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.

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 of 1999 and its amendment A1 of 2003.

2 PRINCIPLES

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

Optimization of the selective system has allowed an improvement in the inhibition of secondary flora that are traditionally found on Baird Parker plates.

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

Rabbi plasma was chosen in light of its excellent specificity towards staphylococcal coagulase and its ability to rapidly form a clot with 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 ± 0,2.

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 per vial, or into multiples of 90 mL.
- 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 in order 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 lyophilsate 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 lyophilsate with 10 mL of sterile water.
- Add aseptically 10 mL of the specific supplement EASY *STAPH* (BS091) to 90 mL of base medium (BM189).
- 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.

NOTE:

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

5 INSTRUCTIONS FOR USE

Respect all 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 standards.

Pour plate inoculation

- Transfer 1 mL of the inoculum and its serial dilutions to empty, sterile 90mm Petri plates.
- Pour roughly 15 mL of complete medium previously prepared and held at 44°C.
- 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

NOTE : For laboratory management reasons, plates can be incubated for up to 72 hours.

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 90mm plates.
- For reasons of laboratory management and organization, plates can be incubated up to 72 hours using the Spiral technology and up to 48 hours using manual surface inoculation.

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 net, stable and well visible.

See ANNEX 1: PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated base medium : white/cream powder, free-flowing and homogeneous.
Supplement EASY *STAPH* : white to pink pellet, giving rise after reconstitution to 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 aureus	WDCM 00032	$P_R \geq 50$ %	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 medium : 2-30 °C.
Supplements EASY *STAPH* : 2-8 °C.
Kits : 2-8 °C.
Pre-poured medium 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 medium
500 g bottle ---------- BK216HA
5 kg drum ---------- BK216GC

EASY *STAPH* Supplement
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 medium in Petri plates (Ø 90 mm)
20 plates ---------- BM18708

Pre-poured, complete medium in Petri plates (Ø 90 mm)
120 plates ---------- BM19008

10 BIBLIOGRAPHY

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NF EN ISO 6888-2. October 1999. 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.

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*_V5
Creation date : 12-2015

Updated : 02-2021
Origin of revision : Addition of enumeration record according to NF EN ISO 7218.

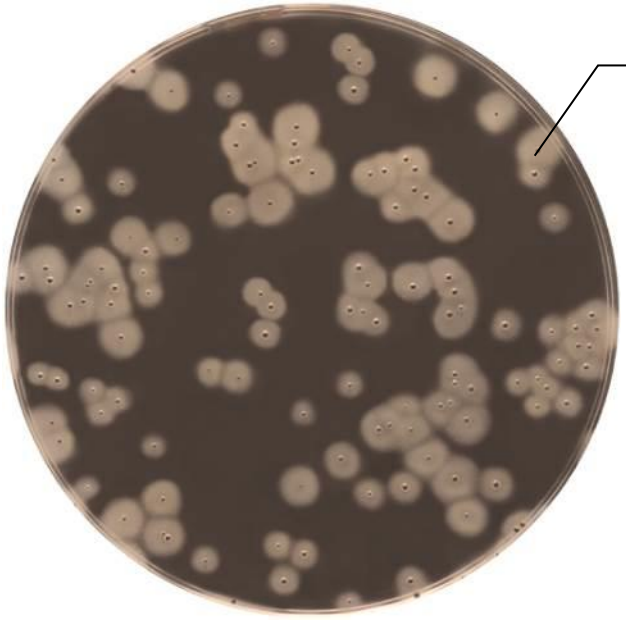
ANNEX 1 : PHOTO SUPPORT

EASY *STAPH* Agar

Enumeration of coagulase positive *Staphylococci*.

Results :

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



Staphylococcus aureus

Characteristic colonies:
surrounded by a halo of fibrin