INTERNATIONAL STANDARD

ISO 6579-1

First edition
2017-02

AMENDMENT 1
2020-03

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1: Detection of *Salmonella* spp.

AMENDMENT 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella —

Partie 1: Recherche des Salmonella *spp.*

AMENDEMENT 1: Extension de la plage de températures pour l'incubation, amendement du statut de l'Annexe D, et correction de la composition des milieux MSRV et SC

Reference number
ISO 6579-1:2017/Amd.1:2020(E)

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Published in Switzerland

Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1: **Detection of *Salmonella* spp.**

AMENDMENT 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC

Foreword, fifth bullet

Replace the bullet with the following:

— The temperature range for incubation of non-selective and selective media has been extended from 37 °C ± 1 °C to 34 °C to 38 °C without further tolerance.

4.3, second paragraph

Replace the paragraph with the following:

The RVS broth or the MSRV agar is incubated at 41,5 °C for 24 h and the MKTTn broth between 34 °C and 38 °C for 24 h.

4.4, last paragraph

Replace the paragraph with the following.

The XLD agar is incubated between 34 °C and 38 °C and examined after 24 h. The second selective agar is incubated according to the manufacturer's instructions.

6.3

Replace the text with the following:

6.3 Incubator, capable of operating in the range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.

6.6

Replace the text with the following:

6.6 Water bath, capable of operating in the range 34 °C to 38 °C.

NOTE The range 34 °C to 38 °C for incubation of media includes the use of water baths set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.

9.3.2, fifth paragraph

Replace the paragraph with the following:

Incubate the inoculated MKTTn broth between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

9.4.2, fifth paragraph

Replace the paragraph with the following:

Incubate the XLD plates inverted between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

9.4.3, second paragraph

Replace the paragraph with the following:

Incubate the XLD plates inverted between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

9.5.3.2, first paragraph

Replace the paragraph with the following:

Streak the agar slant surface and stab the butt. Incubate between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

9.5.3.3, first paragraph

Replace the paragraph with the following:

Streak the agar slant surface. Incubate between 34 °C and 38 °C (6.3) for up to 24 h.

9.5.3.4, first paragraph

Replace the paragraph with the following:

Inoculate just below the surface of the liquid medium. Incubate between 34 °C and 38 °C (6.3) for 24 h ± 3 h.

9.5.3.5, first line of fourth paragraph

Replace the first line of the fourth paragraph with the following:

Add one drop of toluene and shake the tube. Place the tube in a water bath and incubate between 34 °C and 38 °C (6.6) for several minutes (approximately 5 min).

9.5.3.5, fifth paragraph

Replace the paragraph with the following:

Replace the tube in the water bath and incubate between 34 °C and 38 °C (6.6) for up to 24 h.

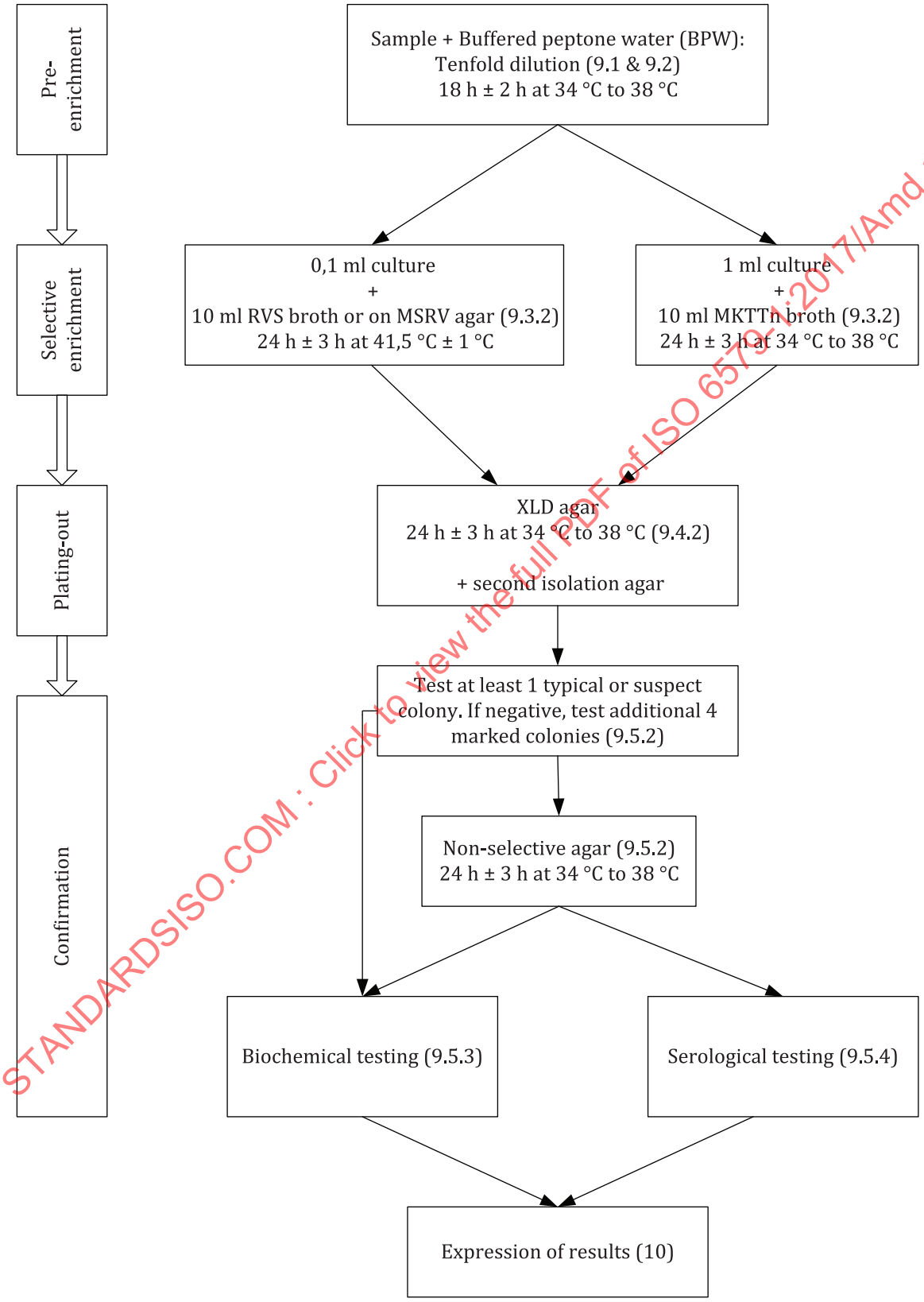
9.5.3.6, third paragraph

Replace the paragraph with the following:

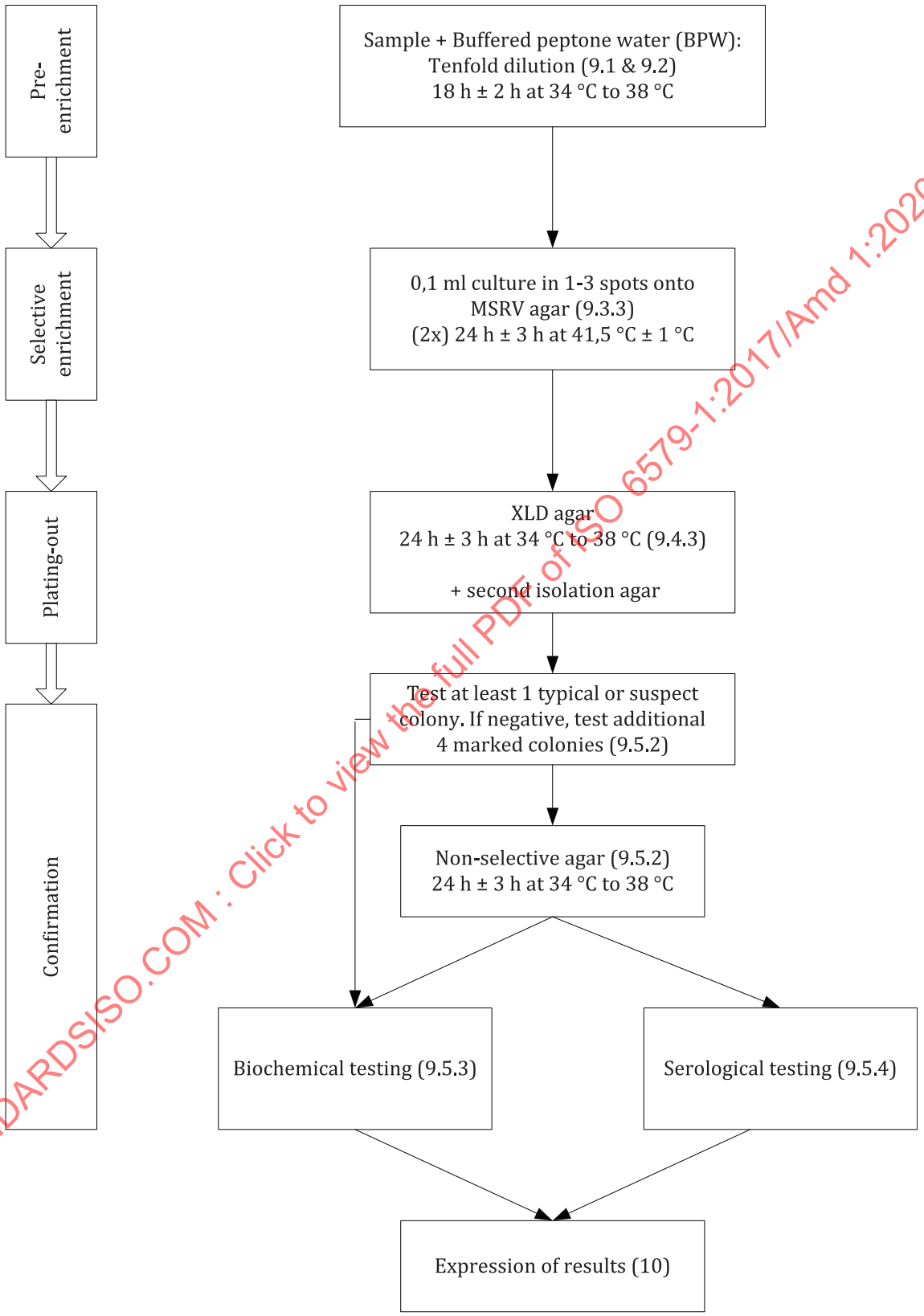
Incubate between 34 °C and 38 °C (6.3) for 24 h ± 3 h. After incubation, add 1 ml of the Kovacs reagent (B.12.2).

Annex A

Replace Figure A.1 with the following figure:



Replace Figure A.2 with the following figure:



Annex B, B.4

Replace the text with the following:

B.4 Modified semi-solid Rappaport-Vassiliadis (MSRV) agar

NOTE See Reference [12].

B.4.1 Solution A

B.4.1.1 Composition

Enzymatic digest of animal and plant tissue	5,0 g
Acid hydrolysate of casein	5,0 g
Sodium chloride	8,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,6 g
Water	1 000 ml

B.4.1.2 Preparation

Dissolve the components in the water by heating to about 70 °C, if necessary.

The solution shall be prepared on the day of preparation of the complete MSRV agar.

B.4.2 Solution B

B.4.2.1 Composition

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	400,0 g
Water	1 000 ml

B.4.2.2 Preparation

Dissolve the magnesium chloride in the water.

As this salt is very hygroscopic, it is advisable to dissolve the entire contents of $MgCl_2 \cdot 6H_2O$ from a newly opened container according to the formula. For instance, 250 g of $MgCl_2 \cdot 6H_2O$ is added to 625 ml of water giving a solution of total volume of 788 ml and a mass concentration of about 31,7 g per 100 ml of $MgCl_2 \cdot 6H_2O$.

The solution may be kept in a dark glass bottle with tight stopper at room temperature for at least two years.

B.4.3 Solution C

B.4.3.1 Composition

Malachite green oxalate	0,4 g
Water	100 ml

B.4.3.2 Preparation

Dissolve the malachite green oxalate in the water.

The solution may be kept in a dark glass bottle at room temperature for at least eight months.

B.4.4 Base medium

B.4.4.1 Composition

Solution A (B.4.1)	1 000 ml
Solution B (B.4.2)	80 ml
Solution C (B.4.3)	10 ml
Agar[a]	3,0 g

[a] It may be necessary to determine experimentally the concentration of agar needed for the optimal swarming of *Salmonella* (e.g. when using a batch of agar with unknown gel strength).

B.4.4.2 Preparation

Add to 1 000 ml of solution A, 80 ml of solution B, and 10 ml of solution C and mix by agitation.

Add and suspend the agar.

Adjust the pH, if necessary, so that after sterilization, it is 5,2 (5,1 to 5,4) at 20 °C to 25 °C.

Heat to boiling with agitation. **Do not autoclave.**

Do not hold the medium at high temperatures longer than necessary.

Cool the medium to 47 °C to 50 °C (6.5).

B.4.5 Novobiocin solution

B.4.5.1 Composition

Novobiocin sodium salt	0,05 g
Water	10 ml

B.4.5.2 Preparation

Dissolve the novobiocin sodium salt in the water.

Sterilize by filtration through a filter with a pore size of 0,2 µm.

The solution may be stored for up to four weeks at 5 °C (6.8) or in small portions (e.g. of 2 ml) at –20 °C (6.9) for up to one year.

B.4.6 Complete medium

B.4.6.1 Composition

Base medium (B.4.4)	1 090 ml
Novobiocin solution (B.4.5)	2,2 ml

B.4.6.2 Preparation

Aseptically, add 2,2 ml of the novobiocin solution (B.4.5) to 1 090 ml of base medium (B.4.4) at 47 °C to 50 °C (6.5). Mix carefully. The final concentration of novobiocin in the complete medium is 10 mg/l.

The final pH shall be 5,2 (5,1 to 5,4) at 20 °C to 25 °C.

Pour the medium into sterile Petri dishes (6.14) up to a volume of 15 ml to 20 ml in dishes with a diameter of 90 mm.

Allow the medium to solidify before moving and handle with care.

Store the plates, **with surface upwards**, and protected from drying for up to two weeks at 5 °C (6.8) in the dark.

Do not invert the plates as the semi-solid agar is too liquid to do so.

Any plates in which the semi-solid agar has liquefied or fragmented shall not be used.

Immediately, before use and only if visible moisture is apparent, dry the surface of the agar plates carefully, for example, by placing them with the lids off and the agar surface **upwards** in a laminar air flow cabinet. Take care not to overdry the medium.

NOTE 1 The composition of MSRV agar, as described in Reference [12], contains 20 mg/l of novobiocin. However, from a scientific point of view, 10 mg/l novobiocin is preferred. Studies have shown larger migration zones on MSRV agar with a lower concentration of novobiocin[23] and the (negative) influence of novobiocin on bacterial motility[22].

NOTE 2 The final medium composition is enzymatic digest of animal and plant tissue 4,6 g/l, acid hydrolysate of casein 4,6 g/l, sodium chloride (NaCl) 7,3 g/l, potassium dihydrogen phosphate (KH_2PO_4) 1,5 g/l, anhydrous magnesium chloride ($MgCl_2$) 10,9 g/l, or magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) 23,3 g/l, malachite green oxalate 0,04 g/l, novobiocin sodium salt 0,01 g/l, and agar 2,7 g/l.

B.15, Table B.1

Replace the third row of Table B.1 with the following:

MKTTn broth	Productivity	24 h ± 3 h/ 34 °C to 38 °C	*Salmonella* Typhimurium[c,d] *Salmonella* Enteritidis[c,d] + *Escherichia coli*[d] + *Pseudomonas aeruginosa*	00031 00030 00012 or 00013 00025	> 10 characteristic colonies on XLD agar or other medium of choice
	Selectivity		*Escherichia coli*[d]	00012 or 00013	Partial inhibition ≤ 100 colonies on TSA
			Enterococcus faecalis[d]	00009 or 00087	< 10 colonies on TSA

B.15, Table B.1

Replace the sixth row of Table B.1 with the following:

XLD agar	Productivity	24 h ± 3 h/ 34 °C to 38 °C	*Salmonella* Typhimurium[c,d] *Salmonella* Enteritidis[c,d]	00031 00030	Good growth (2) of colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
	Selectivity		*Escherichia coli*[d]	00012 or 00013	Growth or partial inhibition (0–1) of yellow colonies
			Enterococcus faecalis[d]	00009 or 00087	Total inhibition (0)