TECHNICAL DATA SHEET

BAIRD-PARKER RPF AGAR

ENUMERATION AND CONFIRMATION OF COAGULASE POSITIVE STAPHYLOCOCCI

1 INTENDED USE

Baird Parker RPF (RPF = Rabbit Plasma Fibrinogen) Agar is used for the direct detection and enumeration of coagulase positive staphylococci. The medium has the advantage of considerably reducing the number of confirmation tests for the presence of coagulase positive staphylococci particularly when atypical colonies are observed on other selective media. The medium allows the simultaneous enumeration and confirmation to be performed in a single operation.

The type-formula of the agar corresponds to the composition defined in the food microbiology directives NF EN ISO 6888-2, NF EN ISO 6888-3 and NF V08-057-1. It also complies with the directives used for water control NF T90-412.

2 HISTORY

The production of free coagulase considered to be the principal characteristic for recognizing the pathogenicity of staphylococci, in particular *Staphylococcus aureus*, was at the origins of the development of Baird Parker with Rabbit Plasma Fibrinogen. Initial formulations of the incorporation of plasma in solid culture media revealed inconsistencies between the results obtained by the coagulase tube test and the formation of a characteristic halo of fibrin around colonies. The differences arose from the fact that certain strains possessed a coagulase that also activated the plasminogen-plasmin system as well, resulting in fibrinolysis and the disappearance of the fibrin halo. In order to overcome this difficulty, it was recommended to add soybean trypsin inhibitor.

In order to favour the detection of coagulase produced by *Staphylococcus aureus*, Devoyod *et al.* studied in 1976, the incorporation of pork plasma into Baird-Parker medium. Hauschild subsequently improved the performance of Devoyod's medium through the inclusion of bovine fibrinogen and a trypsin inhibitor, with a correlative decrease in the amount of plasma. In 1983, Beckers *et al.* modified Hauschild's medium, replacing the pork plasma with rabbit plasma, classically used in the coagulase tube test. These authors also inoculated the medium in depth, rather than the previously used double layer technique of Devoyod. Finally, the formulation was improved in 1986 by Sawhney, after completing studies relative to the toxicity of potassium tellurite towards *Staphylococcus aureus* in a rabbit plasma fibrinogen medium.

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.

Rabbit plasma was chosen for its excellent specificity towards staphylococcal coagulase and by its aptitude to rapidly produce clot formation by forming staphylothrombin from prothrombin. The rabbit plasma is reinforced with bovine fibrinogen. Staphylothrombin acts by cutting the A and B fibrinopeptides of fibrinogen, thereby initiating the polymerization process that results in the appearance of fibrin halos surrounding the colonies.

Soybean trypsin inhibitor prevents fibrinolysis.

The black coloration of staphylococcal colonies is due to the reduction of potassium tellurite to telluride. In addition, the presence of tellurite favors the inhibition of contaminating Gram-positive microflora.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of Baird Parker-Rabbit Plasma Fibrinogen agar :

- Tryptone ..... 9,0 g
- Meat extract..... 4,5 g
- Yeast extract ..... 0,9 g
- Sodium pyruvate ..... 9,0 g
- Glycine ..... 10,8 g
- Lithium chloride ..... 4,5 g
- Bacteriological agar..... 13,5 g
- Bovine fibrinogen ..... 5,0 g
- Rabbit plasma, EDTA..... 25 mL
- Trypsin inhibitor..... 25 mg
- Potassium tellurite..... 25 mg

pH of the complete, ready-to-use media at 25 °C : 7,2 ± 0,2.

Pour 58 g of dehydrated base BK055

- 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

For one vial of supplement BS034
(Qsp 100 mL)

- Bovine fibrinogen .....0,5 g
- Rabbit plasma, EDTA .....2,5 mL
- Trypsin inhibitor.....2,5 mg
- Potassium tellurite.....2,5 mg

For one vial of supplement BS038
(Qsp 500 mL)

- Bovine fibrinogen .....2,5 g
- Rabbit plasma, EDTA .....125 mL
- Trypsin inhibitor .....125 mg
- Potassium tellurite.....125 mg

5 PREPARATION

Preparation of the culture medium :

- Suspend 54,9 g of dehydrated medium (BK055) in 900 mL of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in flasks or vials by adding 90 mL, or multiples of 90mL per flask.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47°C.

✓ **Reconstitution : 54,9 g for 900 mL**

✓ **Sterilization : 15 min at 121 °C**

Use with the ready-to-melt base medium :

- Melt the base medium found in the kits or if prepared in advance for the minimum amount of time necessary in order to achieve total liquefaction.
- Cool and maintain at 44-47°C.

Rehydration of lyophilized supplements :

- Dissolve the lyophilsate by adding aseptically the volume of sterile distilled water as follows : .
  - Qsp 100 mL of complete medium (BS034, BS045) : 10 mL of sterile, distilled water
  - Qsp 500 mL of complete medium (BS038) : 50 mL of sterile, distilled water.
  - Qsp 200 mL of complete medium - specific to kit B7010 (BS075) : 10 mL of sterile distilled water.

- The dissolution will take place faster if the water is preheated (do not exceed 44 °C).

- Turn end-over-end to dissolve. Avoid frothing the solution. If complete dissolution is not achieved immediately, it is possible to use a mechanical mixer (type Vortex) to speed the reaction. The supplement should be completely dissolved before adding to Baird-Parker Agar base.

Preparation of complete media

- For 90 mL of base media included in the kit BT005 or prepared from dehydrated base, add aseptically 10 mL of reconstituted Rabbit Plasma Fibrinogen Supplement (BS034, BS045 or BS038).
- For 190 mL of specific base included in the kit BT010 (BM158), add aseptically 10 mL of the specific Rabbit Plasma Fibrinogen supplement from the kit (BS075).
- Mix well.
- Use immediately for the inoculation of pour plates or to prepare plates for surface inoculation.

✓ **Kit BT005**
Add 10 mL of BS045 to 90 mL of BM040

✓ **Kit BT010**
Add 10 mL of BS075 to 190 mL of BM158

NOTE :
The complete media cannot be kept for prolonged periods at 44-47 °C.

6 INSTRUCTIONS FOR USE

Enumeration of coagulase positive staphylococci, Food microbiology (NF EN ISO 6888-2)

- Transfer 1 mL of the sample to analyze and its tenfold dilution series into sterile 90mm diameter Petri dishes.
- Pour 10 to 15 mL of complete medium previously reconstituted.
- Homogenize by swirling.
- Let solidify or a cool surface.
- Incubate at 35 ± 1 °C or at 37 ± 1 °C for 24 ± 2 hours. If necessary, prolong the incubation for another 18 to 24 hours.

✓ **Inoculation :**
1 mL in depth (pour plates)

✓ **Incubation :**
24 to 48 h at 35-37 °C

Confirmation of pathogenic staphylococci, Food microbiology (NF EN ISO 6888-3)

- Pour the complete, prepared media into sterile Petri dishes or use the ready-to-use plates (BM067).
- Inoculate a loop from each specific tube of enrichment media (Giolitti & Cantoni).
- Incubate at 37 ± 1 °C for (24 ± 2) hours and prolong if necessary another 24 hours.

✓ **Inoculation :**
By streak plate

✓ **Incubation :**
24 to 48 h at 37 ± 1 °C

NOTE :
- To confirm the colonies within the context of the norm NF V08-057-1, pick characteristic colonies with a needle onto RPF agar.
- Incubate 24 h at 37 °C.

Enumeration of pathogenic staphylococci, Water microbiology and swimming pool water (NF T90-412)

- Pour the complete, prepared media into sterile Ø 55 mm Petri dishes or use the ready-to-use plates (BM159).
- Filter through a membrane the volume of water to be tested.
- Place the membrane on the solidified agar, filtered side up, making sure no bubbles form between the membrane and the agar surface.
- Invert the plate and incubate at 36 ± 2°C.
- Record the first results after 21 ± 3 hours. Delicately raise the membrane in order to observe the presence of coagulation zones on the agar.
- Record definitive results after (44 ± 4) hours.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
44 ± 4 h at 36 ± 2°C

NOTE :
The disappearance of opaque zones can occasionally take place at or beyond 48 hours.

7 RESULTS

Coagulase positive staphylococci are characterized by the formation of gray or black colonies surrounded by an opaque halo of fibrin that is clearcut, stable and well visible. Use of the RPF technique eliminates the need for confirming the results by the coagulase tube test. Other microorganisms can develop by forming gray to black colonies but they do not present the positive coagulase reaction (absence of an opaque halo surrounding the colonies).

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base medium : cream-white powder, free-flowing and homogeneous.
RPF supplement : white to rosy pellet, after reconstitution giving an amber, limpid and a slightly opalescent solution.
Complete prepared media : amber agar.

Typical culture response after 48 hours of incubation on complete media at 37°C (NF EN ISO 11133, NF T90-412) :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Staphylococcus aureus	WDCM 00034	$P_R \geq 50$ %	Black colonies, with an opaque halo
Staphylococcus aureus	WDCM 00035	$P_R \geq 50$ %	Black colonies, with an opaque halo
Staphylococcus epidermidis	WDCM 00036	Slowed, score 0-1	Black or gray colonies, without an opaque halo
Staphylococcus saprophyticus	WDCM 00159	Slowed, score 0-1	Black or gray colonies, without an opaque halo
Escherichia coli	WDCM 00013	Inhibited, score 0	-
Enterococcus faecalis	WDCM 00176	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Freeze-dried Rabbit Plasma Fibrinogen supplements : 2-8 °C.
Kits : 2-8 °C.
Pre-poured media in Petri dishes : 2-8 °C
The expiration dates are indicated on the labels.

Base media prepared in vials (*) : 180 days at 2-8 °C.
Rehydrated freeze-fried supplement (*) : 8 days at 2-8 °C. Pre-heat before use at 37 °C.
Complete media prepared in plates : 30 days at 2-8 °C.
Complete media prepared in vials (*) : Use immediately.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media
500 g bottle BK055HA
5 kg drum BK055GC

Rabbit Plasma Fibrinogen Supplement
Package of 8 vials qsp 100 mL BS03408
Package of 1 vial qsp 500 mL BS03808

Kit (6 x 100 mL)
6 vials of 90 mL of base media and 6 RPF freeze-dried supplements BT00508

Kit (6 x 200 mL)
6 vials of 190 mL of base media and 6 RPF freeze-dried supplements (BS075) BT01008

Complete media, pre-poured in Petri dishes (Ø 90 mm)
20 plates BM06708

Complete media, pre-poured in Petri dishes (Ø 55 mm)
20 plates BM15908

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels 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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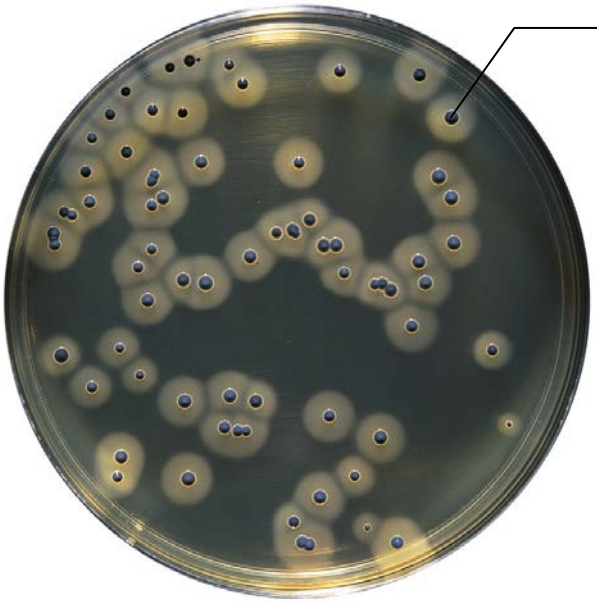
ANNEX 1 : PHOTO SUPPORT

BAIRD-PARKER RPF Agar

Enumeration and confirmation of coagulase positive staphylococci.

Results :

Growth obtained after 24 hours of incubation at 37°C.



Staphylococcus aureus

Characteristic colonies : Gray to black colonies surrounded by an opaque fibrin halo.