RAPID'*Staph*/Agar

356-4704
356-3960

DEFINITION

Medium used for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species) at 37°C **in 24h** in products intended for human or animal consumption and environmental samples. Positive results can be confirmed with a Pastorex™ *Staph* Plus latex test or on a pre-poured Baird Parker + RPF agar plate.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*Staph* method has been certified NF VALIDATION for the enumeration of coagulase positive Staphylococci (*Staphylococcus aureus* and other species) for **all food products destined for human and animal consumption and for environmental samples,** according to NF EN ISO 16140.

BRD 07/09-02/05
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

- End of NF VALIDATION: please see the certificate BRD 07/09 - 02/05 . This certificate is available from Bio-Rad representative or AFNOR Certification.

AOAC-RI VALIDATION

RAPID'*Staph* is validated by the AOAC Research Institute under the "Performance Methods Tested" status, under **Certificate n° 080602.**

STANDARDS

FOOD MICROBIOLOGY

• U.S. Department of Health and humanServices U.S. Food and Drug

Administration Center for Food Safety & Applied Nutrition - Bacteriological, Analytical, on-line Manual, January 2003.

• NF EN ISO 6888-1 (October 1999): Food microbiology - Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species)

- Part 1: Technique using Baird-Parker agar medium. (IC: V08-014-1).

• NF EN ISO 6888-3 (March 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.

PRINCIPLE

The RAPID'*Staph* medium is based on a Baird Parker formula optimized for the detection and enumeration of *Staphylococcus aureus* in 24h.
The principle of the medium relies on the capacity of *Staphylococcus aureus* to reduce tellurite (black colonies) and provoke proteolysis of egg yolk (clear halo around colonies).

PRESENTATION

• Base
Dehydrated
500 g **code 356-4704**

• Complete
Pre-poured
90 mm x 20 dishes **code 356-3960**

STORAGE

- Pre-poured : + 2° to 8°C.
- Dehydrated : + 15° to 25°C, 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° to 8°C, in a dark place

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RAPID'*Staph*Agar

THEORETICAL FORMULA

Peptone	10 - 20 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

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- **Egg yolk with potassium tellurite**
  5 ml x 1 vial **code 355-4201**
  25 ml x 1 flask **code 355-4205**

- **Pastorex™ *Staph* Plus**
  50 tests **code 355-6356**
  50 tests x 5 **code 355-6353**

- **Baird Parker +RPF**
  Dehydrated (non-supplemented base)
  500 g **code 356-4814**
  RPF supplement
  10 lyophilized vials
  code 356-46 18
  Ready-to-use
  90 ml x 6 vials Baird Parker Base
  + 6 lyophilized supplements
  code 357-8618
  Pre-poured
  90 mm x 20 **code 356-3996**
  See corresponding Technical Sheet(s).

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)
- Sterile pipettes (0,1 ml, 1 ml, etc)
- Sterile spreaders
- Water-bath, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

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 : 57 g/l

500 g of powder makes 8.7 liters of medium.

PREPARATION OF COMPLETE MEDIUM

Using dehydrated medium:

- At the moment of use, add the following solutions to 90 ml of this base, previously melted and cooled to between 44° and 47°C:
  - 5 ml egg yolk with potassium tellurite
  - Mix thoroughly
  - Pour into Petri dishes (thickness ~ 4 mm) and leave to solidify on a level surface.

PROTOCOL

Preparation of samples

According to the standards applicable to the product concerned.

Inoculation and incubation

- 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 h (± 2h).

If for some products it is necessary to proceed with the estimation of small numbers, spread 1 ml of SM over 3 dishes of Ø = 90 mm (~ 0.33 ml/dish) or over 1 dish of Ø = 140 mm.

READING AND INTERPRETATION

Counting/Confirmation of colonies (CFU)

After incubation, counting the 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).

In some rare cases, some *Staphylococcus aureus* do not give characteristic colonies.

From the plates, collect 3 typical colonies and confirm :

- On Latex PASTOREX® STAPH+ (**codes 355-6356** and **355-6353**)
- or spot collected colonies on pre-poured Baird Parker + RPF agar. You can confirm up

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to 12 colonies on one plate of pre-poured Baird Parker + RPF agar (**code 356-4814**, **code 357-8613** and **code 356-3996**) *(see corresponding Technical Sheet(s)).*

Expression of results/Calculations
For calculation method, refer to standard NF 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 45 minutes.
- Do not add the egg yolk to the potassium tellurite, sodium pyruvate and LGlycine in a base medium at a temperature exceeding 47°C.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains.

STRAINS	Results after 24-48H 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 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 T.C.S. agar.**

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.

KEY WORDS

RAPID'*Staph* / *Staphylococcus aureus* / Food product / Enumeration / Coagulase / Medium.

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