isolation and enumeration of coagulase positive Staphylococci from food and other materials.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Beef extract	5.000
Yeast extract	1.000
Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Sulphamethazine	0.050
Agar	20.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Baird Parker Agar was developed by Baird Parker [1, 2] by moditying the Tellurite-glycine formulation of Zebovitz et al [3] for isolation and enumeration of coagulase positive strains of Staphylococci in food and other material since high correlation has been found between the coagulase test and the presence of clear zone of lypolysis in this medium, which is due to the lecithinase activity of Staphylococci that breakdown, the egg yolk. On the other hand, studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, and is not hold good for coagulase nagetive. Staphylococci. The medium was found to be more selective to *Staphylococcus aureus* than other media [4-6]. Later on 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]. Fibrinogen Plasma Trypsin Inhibitor supplement (MS1195) 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 3 5°C. Reduction in tellurite is necessary because of absence of egg yolk emulsion. This result 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. 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.

Casein enzymic hydrolysates, peptic digest of animal tissue, beef, meat and yeast extract are sources of nitrogen, carbon, sulphur and vitamins. Sodium pyruvate not only protects injured cells and helps recovery but also stimulates *Staphylococcus aureus*growth without destroying selectivity. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus*. The tellurite additive is toxic to egg yolk-clearing strains other than S. aureus and imparts a black color to the colonies. Glycine, pyruvate enhances growth of *Staphylococcus*. With the addition of egg yolk, the medium becomes yellow, opaque. The egg yolk additive, in addition to provide enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). 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.

When testing the medium, inoculate the material to be examined (0.1 ml per plate of diameter 90-100 mm), incubate at 37°C and take the first reading after 24-26 hours. The colonies of *Staphylococcus aureus* are black and shiny, with a fine white rim, surrounded by a clear zone. Incubate at 37°C for another 24 hours and perform the coagulase test on the colonies with the above characteristics, which have developed during the further incubation period.

Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones. The basal medium, without the egg yolk or the tellurite, is perfectly stable. Colonies of some contaminating organisms may digest the coagulase halo reaction. Other bacteria may grow on this media but biochemical test will differentiate coagulase positive Staphylococci from the other organisms.

Methodology

Suspend 63.05 grams of powder media in 950 ml distilled water. Shake well and heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (MS2045) and 3 ml sterile 3.5% Potassium Tellurite solution (MS2047) or 50 ml Egg Yolk Tellurite Emulsion (MS2046). Mix well and pour into sterile Petri plates.

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

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of Egg Yolk Tellurite Emulsion (MS2046): Yellow coloured opaque gel forms in Petri plates.

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH range 6.80-7.20

Cultural Response/Characteristics

DM2140: Cultural characteristics observed with added Egg Yolk Tellurite Emulsion (MS2046) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Bacillus subtilis ATCC 6633	50-100	none-poor	Negative	
Escherichia coli ATCC25922	50-100	none-poor	negative	large brown black
Micrococcus luteus ATCC10240	50-100	fair-good	Negative	very small, brown-black
Proteus mirabilis ATCC25933	50-100	none-poor	Negative	brown-black w/ o swarming
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	positive,halo or clear zone around the colony	grey-black shiny
Staphylococcus epidermidis ATCC 12228	50-100	fair-good	negative	Black

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Baird-Parker A. C., 1962, J. Appl. Bacteriol., 25:12.
2. Baird-Parker A. C. and Davenport E., 1965, J. Appl. Bacteriol., 28:390.
3. Zebovitz E., Evans J. B. and Niven C.F., 1955, J. Bacteriol., 70:686.
4. Tardio and Baer, 1971, J. Assoc. Off. Anal. Chem., 54:728.
5. Baer, 1971, J. Assoc. Off. Anal. Chem., 54:732.
6. Assoc. off. Anal. Chem., 1971, 54:401.
7. Horwitz (Ed.), 2000, Official methods of analysis of AOAC International, 17th Ed., Vol. I., AOAC International, Gaithersburg, Md.
8. The United States Pharmacopoeia, 2008, USP3 1, The United States Pharmacopeial 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. Bacteriol., 27:78.
11. Beckers N. J. et al, 1984, Can. J. Microbiol., 30:470.

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