Baird-Parker base for RPF/Agar

357-8618
356-4814
356-4618
356-3996

DEFINITION

Selective medium used with the addition of RPF for direct enumeration (without 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

- **NF EN ISO 6888-2/A1 (December 2003):** Food microbiology - Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) - Part 2: Technique using agar medium with rabbit plasma and fibrinogen

- **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 145A (1997):** Milk and milk-based products - Enumeration of coagulase-positive *Staphylococcus aureus* - Technique enumeration of colonies

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 complete medium (RPF supplement + Baird-Parker base for RPF) relies on the ability of coagulase-positive staphylococci to reduce tellurite (gray to black colonies), and to transform the plasma fibrinogen to fibrin as a result of their coagulase activity (whitish opaque halo around colonies).
Due to its lithium chloride and potassium tellurite content the complete medium inhibits other bacteria.

PRESENTATION

- **Pre-poured**
  20 dishes x 90 mm **code 356-3996**

- **Ready to use**
  90 ml x 6 bottles of Baird-Parker base agar + 6 Freeze-dried supplements
  code 357-8618

- **Dehydrated (Base)**
  500 g **code 356-4814**

- **RPF Supplement**
  10 vials **code 356-4618**

STORAGE

- Ready to use: +2-8°C, in a dark place
- Dehydrated: +15-25°C, in carefully-sealed bottles in a cool, dry place
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Baird-Parker base agar (not supplemented)

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

Final pH (25°C) = 7.2 ± 0.2

Freeze-dried supplement (per bottle)

Rabbit plasma	2.5 ml
Bovine fibrinogen	0.375 g
Trypsin inhibitor	2.5 mg
Potassium tellurite	2.5 mg

Ready to use Baird-Parker + RPF

Peptone	10 g
Yeast extract	1 g
Meat extract	5 g
Lithium chloride	5 g
L-Glycine	12 g
Sodium pyruvate	10 g
Agar	14 g
Rabbit plasma	25 ml
Bovine fibrinogen	3.75 g
Trypsin inhibitor	25 mg
Potassium tellurite	25 mg
Distilled water	1,000 ml

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
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EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Vortex-type shaker
- 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, etc)
- Sterile spreaders
- Water-bath, precise to ±1°C
- Thermostatically-controlled incubators or incubation room, precise to ±1°C
- All usual laboratory equipment

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- **Sterile distilled water for reconstitution of RPF supplement** (code 355-4155)

See corresponding Technical Sheet(s)

RECONSTITUTION OF RPF SUPPLEMENT
- Under aseptic conditions, slowly add 10 ml of sterile distilled water preheated at 37°C* to the bottle of freeze-dried supplement.
- **Shake the bottle with a vortex if necessary** to ensure it is completely dissolved.
- Take care to avoid frothing. If necessary, place the bottle in an incubator at 37°C (± 1°C) until the lyophilized substance is completely dissolved.

* Helps dissolve lyophilised reagent.

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, swirling 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 ratio: 57 g/l
500 g of powder makes 8.7 liters of medium.

NB: This preparation can be used with or without the RPF supplement (See corresponding Technical Sheet).

PREPARATION OF COMPLETE MEDIUM
• Using dehydrated medium
At the moment of use, add the contents of a bottle of reconstituted RPF supplement to 90 ml of this Baird Parker base, previously melted and cooled to between 44-47°C.
Mix thoroughly.
Pour into Petri dishes (thickness ~ 4 mm) and leave to solidify on a level surface.

• Using ready-to-use medium
Under aseptic conditions, add the contents of a bottle of reconstituted RPF supplement to 90 ml of Baird Parker base agar for RPF, cool and maintain at between 44-47°C (= complete medium). Mix thoroughly.
A bottle of reconstituted RPF supplement complements 90 ml of Baird-Parker base agar supplemented with L-Glycine and sodium pyruvate.

PROTOCOL
• Preparation of samples
According to standards applicable to the product concerned.

• Inoculation and incubation
Food products
- Using sterile pipettes, transfer 1 ml of the test specimen (liquid product) or 1 ml of stock suspension (other products) and/or 1 ml of its decimal dilutions to sterile Petri dishes.
- Quickly pour out about 10 ml of complete medium.
- Homogenize and leave to cool on a cold, level surface.
- Once completely solidified, turn over the dishes and incubate at 37°C (± 1°C) for 18-24 hours or 48 hours if necessary.

NB: Surface inoculation is also possible, according to the standards for the product concerned.

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 the incubation period, proceed with the count of characteristic colonies. Coagulase positive staphylococci form gray to black colonies surrounded by a whitish opaque halo, indicating coagulase activity.

NB:
- *As the rabbit plasma fibrinogen agar is based on a coagulase reaction, it is not necessary to confirm this activity.*
- *Retain only plates containing at least 15 characteristic colonies and fewer than 300 colonies in all.*

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- *Depending on the method of calculation, plates containing fewer than 15 colonies, or no colonies, may be retained (estimation of small numbers).*

• Expression of results/Calculation

Refer to standard ISO 7218 and to the specific standard for the method of calculation.

PRECAUTIONS

- Avoid any prolonged overheating of the medium during melting.
- The medium may present a frothy appearance after gelling in bottles. It nevertheless conserves all its qualities as soon as this disappears after fusion and shaking.
- The time lapse between the end of preparation of the stock solution (or the 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.
- 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/RPF/*Staphylococcus*/Food products/Enumeration/Fibrinogen/Coagulase/Medium

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