

## Baird Parker HiCynth™ Agar Base

MCD043

Baird Parker HiCynth™ Agar Base is recommended for the isolation and enumeration of coagulase positive staphylococci from food and other materials.

### Composition\*\*

Ingredients	Gms / Litre
HiCynth™ Peptone No.2*	10.000
HiCynth™ Peptone No.7*	6.000
Glycine	12.000
Sodium pyruvate	10.000
Lithium chloride	5.000
Agar	20.000
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

\*Chemically defined peptones

### Directions

Suspend 63 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (FD045) and 3 ml sterile 3.5% Potassium Tellurite solution (FD047) or 50 ml Egg Yolk Tellurite Emulsion (FD046). For additional selectivity, if desired add rehydrated contents of 1 vial of BP Sulpha Supplement (FD069). Alternatively 1 vial of Fibrinogen Plasma Trypsin Inhibitor Supplement (FD195) may be used per 90 ml medium in place of Egg yolk Tellurite Emulsion (FD046) for identification of coagulase, positive Staphylococci. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Baird Parker HiCynth™ Agar Base is a modification of Baird Parker Agar Base, originally developed by Baird Parker (1, 2) from the Tellurite-glycine formulation of Zebovitz et al (3) for isolation and enumeration of Staphylococci in food and other material since it allows a good differentiation of coagulase positive strains. A 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 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, whereas other Staphylococci cannot always do so. The medium 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). Fibrinogen Plasma Trypsin Inhibitor supplement (FD195) dissolved in 10 ml sterile distilled water is 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. 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.

Baird Parker HiCynth™ Agar Base is prepared by replacing animal peptones in Baird Parker Agar Base with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. HiCynth™ Peptone No.2 and HiCynth™ Peptone No.7 acts as sources of nitrogen, carbon, long chain amino acids, 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 colour to the colonies. Glycine, sodium 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.

## Quality Control

### Appearance

Cream to yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 2.0% agar gel.

### Colour and Clarity of 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

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

### pH

6.80-7.20

### Cultural Response

Cultural response was observed after an incubation at 35-37°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
<b>Cultural response</b>					
<i>Staphylococcus aureus</i> ATCC 6538	50 -100	luxuriant	≥50 %	grey-black shiny	Positive, opaque zone around the colony
<i>Staphylococcus aureus</i> ATCC 25923	50 -100	luxuriant	≥50 %	grey-black shiny	Positive, opaque zone around the colony
<i>Proteus mirabilis</i> ATCC 25933	50 -100	good - luxuriant	≥50 %	brown - black	Negative
<i>Micrococcus luteus</i> ATCC 10240	50 -100	poor - good	30 -40 %	shades of brown-black (very small)	Negative
<i>Staphylococcus epidermidis</i> ATCC 12228	50 -100	poor - good	30 -40 %	black	Negative
<i>Bacillus subtilis</i> ATCC 6633	50 -100	none - poor	0 -10 %	dark brown matt	Negative

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<i>Escherichia coli</i> ATCC 8739 50 -100	none- poor	0 -10 %	large brown black	Negative
<i>Escherichia coli</i> ATCC 25922 50 -100	none- poor	0 -10 %	large brown black	Negative
<i>Escherichia coli</i> NCTC 9002 50 -100	none- poor	0 -10 %	large brown black	Negative

## Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

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Revision : 00/ 2015



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