

Agar medium O (Baird-Parker) Baird-Parker agar medium (V)

356-3991
356-4814

DEFINITION

Medium used for the enumeration (with confirmation of colonies) of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) in products intended for human or animal consumption at 37°C.

Equivalent USP 30/NF 25: Medium V

STANDARDS

- **European Pharmacopeia 6.0** - Biological methods - **2.6.13.**: Microbiological test of non-sterile products (Detection of specified micro-organisms)

- **USP 30/NF 25 US Pharmacopeia and National Formulary (2007)**: Microbial Limit Tests (61) - Microbiological Tests

PRINCIPLE

The principle of the medium relies on the ability of *Staphylococcus aureus* to reduce tellurite (black colonies), to provoke proteolysis of egg yolk (clear halo around colonies), and to render the proteolysis zone opaque (lipase activity). Lithium chloride and potassium tellurite make this medium inhibit other bacteria. Sulfamethazine must be added to the medium for testing products highly contaminated with *Proteus*.

PRESENTATION

Base

- Dehydrated
500 g **code 356-4814**

Complete

- Pre-poured
90 mm x 20 plates **code 356-3991**

STORAGE

- Pre-poured: +2-8°C
- Dehydrated: +15-25°C, in carefully-sealed bottles in a cool, dry place
- Expiration date and batch number are shown on the package.
- Petri dishes (complete medium) prepared by user: 5 days maximum at +2-8°C, in a dark place

TYPICAL FORMULA

Base

Pancreatic casein peptone	10 g
Yeast extract	1 g
Meat extract	5 g
Lithium chloride	5 g
L-Glycine	12 g
Sodium pyruvate	10 g
Agar	16 g
Distilled water	1,000 ml
Final pH (25°C) = 7.2 ± 0.2	

Complete

Pre-poured	
Pancreatic casein peptone	10 g
Yeast extract	1 g
Meat extract	5 g
Lithium chloride	5 g
Agar	16 g
L-Glycine	12 g
Sodium pyruvate	10 g
Potassium tellurite	0.1 g
Egg yolk emulsion	10 ml
Sulfamethazine	0.05 g
Distilled water	1,000 ml
Final pH (25°C) = 7.2 ± 0.2	

NB: the formula has been adapted to attain the required performance criteria.

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- **Egg yolk emulsions with potassium tellurite**
5 ml x 1 vial (code 355-4201)
25 ml x 1 bottle (code 355-4205)
- **Sulfamethazine 0.2%**
2.5 ml x 1 vial (code 356-2682)

• Diluent(s)

• Distilled water

See corresponding Technical Sheet(s)

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EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Vortex-type shaker
- 125 ml bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (0.1 ml, 1 ml...)
- Sterile spreaders
- Water-bath, precise to ±1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use.

Dissolve 57 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix until a homogenous suspension is obtained. Heat gently, shaking frequently, then bring to the boil until completely dissolved. Dispense 90 ml of medium per bottle. Sterilize in autoclave at 121°C (± 1°C) for 15 minutes.

Reconstitution: 57 g/l
500 g of powder makes 8.7 liters of medium.

PREPARATION OF COMPLETE MEDIUM Using dehydrated medium

- At moment of use, add the following solutions to 90 ml of this base, previously melted and cooled to between 44-47°C:
 - 5 ml egg yolk with potassium tellurite
 - 2.5 ml of 0.2% sulfamethazine if necessary
- Mix thoroughly
- Pour into Petri dishes (thickness ~ 4 mm) and leave to solidify on a level surface.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned

• Inoculation and incubation

- Spread 0.1 ml of the sample to be analyzed, or 0.1 ml of stock suspension (other products) and/or 0.1 ml of its decimal dilutions over the surface of the *dried* agar.
- Turn the dishes over and incubate at 37°C (± 1°C) for 24 hr (± 2hr), then re-incubate for a further 24 hr (± 2 hr).

READING AND INTERPRETATION

• Counting/Confirmation of colonies (UFC)

After each period of incubation, count the typical colonies. Presumptive coagulase-positive *staphylococci* form black colonies on this opaque medium, with:

- a clear halo around the colony, corresponding to a zone of proteolysis (lightening of egg yolk).
- opaque zones that may appear later in the clear halo. These are due to the action of lipases.

From plates containing between 15 and 150 typical and/or non-typical colonies, collect 3 to 5 colonies and inoculate in tubes of Brain-Heart broth (code 355-3664). After 24 hours (± 2 hr) of incubation at 37°C (± 1°C), carry out detection of coagulase with rabbit plasma (code 355-6352)

See corresponding Technical Sheet

NB: confirmation possible (non-standard) with Latex Pastorex® Staph+ (codes 355-6356 and 355-6353) (see corresponding Technical Sheet)

• Expression of results/Calculations

For calculation method, refer to standard NF ISO 7218 and the specific standard

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or 10. dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Do not add the egg yolk to the potassium tellurite, sulfamethazine, sodium pyruvate and L-Glycine in a base medium at a temperature exceeding 47°C.
- Comply with Good Laboratory Practice.

QUALITY CONTROL

In view of the current harmonization of pharmacopeias, we recommend that you refer to the certificates of analysis for procedures relating to the quality control (performance and selectivity) of media produced by Bio-Rad.

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Baird-Parker/*Staphylococcus aureus*/Food products/Enumeration/Coagulase/Medium

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BIBLIOGRAPHY

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• **SMITH B.A., BAIRD PARKER A.C. (1964):** The use of sulfamethazine for inhibiting *Proteus* spp. on Baird Parker's isolation medium for *Staphylococcus aureus*. Journal of Applied Bacteriology 27: 78