Baird-Parker/Agar

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 and swimming pool waters.

STANDARDS

FOOD MICROBIOLOGY

- **US Department of Health and Human Services US Food and Drug Administration Center for Food Safety and Applied Nutrition.** Bacteriological, analytical, on-line Manual, January 2003

- **NF EN ISO 6888-1/A1 (January 2004):** Food microbiology - Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker agar medium

- **NF EN ISO 6888-3 (June 2003):** Food microbiology - Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species - Part 3: Detection and MPN method for small numbers

- **FIL 60B (1990):** Dried milk products - Enumeration of *Staphylococcus aureus* - Most Probable Number technique

- **FIL 145 (1990):** Dried Milk - Enumeration of *Staphylococcus aureus* - Technique of colony count at 37℃

- **FIL 138 (1986):** Milk and milk products - Enumeration of *Staphylococcus aureus* - Technique of colony count at 37℃

- Official methods of specimen collection and bacteriological analysis of ice-cream (French government decree of August 30, 1968 published in the Official Journal on 21 September 1968)

- Methods of analysis of pasteurised milk (French government decree of January 3, 1985 published in the Official Journal on 17 February 1985 and modifying the decree of 21 June 1982 appearing in the Official Journal dated 11 July 1982)

WATER

- **NF T90-421 (August 2006):** Bacteriological test for water in swimming pools

- **XP T90-412 (June 2006):** Water quality - Detection and enumeration of pathogenic *Staphylococci* - Method by membrane filtration

- **NF T90-461/A2 (May 2007):** Water quality - Microbiology - Culture media quality control

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).
Due to its lithium chloride and potassium tellurite content this medium inhibits other bacteria.
Sulfamethazine must be added to the medium to test products highly contaminated with *Proteus*.

PRESENTATION

- **Base**
  500 g **code 356-4814**

- **Complete**
  90 mm x 20 plates **code 356-3991**

STORAGE

- Pre-poured: +2-8℃
- Ready to use: +15-25℃
- Dehydrated: +15-25℃, in carefully-sealed bottle 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℃, in a dark place

THEORETICAL FORMULA

Base medium (Dehydrated)

Pancreatic casein peptone	10 g
Yeast extract	1 g
Meat extract	5 g
Lithium chloride	5 g
Agar	14 g
Distilled water	1,000 ml
L-Glycine	12 g
Sodium pyruvate	10 g

Final pH (25℃) = 7.2 ± 0.2

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Baird-Parker/Agar

Complete medium (Pre-poured)

Pancreatic casein peptone	10 g
Yeast extract	1 g
Meat extract	5 g
Lithium chloride	5 g
Agar	14 g
L-Glycine	12 g
Sodium pyruvate	10 g
Potassium tellurite	0,1 g
Egg yolk	10 ml
Sulfamethazine	0.05 g
Distilled water	1,000 ml

Final pH (25°C) = 7.2 ± 0.2

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 / 55 mm)
- Filtration apparatus
- Filter membranes (Ø = 47 mm, ≤ 0.45 µm)
- Tweezers for handling membranes
- Sterile pipettes (0.1 ml, 1 ml…)
- Sterile spreaders
- Water-bath, precise to ±1°C
- Autoclave
- All usual laboratory equipment

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- **Distilled water**

- **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%/Additive**
  2.5 ml x 1 vial (code 356-2682)

- **Baird-Parker + RPF/Agar**
  90 mm x 20 plates (code 356-3991)
  90 ml x 6 bottles
  + 6 vials of supplement (code 357-8618)

- **Brain-Heart Infusion/Broth**
  10 ml x 25 tubes (code 355-3664)
  500 g (code 356-4014)

- **Rabbit plasma**
  package for 20 reactions **(code 355-6352)**

- **Latex Pastorex® Staph+ Kit**
  50 tests (code 355-6356)
  5 x 50 tests (code 355-6353)

See corresponding Technical Sheet(s)

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.

NB: This preparation can be used with the RPF supplement (See corresponding Technical Sheet).

PREPARATION OF COMPLETE MEDIUM

At moment of use, add the following solutions to 90 ml of the dehydrated 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 standards applicable to the product concerned.

• Inoculation and incubation

Food products

- 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 (± 2 hr), then re-incubate for a further 24 hr (± 2 hr).

Water testing

Membrane filtration method: filter 100 ml of water sample and deposit the membrane on the surface of a pre-poured dry Petri dishes, avoiding the formation of any air bubbles. Then, incubate at 36 ± 2°C for 44 ± 4 hours.

READING AND INTERPRETATION

• Counting/Confirmation of colonies (UFC)

After each period of incubation, proceed with counting of 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.

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Remark for water testing: reading of halo and opaque zone may be difficult due to the presence of the membrane. Therefore, raise carefully the membrane with tweezers .

From plates containing between 15 and 150 typical and/or atypical colonies, collect 3 to 5 colonies and inoculate in tubes of Brain-Heart Infusion broth (code 355-3664, 356-4014).
After 24 hours (± 2 hr) of incubation at 36°C (± 2°C), carry out detection of coagulase with Rabbit Plasma (code 355-6352).

• Other possible confirmation of colonies for Water Testing
According to XP T90-421, direct confirmation of colonies could also be performed using:
- **Latex Pastorex® Staph+ Kit** (code 355-6356, 355-6353)
- or by transferring the membrane from Baird Parker on a **Baird-Parker + RPF** agar plate which will be then incubate 21 hours (± 3 hr), and 44 hours (± 4 hr), at 36°C (± 1°C).
  See corresponding Technical Sheet

• Expression of results/Calculation
For the calculation method, refer to standard ISO 7218 and the specific standard.

PRECAUTIONS
- The time lapse between the end of preparation of the stock solution (or 10^{-1} 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 OF MANUFACTURER
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.

PERFORMANCES/QUALITY CONTROL OF THE TEST
The growth performances of the media are verified with the following strains:

STRAINS	Result after 24-48 hr incubation at 37°C	
Staphylococcus aureus ATCC 6538	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	PR* ≥ 0.5
Staphylococcus aureus ATCC 25923	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	PR* ≥ 0.5
Staphylococcus aureus ATCC 9144	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	Fertility yield R = [66- 150%]
Staphylococcus epidermidis ATCC 12228	**Tellurite reduction**	Gray/black colonies
	Halo	Negative
	Growth	Poor to good
Escherichia coli ATCC 25922	No growth	

*** PR = Total colony count obtained on 2 plates of Baird-Parker/total colony count on 2 plates of TCS agar.**

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

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