RAPID'*Salmonella*Agar

356-3961
356-3963
356-4705

DEFINITION

RAPID'*Salmonella* agar is a chromogenic medium used for the detection of *Salmonella* spp. in the analysis of food products for human, animal consumption and in environmental samples.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*Salmonella* method has been certified NF VALIDATION as alternative to reference method NF EN ISO 6579, according to the ISO 16140 protocol, for the **detection of *Salmonella* spp.** in all food products for human and animal consumption (short protocol and double enrichment protocol) and in environmental samples (Short protocol only and primary production stage samples excluded).

BRD: 07/11–12/05
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
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AOAC-RI VALIDATION

The RAPID'*Salmonella* method is validated by the AOAC Research Institute under the "Performance Tested Methods" status, under **Certificate n° 050701.**

NORDVAL VALIDATION

The RAPID'*Salmonella* method (short protocol and double enrichment protocol but with RVS enrichment time 24h±2 only) is NordVal validated as an alternative method to the reference standard EN ISO 6579, according to the ISO 16140 protocol, for the **detection of *Salmonella* spp.** in all food products for human, animal consumption and in environmental samples (Short protocol only and primary production stage samples excluded).

STANDARDS

FOOD MICROBIOLOGY

• **ISO 6579 (July 2002):** Food microbiology - Horizontal method for the detection of *Salmonella* spp.

PRINCIPLE

RAPID'*Salmonella* agar allows the presumptive identification of *Salmonella* spp., by detecting C8-esterase activity. Simultaneous screening of β-glucosidase activity permits the differentiation of salmonella colonies from those of other enterobacteria.

After incubation, salmonella appear as readily identifiable typical magenta colonies whereas non-salmonella grow as blue or non colored colonies.

RAPID'*Salmonella* agar permits detection of motile and non-motile salmonella, as well as lactose-positive *Salmonella, Salmonella* Typhi and *Salmonella* Paratyphi.

PRESENTATION

• **Pre-poured**

90 mm x 20 dishes	**code 356-3961**
90 mm x 120 dishes	**code 356-3963**

• **Dehydrated**

500g	**code 356-4705**

STORAGE

• Pre-poured: at + 2° to 8°C in a dark place.
• Dehydrated: at + 2° to 8°C, in carefully sealed bottles in a cool, dry place.
• Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Nutritive Mix	14.5 g
Selective Agents	14 g
Chromogenic Mix	2.3 g
Agar	12.7 g
Distilled water qsp	1000 mL

Final pH = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

• Buffered Peptone Water : 6 vials of 225 ml (ex. code **355-4179**), 500 g (eg. code **356-4684**),5 bags of 2,3 L (eg. code **355-5789**) 2 bags of 5 l (eg. code **355-5790**)

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RAPID'*Salmonella*Agar

Selective supplement:
• 100 RAPID'*Salmonella* capsules (code **356-4710**), 100 x Quantity for 250 ml.
• 100 RAPID'*Salmonella* capsules - 10 times concentrated (code **356-4709),** 100 x QSP 2.5 Litres
• RAPID'*Salmonella* Supplement - (code **356-4712),** 1 x QSP 100 analyses

EQUIPMENT REQUIRED (NOT SUPPLIED)

(Non exhaustive list)
- Scales
- Sterile weighing bags
- Stomacher
- Hotplate
- Magnetic stirrer
- Sterile Petri dishes (∅ = 90mm)
- Sterile Pasteur pipettes or inoculating loops
- Water-bath
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

"Always shake well before use"

Dissolve 43.5 g of powder in 1 litre of distilled water and mix until a homogenous suspension is obtained.
Heat gently, agitating frequently, then brings to the boil for less than 1 minute.
DO NOT PROLONG HEATING.
DO NOT AUTOCLAVE.
Cool down the medium to 50°C.
Dispense in Petri dishes and leave to dry.

Reconstitution ratio: 43.5 g / litre
500g of powder make 11.5 litres of medium.

PROTOCOLS

STANDARD METHOD NF EN ISO 6579

Preparation of samples
According to standards for the product concerned.

Enrichment
According to standards for the product concerned.

Inoculation and incubation
Streak in the usual way 10 µL of the enrichment broth at end of incubation onto RAPID'*Salmonella* agar and X.L.D. agar (codes **354-1751** and **356-9124**).
Incubate at 37°C ± 1°C for 24 ± 3 hours for X.L.D. agar and 24 ± 2 hours for RAPID'*Salmonella* agar.

Reading
Salmonella form magenta colonies on RAPID'*Salmonella* agar plate.

NF VALIDATION CERTIFIED RAPID'*SALMONELLA* METHODS

▪ RAPID'*Salmonella* method - Short protocol
Preparation of samples
Dilute η g or η mL of sample in 9 x η mL of Buffered Peptone Water (eg. Codes **355-4179**, **355-5789**, **356-4684** and **355-5790**).
e.g.: dilute 25 g or 25 mL of sample in 225 mL of Buffered Peptone Water broth to obtain a 1/10 dilution.
Specific preparations (cocoa, acid foods,…) are described in ISO 6579 standard.
Homogenise with an agitator like a Stomacher.
Open a RAPID'*Salmonella* Capsule (code **356-4710**) and pour its content directly in the broth.
Homogenize by strong shaking.

Note: Either the whole capsule or its contents only can be added before the stomacher phase. In order to make handling easier, we recommend opening the capsule and pouring out the contents (see PRECAUTIONS FOR USE).

Note: In the context of NF VALIDATION mark, no samples of over 25 grams were tested.

Sample preparation with addition of the selective supplement as a concentrated solution, to the enrichment broth

The capsule contents and RAPID'*Salmonella* Supplement boxes (code **356-4712)** can be diluted in Buffered Peptone Water first or Sterile Distilled Water for incorporation in liquid form.

- Dilute η g or η mL of the sample in 9 x η ml of Buffered Peptone Water. (ex. codes **355-4179**, **355-5789**, **356-4684 and 355-5790**).
- Homogenise in a Stomacher blender.

- Where RAPID'*Salmonella* QSP 250 ml capsules (code **356-4710**) are used: Open n capsules and pour the contents directly into n x 10 ml Buffered Peptone Water to obtain a concentrated supplement solution.
Add η x 0.4ml of the concentrated supplement solution to the sample to be analysed.
Homogenise by agitating vigorously.

- Where RAPID'*Salmonella* QSP 2.5 Litre capsules are used (code **356-4709**): Open n

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capsules and pour their contents directly into an empty recipient. Fill with n x 10 ml Buffered Peptone Water or with n x 10 ml of Sterile Distilled Water.
Homogenise by agitating vigorously to obtain a red concentrated solution.
Add η x 0.04ml of the concentrated supplement solution to the sample diluted in the Buffered Peptone Water.
Homogenise by agitating vigorously.

- Where RAPID'*Salmonella* Supplement - (code **356-4712),** 1 x QSP 100 analyses are used: Open the box and fill with 100 ml of Buffered Peptone Water or Sterile Distilled Water. Homogenise by agitating vigorously to obtain a red concentrated solution.
Add η x 0.04ml of the concentrated supplement solution to the sample diluted in the Buffered Peptone Water.
Homogenise by agitating vigorously.

Example for a 10g sample:
- Dilute the 10g sample in 90 ml Buffered Peptone Water.
- Homogenise using a Stomacher type blender.
- Dilute 1 RAPID'*Salmonella* QSP 250 ml capsule (code 356-4710) in 10 ml Buffered Peptone Water to obtain a concentrated supplement solution
- Add 4 ml of concentrated supplement solution to the 90 ml Buffered Peptone Water diluent + sample in order to achieve the correct capsule dilution ratio.

N.B.: The concentrated solution, once reconstituted with Buffered Peptone Water or Sterile Distilled Water can be stored for 1 week at ambient temperature, or at +2-8°C.

Note: In the context of NF VALIDATION mark, no samples of over 25 grams were tested.

Enrichment
Incubate at 41.5°C ± 1°C for 18 ± 2 hours.

Note: After incubation, the enrichment broth may be stored in a refrigerator (2°-8°C) for 72 hours, before inoculating RAPID'*Salmonella*.

Inoculation and incubation
Using a sterile loop, collect 10 µL of enrichment broth at end of incubation and inoculate RAPID'*Salmonella* plate by streaking in the usual way.
Incubate at 37°C ± 1°C for 24 ± 2 hours.

Reading
Salmonella form magenta colonies on RAPID'*Salmonella* agar plate.

▪ RAPID'*Salmonella* method – Double enrichment protocol

Preparation of samples
Dilute η g or η mL of sample in 9 x η mL of Buffered Peptone Water (eg. Codes **355-4179**, **355-5789**, **356-4684** and **355-5790**).
e.g.: dilute 25 g or 25 mL of sample in 225 mL of Buffered Peptone Water broth to obtain a 1/10 dilution.
Specific preparations (cocoa, acid foods,…) are described in ISO 6579 standard.
Homogenise with an agitator like a Stomacher.

Note: In the context of NF VALIDATION mark, no samples of over 25 grams were tested.

Enrichment
Incubate at 37°C ± 1°C for 18 ± 2 hours.

Transfer 0,5 mL of culture from the non-selective enrichment to 10 mL of RVS broth (eg. codes **356-4324** and **355-5773**), previously brought to incubation temperature.
Incubate at 41,5°C ± 1°C for 6 to 26 hours.
In the case of the NF VALIDATION certified method, enrichment in RVS broth takes:
- 6 hours to 26 hours for seafood products, vegetables, dairy products and egg products
- 24 ± 2 hours for meat products and animal feedstuffs.

Inoculation and incubation
Using a sterile loop, collect 10 µL of enrichment broth at end of incubation and inoculate RAPID'*Salmonella* plate by streaking in the usual way.
Incubate at 37°C ± 1°C for 24 ± 2 hours.

Reading
Salmonella form magenta colonies on RAPID'*Salmonella* agar plate.

CONFIRMATION OF POSITIVE RESULTS

In the context of the NF VALIDATION mark, all samples identified as positive must be confirmed in one of the following ways:

- Using the conventional tests described in the standardized methods by CEN or ISO (including the purification step)
- Using nucleic probes as described in ISO 7218 standard (eg. iQ-Check™ *Salmonella*

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II, code **357-8123)** using isolated colonies (with or without purification step).

- Evaluation of oxidase activity (oxidase test, code **355-3834**), followed by omnivalent Omni-O test (A60) (code **356-0781**) using 1 to 3 isolated suspect colonies. If reaction is positive to the Omni-O test, proceed with an ONPG biochemical test (code **355-3822**). Salmonella are negative to oxidase test, positive to Omni-O test (A60) and negative to ONPG test, with the exception of lactose-positive *salmonella* which are ONPG+.

- Performing a latex agglutination test : SALMONELLA LATEX (code **355-6710**) test on an isolated colony. *Salmonella* of groups B to E and G are positive to the latex test, or performing a Salmonella Confirm Latex test, using an isolated colony (code **355-6711**). Oxoid Salmonella Latex test was also validated.

- Use of **any other NF VALIDATION certified method based on a different principle** from that of RAPID'*Salmonella.* The validated protocol of the second method must be respected in its entirety, i.e. all steps preceding the intermediary stage used as departure point for confirmation must be common to both methods.

In the event of discordant results (presumptive positive with RAPID'*Salmonella method, negative with confirmation method and especially by the Latex test) the laboratory must follow the necessary steps to ensure validity of the result obtained. .*

LIMITATION OF USE

• ONPG confirmation test excludes confirmation of lactose positive *Salmonella*.
• Although the most prevalent *Salmonella* strains can be detected by Salmonella Confirm Latex kit, it must be noted that during the NF VALIDATION extension of RAPID'*Salmonella* method, Salmonella Confirm Latex kit not allowed the detection of 41 of the 150 tested strains.
• Some strains of *Salmonella* (a few are part of Dublin serovar and *S. Bongori* specie) can show a weak magenta color due to a low esterase activity.

PRECAUTIONS

- Respect of Good Laboratory Practice (eg. EN ISO 7218).
- If the whole capsule is added to the Buffered Peptone Water, sterile tweezers must be used to add the capsule to the bag. We recommend that you check that the capsule actually opened during the stomaching stage.
- If the capsule contents are handled with the fingers, it cannot be added to the enrichment broth due to the risk of contamination.
- The RAPID'*Salmonella* capsule and RAPID'*Salmonella* Supplement - (code **356-4712)** contain selective agents and an excipient. Selective agents dissolve very well. The excipient however, remains in suspension and may create a deposit when the contents of the capsule are diluted in a small quantity of Buffered Peptone Water or Sterile Distilled Water. Always shake well therefore before using the concentrated solution.
- End of NF VALIDATION: please see the certificate BRD: 07/11–12/05. This certificate is available from Bio-Rad representative or AFNOR Certification

REMARKS

As with all chromogenic media, it is essential to **carry out streak inoculation** so as to obtain correctly isolated colonies.

QUALITY CONTROL

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.

STRAINS	Results after 24 hours culture at 37°C
Salmonella Enteritidis ATCC 13076	Magenta colonies
Salmonella Typhimurium ATCC 14028	Magenta colonies
Escherichia coli ATCC 25922	Partial or total inhibition Colourless colonies
Enterococcus faecalis ATCC 19433	Total inhibition

KEY WORDS

RAPID'*Salmonella* / *Salmonella* / Food products / Detection / Enumeration / Chromogenic / Medium.

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