BAIRD-PARKER AGAR

DETECTION AND ENUMERATION OF Staphylococcus aureus

1 INTENDED USE

Baird-Parker Agar with Egg Yolk Tellurite is a selective medium for the detection and enumeration of Staphylococcus aureus in biological samples, pharmaceutical products, cosmetics, foods and water.

2 HISTORY

The formula, developed by Baird-Parker in 1962, was found to be particularly appropriate for the enumeration of coagulase positive staphylococci. In 1964, Smith and Baird-Parker showed that adding sulfamethazine to the medium inhibited the growth of Proteus and in 1971, Tardio and Baer observed, that among 18 selective isolation media tested, that the Baird-Parker formulation was less inhibitory than Vogel-Johnson medium, used previously with some frequency.

3 PRINCIPLES

The growth of staphylococci is favored by sodium pyruvate and glycine.

Accompanying microflora is inhibited by lithium chloride and potassium tellurite (added extemporaneously), as well as a high concentration of glycine.

The addition of sulfamethazine after autoclaving inhibits most Proteus and thus limits the invasion of the medium by this species.

Enrichment with egg yolk aids in identification by showing the action of lecithinase.

The characterization of Staphylococcus aureus, black colonies due to the reduction of tellurite to telluride and surrounded by clear halos, may be complemented by the coagulase test and optionally by the desoxyribonuclease and phosphatase tests.

4 PREPARATION

Preparation of dehydrated medium :

- Suspend 58.0 g of dehydrated base medium (BK055) in 950 mL of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in flasks by adding 95 mL per flask.
- Sterilize in an autoclave at 121°C for 15 minutes.

Preparation of medium in Petri dishes :

- Melt the medium (if prepared in advance).
- Cool and maintain at 44-47°C.
- Aseptically add 5 mL of Egg Yolk Tellurite Enrichment (BS060) to 95 mL of base and, if necessary, 1 mL of reconstituted Sulfamethazine Selective Supplement (BS028) when Proteus is suspected.
- Mix rapidly and thoroughly.
- Pour into sterile Petri dishes.
- Let solidify on a cool surface.
- Dry the plates in an incubator with the covers partially removed.

Reconstitution: 58.0 g for 950 mL
Sterilization: 15 min at 121 °C
Add 5 mL of Egg Yolk Tellurite enrichment in 95 mL of medium
5 Instructions for use

Microbiology of food stuffs

- Transfer 0.1 mL of the sample to analyze and its serial tenfold dilutions to the plates prepared as above or onto complete ready-to-use plates (BM018 or BM091) brought to room temperature.
- Spread the inoculum on the surface of the agar with a sterile triangle.
- Incubate at 37 °C (ordinary temperature) for 24 hours ± 2 hours and extend for 48 hours ± 2 hours.

Note: The temperature is agreed between the interested parties and is indicated in the test report.

Water quality

- Aseptically filter through a membrane a known volume of the sample to test.
- Deposit the membrane on the surface of the agar, filtered side up and making sure that the membrane and agar are in close contact.
- Incubate at 36 ± 2 °C for 21 hours ± 3 hours and extend to 44 hours ± 4 hours.

Cosmetics

- Transfer 0.1 mL of the sample to analyze and its serial tenfold dilutions to the plates prepared as above or onto complete ready-to-use plates (BM018 or BM091) brought to room temperature.
- Spread the inoculum on the surface of the agar with a sterile triangle.
- Incubate at 32.5 ± 2.5 °C (ordinary temperature) for 24 to 48 hours.

6 Results

Staphylococcus aureus is characterized by the formation of black colonies (reduction of tellurite to telluride), which are shiny, convex and surrounded by clear zones (egg yolk reaction) of 2 to 5 mm in diameter, resulting from proteolysis. An opaque zone due to the action of lecithinase may appear inside the halo. Other bacteria are, in principle, inhibited.

See ANNEX 1: PHOTO SUPPORT

Nevertheless, it is possible to observe brown colonies of micrococi, white colonies of yeast and matte brown colonies of Bacillus or Proteus.

For enumeration, count plates containing between 15 to 150 characteristic colonies for surface inoculation and between 10 to 100 characteristic colonies after membrane filtration.

Both characteristic and/or non-characteristic colonies must be verified with the coagulase tube test (refer to the technical data sheets on the Coagulase Rabbit Plasma, BR002 or on the BAIRD-PARKER RPF agar BM067, BM159, BK055, BS034 and BS038) in order to confirm their pathogenicity. Coagulase negative staphylococci are usually inhibited. If a culture appears, however, typical clear zones are absent.

7 Typical composition

The typical composition can be adjusted to obtain optimal performance.

For 1 liter of medium:
- Tryptone ................................................................. 10.0 g
- Meat extract .......................................................... 5.0 g
- Yeast extract .......................................................... 1.0 g
- Sodium pyruvate .................................................... 10.0 g
- Glycine ................................................................. 12.0 g
- Lithium chloride .................................................... 5.0 g
- Bacteriological agar ............................................... 15.0 g
- Egg Yolk Emulsion ............................................... 47.0 mL
- Potassium tellurite (3.5%) ...................................... 3.0 mL

pH of ready-to-use medium at 25 °C : 7.2 ± 0.2.
8 QUALITY CONTROL

Dehydrated medium: cream-white powder, free-flowing and homogeneous.
Prepared medium: yellowish opaque agar.

Typical culture response after 48 hours of incubation at 37°C (XP CEN ISO/TS 11133):

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Growth (Productivity ratio: $P_0$)</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>$P_0 \geq 50%$</td>
<td>Black with halo</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>$P_0 \geq 50%$</td>
<td>Black with halo</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Slowed, score 0–1</td>
<td>Black without halo</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Inhibited, score 0</td>
<td></td>
</tr>
</tbody>
</table>

9 STORAGE

Dehydrated base medium (without Egg Yolk Tellurite): 2-30 °C.
Prepared medium from dehydrated base in flasks (benchmark value): 6 months at 2-8 °C.
Prepared complete medium in plates: 5 days at 2-8 °C.

Pre-poured media in Petri plates, Sterile Egg Yolk Tellurite, Sulfamethazine 25 mg Selective Supplement, Coagulase Rabbit Plasma: Store between 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

10 PRESENTATION

Pre-poured media in Petri plates (Ø 90 mm):
20 plates .................................................................................................................. BM01808
120 plates .................................................................................................................. BM09108

Dehydrated base medium (without Egg Yolk Tellurite):
500 g bottle ................................................................................................................ BK055HA
5 kg drum .................................................................................................................. BK055GC

Egg Yolk Tellurite enrichment:
10 x 50 mL vials ........................................................................................................ BS06008

Sulfamethazine 25 mg Selective Supplement:
10 vial pack ................................................................................................................ BS02808

11 BIBLIOGRAPHY


European Pharmacopoeia. 2.6.13. Microbiological examination of non-sterile products test for specified microorganisms. Recommended solution and culture media.


**12 ADDITIONAL INFORMATION**

*Benchmark value refers to the expected shelf life when prepared under standard laboratory conditions following manufacturer’s instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.

The information provided on the package take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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Grounds for revision : General revision (§ 5 : Instruction for use; § 8 : Control strains; § 22 : Bibliography)
ANNEX 1: PHOTO SUPPORT

**Baird Parker medium with Egg yolk tellurite supplement**

Detection and enumeration of *Staphylococcus aureus*

**Reading:**

Incubation 48 hours at 37°C (in surface)

*Staphylococcus aureus*

Characteristics: Shiny, black colonies surrounded by an inner opaque (lipase reaction) halo and clearing zones (protease reaction).