# Baird Parker Agar with Sulpha

**M1140**

## Intended use

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 |
|---|---|
| Tryptone | 10.000 |
| HM peptone B # | 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

# Equivalent to Beef extract

## Directions

Suspend 63.05 grams in 950 ml purified / distilled water. 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 (FD045) and 3 ml sterile 3.5% Potassium Tellurite solution (FD047) or 50 ml Egg Yolk Tellurite Emulsion (FD046). Mix well and pour into sterile Petri plates.

## Principle And Interpretation

Baird Parker Agar was developed by Baird Parker (3,4) from the Tellurite-glycine formulation of Zebovitz et al (16) 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 (13, 5, 1). 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 (14). Recently, ISO committee has also recommended this medium for the isolation and enumeration of Staphylococci (8).

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 (6). Fibrinogen Plasma Trypsin Inhibitor supplement (FD195) 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. Smith and Baird-Parker (11) found that the addition of 50 mg/l Sulphamethazine in the medium, suppresses the growth and swarming of *Proteus* species.

Tryptone, HM peptone B 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. The basal medium, without the egg yolk or the tellurite, is perfectly stable.

## Type of specimen

Food and dairy samples

## Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,12,15). After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones.
2. Colonies of some contaminating organisms may digest the coagulase halo reaction.
3. Other bacteria may grow on this media but biochemical test will differentiate coagulase positive Staphylococci from the other organisms.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## 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 : Light amber coloured clear to slightly opalescent gel. After addition of Egg Yolk Tellurite Emulsion (FD046) : 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**

M1140: Cultural characteristics observed with added Egg Yolk Tellurite Emulsion (FD046) after an incubation at 35-37°C for 24-48 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Lecithinase activity | Colour of colony |
|---|---|---|---|---|---|
| **Cultural Response** | | | | | |
| *Bacillus subtilis* subsp. *spizizenii* ATCC 6633 (00003*) | 50-100 | none-poor | <=10% | negative | |
| *Escherichia coli ATCC 25922* (00013*) | 50-100 | none-poor | <=10% | negative | large brown |
| *Micrococcus luteus ATCC 10240* | 50-100 | fair-good | 30-40% | negative | black very small, brown-black |

| | | | | | |
|---|---|---|---|---|---|
| *Proteus mirabilis ATCC 25933* | 50-100 | none-poor | <=10% | negative | brown-black w/ o swarming |
| *Staphylococcus aureus* subsp.*aureus ATCC 25923* (00034*) | 50-100 | good-luxuriant | >=50% | positive,halo or clear zone around the colony | grey-black shiny |
| *Staphylococcus epidermidis ATCC 12228* (00036*) | 50-100 | fair-good | 30-40% | negative | black |

Key : *Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

## Reference

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