TECHNICAL DATA SHEET

EASY *STAPH* AGAR

ENUMERATION OF COAGULASE POSITIVE *STAPHYLOCOCCI*

1 INTENDED USE

EASY *STAPH* is an alternative method for the enumeration of coagulase positive *Staphylococci* in food products, in animal feeds and in environmental samples. Devoid of the need for confirmation, the method allows count of pathogenic *Staphylococci* in 22 hours instead of the usual 48 hours in the case of the standardized method NF EN ISO 6888-2.
The medium support the use of surface inoculation, in depth or by the Spiral method.
This method is certified NF VALIDATION according to the validation protocol EN ISO 16140-2:2016, for all food products and for industrial production environmental samples.

BKR 23/10 - 12/15
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification **http://nf-validation.afnor.org**

Refer to the certificate available on the NF VALIDATION website for the end of validity date of the method.

The reference method used for the validation is the standard NF EN ISO 6888-2: 2021.

2 PRINCIPLES

The base medium has been specially formulated to allow the development of coagulase positive *Staphylococcus* in 22 hours.

Optimization of the selective system allows an improvement in the inhibition of secondary flora that are commonly found on Baird Parker plates.

The freeze-dried EASY STAPH supplement is an optimized Rabbit Plasma Fibrinogen supplement. The composition has been adjusted to favor the development of fibrin halo in 22 hours.

Rabbit plasma was chosen for its excellent specificity for staphylococcal coagulase and its ability to rapidly product a clot by forming staphylothrombin from prothrombin. It is reinforced in bovine fibrinogen. The staphylothrombin acts by cutting the fibrinopeptides A & B of fibrinogen, which initiates a polymerization process that terminates in the appearance of a fibrin halo around the colonies.

A trypsin inhibitor extracted from soy avoids lysis of the fibrin halos.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 L of complete medium

- Peptones ..... 19.4 g
- Activators ..... 21.7 g
- Buffering system ..... 1.4 g
- Selective system ..... 4.5 g
- Bacteriological agar ..... 14.4 g
- Bovine fibrinogen ..... 5.3 g
- Rabbit plasma, EDTA ..... 25 mL
- Trypsin inhibitor ..... 25 mg
- Potassium tellurite ..... 25 mg

pH of the complete ready-to-use medium at 25 °C: 7.3 ± 0.3.

For 68.2 g of dehydrated base (BK216)

- Peptones ..... 21.6 g
- Activators ..... 24.1 g
- Buffering system ..... 1.5 g
- Selective system ..... 5.0 g
- Bacteriological agar ..... 16.0 g

For one vial of supplement BS090
(Qs 100 mL)

- Bovine fibrinogen ..... 0.53 g
- Rabbit plasma, EDTA ..... 2.5 mL
- Trypsin inhibitor ..... 2.5 mg
- Potassium tellurite ..... 2.5 mg

For 190 mL of ready-to-use base (BM185) / kit BT012

- Peptones ..... 4.3 g
- Activators ..... 4.8 g
- Buffering system ..... 0.3 g
- Selective system ..... 1.0 g
- Bacteriological agar ..... 3.2 g

For one vial of supplement (BS086) / kit BT012

- Bovine fibrinogen ..... 1.06 g
- Rabbit plasma, EDTA ..... 5 mL
- Trypsin inhibitor ..... 5 mg
- Potassium tellurite ..... 5 mg

4 PREPARATION

Preparation from dehydrated medium:

- Dissolve 68.2 g of dehydrated medium (BK216) in 1 L of distilled or demineralized water.
- Slowly bring to boiling with constant agitation and maintain throughout the time needed to achieve complete dissolution.
- Divide into vials, 90 mL, or multiples of 90 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain in a molten state at 44-47 °C.

✓ **Reconstitution:**
68.2 g/L

✓ **Sterilization:**
15 min at 121 °C

Rehydration of freeze-dried supplements

- Rehydrate the lyophilisate by adding aseptically a volume of sterile distilled water as shown in the following table:
  - Qsp 100 mL of complete medium (BS090): 10 mL of sterile water
  - Qsp 200 mL of complete medium, **specific to the BT012 kit** (BS086): 10 mL of sterile water
  - Qsp 500 mL of complete medium (BS097): 50 mL of sterile water

- Dissolution will take place quicker if warm water is used for the preparation (use room temperature water or water that has been slightly heated, not going over 44°C).
- Mix the vial well to achieve complete dissolution of the supplement, while avoiding the formation of foam. The dissolution may not occur immediately, and the process can be accelerated by using a Vortex mixer. The product must be completely dissolved before use.

Utilization of the kit BT01208 (Qsp 200 mL)

- Melt the solidified medium provided in the kit for the minimum amount of time necessary to achieve total liquefaction. Cool and maintain in a molten state at 44-47 °C.
- Dissolve the lyophilisate with 10 mL of sterile water.
- Add aseptically 10 mL of the specific supplement EASY *STAPH* (BS086) to 190 mL of base medium (BM185).
- Use immediately after preparation, for the inoculation of plates using a pour plate method or pour into empty, sterile Petri plates for use with surface inoculation.

Utilization of the kit BT01308 (Qsp 100 mL)

- Melt the solidified medium provided in the kit for the minimum amount of time necessary to achieve total liquefaction. Cool and maintain in a molten state at 44-47 °C.
- Dissolve the lyophilisate with 10 mL of sterile water.
- Add aseptically 10 mL of the specific EASY *STAPH* supplement (BS091) to 90 mL of base medium (BM189).
- Use immediately after preparation, for the inoculation of plates by pour plate method or pouring into empty, sterile Petri plates for surface inoculation.

NOTE:
The complete medium cannot be held for an extended period at 44-47 °C.

5 INSTRUCTIONS FOR USE

Respect good laboratory practices.

Refer to NF EN ISO 7218 for plating, colony counting and for calculations and expression of results.

Prepare the initial suspension of the sample and the decimal dilutions according to the rules defined in the corresponding ISO 6887 and/or ISO 18593 standard.

Pour plate inoculation

- Transfer 1 mL of the inoculum and its serial dilutions to empty, sterile 90 mm Petri plates.
- Pour roughly 15 mL of complete medium previously prepared.
- Mix well.
- Let solidify on a cool, flat surface.
- Incubate at 37 ± 1 °C for 24 ± 2 hours.

✓ **Inoculation:**
1 mL pour plates

✓ **Incubation:**
24 h at 37°C

When low numbers are expected, refer to ISO 7218.

Surface inoculation

- On the surface of prepared plates or if using the pre-poured medium (BM187) brought to room termperature, transfer 0.1 mL of the sample to test and its serial dilutions.
- Spread across the surface of the plate with a sterile bent glass rod or “hockey stick”.
- Incubate at 37 ± 1 °C for 24 ± 2 hours.

✓ **Inoculation:**
0.1 mL on surface

✓ **Incubation:**
24 h at 37 ± 1 °C

NOTES:

- The method is validated also with the Spiral inoculation technology. The inoculation can be in logarithmic mode from 50 or 100 µL.

- The counting limits can be decreased by a factor of 10 by inoculating 1.0 mL of the sample or initial suspension to the surface of three 90 mm plates.

- The incubation time is 24 ± 2 hours, however, for reasons of laboratory management and organization, plates can be incubated
  up to 72 hours using the Spiral technology,
  up to 48 hours using manual surface inoculation.

- For surface samples after cleaning, which may contain disinfectant residues, it is recommended to use swabs, sponges or wipes already soaked in neutralizing solution, or to use a diluent containing 10% universal neutralizers.

6 RESULTS

Coagulase positive staphylococci are characterized on the surface as having formed white, grey, or black colonies surrounded by an opaque halo of fibrin that is clear, stable, and clearly visible.

Only count the plates containing less than 100 characteristic colonies.

See ANNEX 1: PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated base media: white/cream powder, free-flowing and homogeneous.
EASY *STAPH* Supplements: white to pinkish pellet, giving after reconstitution a solution that is amber, limpid and slightly opaque.
Complete, prepared medium: amber agar.

Typical culture response after 24h incubation at 37 °C

Microorganisms		Growth (Productivity ration: P_R)	Characteristics
Staphylococcus aureus	WDCM 00034	$P_R \geq 50$ %	Colonies with an opaque halo
Staphylococcus saprophyticus	WDCM 00159	Slowed, score 0-1	Colonies without opaque halo
Escherichia coli	WDCM 00013	Inhibited, score 0	-

8 STORAGE / SHELF LIFE

Dehydrated base media: 2-30 °C.
EASY *STAPH* Supplements: 2-8 °C.
Kits: 2-8 °C.
Pre-poured media in plates: 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base medium in vials (*): 6 months at 2-8 °C.
Reconstituted freeze-dried supplement (*): 8 days at 2-8 °C. Reheat to between 25 and 37 °C before use. The white precipitate observed at 2-8 °C will disappear when heated to between 25 and 37°C.
Prepared, complete medium in plates (*): 1 month at 2-8 °C, shielded from light.
Prepared, complete medium in vials (*): Use immediately.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated base media
500 g bottle..... BK216HA
5 kg drum..... BK216GC

EASY *STAPH* Supplements
8 vials qsf 100 mL..... BS09008
1 vial qsf 500 mL BS09708

Kit (6 x 200 mL)
6 x 190 mL vials of base medium plus 6 freeze-dried supplements..... BT01208

Kit (6 x 100 mL)
6 x 90 mL vials de base medium plus 6 freeze-dried supplements..... BT01308

Pre-poured, complete media in Petri plates (Ø 90 mm)
20 plates..... BM18708
120 plates..... BM19008

10 BIBLIOGRAPHY

Baird-Parker, A.C .. 1962. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. Journal of Applied Bacteriology, **25** : 12-19.

Hauschild, A.H., Park, C.E. and Hilsheimer, R .. 1979. A modified pork plasma agar for the enumeration of *Staphylococcus aureus* in foods. Canadian Journal of Microbiology, **25** : 1052-1057.

Beckers, H.J., van Leusden, F.M., Bindschedler, O. and Guerraz, D 1984. Evaluation of a pour-plate system with a rabbit plasma-bovine fibrinogen agar for the enumeration of *Staphylococcus aureus* in food. Canadian Journal of Microbiology, **30** : 470-474.

NF EN ISO 6888-2. September 2021. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: Technique using rabbit plasma fibrinogen agar medium. Modified by amendment A1 in September 2023.

NF EN ISO 7218. July 2024. Microbiology of the food chain — General requirements and guidance for microbiological examinations.

NF EN ISO 18593. July 2018. Microbiology of the food chain — Horizontal methods for surface sampling.

NF EN ISO 16140-2. Septembre 2016. Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

11 ADDITIONAL INFORMATION

Document code : EASY *STAPH*_V7 (en)
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ANNEX 1: PHOTO SUPPORT

EASY *STAPH* Agar

Enumeration of coagulase positive *Staphylococci*.

Results:

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