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

Part 3: **Guidelines for serotyping of *Salmonella* spp.**

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

Partie 3: Lignes directrices pour la sérotypie des Salmonella *spp.*

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 6579 consists of the following parts, under the general title *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella*:

— *Part 1: Horizontal method for the detection of* Salmonella *spp.*[1)]

— *Part 2: Enumeration by a miniaturized most probable number technique* [Technical Specification][2)]

— *Part 3: Guidelines for serotyping of* Salmonella *spp.* [Technical Report]

1) Under preparation. (Revision of ISO 6579:2002)

2) The main element of the series title has been changed since Part 2 was published. It is intended that upon revision, the main element of the title will be aligned with Part 3.

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Introduction

This part of ISO 6579 gives information on the taxonomy of *Salmonella* spp. and it gives guidance on serotyping of *Salmonella* serovars, based on the White–Kauffmann–Le Minor scheme (see Reference [9]).

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

Part 3:
Guidelines for serotyping of *Salmonella* spp.

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting and typing *Salmonella*, be undertaken only in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials.

IMPORTANT — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 6579 gives guidance on the procedure for serotyping *Salmonella* serovars and is applicable to the serotyping of pure cultures of *Salmonella* spp., independent of the source from which they are isolated.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6579-1, *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Horizontal method for the detection of Salmonella spp.*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
Salmonella
gram-negative, oxidase-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria which generally form colonies of 2 mm to 4 mm in diameter on solid selective media and display biochemical and serological characteristics described when tests are carried out in accordance with this part of ISO 6579

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3.2
serotyping of *Salmonella*
determination of the presence or absence of specific O-antigens, H-antigens and Vi-antigens in an isolate confirmed as *Salmonella* (3.1)

3.3
antigenic formula
combination of numbers and letters representing the O-, H-, and Vi-antigens of an isolate confirmed as *Salmonella* (3.1)

4 Principle

For the serotyping of *Salmonella* spp. the following antigens are determined for isolates biochemically confirmed as *Salmonella* spp.:

O-antigens, H-antigens and Vi-antigens.

NOTE Alternative procedures can be used to confirm the isolate being *Salmonella* spp. provided the suitability of the alternative procedure is verified (see ISO 7218).

5 Culture media and sera

5.1 General

For current laboratory practice, apply ISO 7218.

For the performance testing of media, follow the recommendations of ISO 11133.

5.2 Culture media and reagents

See Annex A.

5.3 Antisera

O-antisera, H-antisera and Vi-antisera are available from various commercial suppliers. Information on relevant polyvalent antisera and monovalent antisera can be found in Annex B.

6 Apparatus

Disposable supplies are an acceptable alternative to reusable glassware if they have similar specifications.

Usual microbiological laboratory equipment and, in particular, the following.

6.1 Incubator, to grow *Salmonella* isolates, capable of operating in the range 34 °C to 38 °C.

NOTE In this part of ISO 6579, the incubation temperature is not a differential parameter. Isolates are cultured to obtain sufficient material to perform the tests on a pure culture. Therefore, culture step is performed at an optimal growth temperature. For *Salmonella* this is generally a temperature between 34 °C and 38 °C.

6.2 Oven (for dry sterilization) or **autoclave** (for wet sterilization). See ISO 7218.

6.3 Refrigerator (for storage of prepared media), capable of operating at 5 °C ± 3 °C.

6.4 Glass slides.

6.5 Sterile inoculation instrument, e.g. needles, wires, wooden sticks, loops (e.g. of 1 µl).

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6.6 Sterile test tubes and **flasks**, of appropriate capacity. Flasks or bottles and test tubes with non-toxic metallic or plastic (screw) caps may be used.

6.7 Sterile Petri dishes, with diameters of approximately 55 mm and 90 mm.

6.8 Water bath, capable of operating at 47-50 °C.

6.9 Water bath (or incubator), capable of operating at 50 °C ± 2 °C.

7 Sample

It is important that the laboratory works with a pure culture which has been biochemically confirmed as *Salmonella* spp.

8 Taxonomy of *Salmonella*

8.1 General

Approximately every 7 years, the WHO Collaborating Centre for Reference and Research on *Salmonella* (Institut Pasteur, Paris) publishes an update of the "Antigenic formulae of the *Salmonella* serovars", which is the basis for assigning serovar names and formulas to isolates of *Salmonella* spp. At the time of publication, the latest version of the White–Kauffmann–Le Minor scheme is that of 2007 (Reference [9]).

NOTE Supplements to the White-Kauffmann-Le Minor scheme are published in *Research in Microbiology*, a publication of the Institut Pasteur (formerly called *Annales de l'Institut Pasteur/Microbiologie*). For instance, supplement no. 47 was published in 2010 and characterises new serovars found between 2003 and 2007 (Reference [10]).

This part of ISO 6579 provides guidance on the serotyping of *Salmonella* serovars.

8.2 Nomenclature

Different nomenclatures have been used (or are still in use) for *Salmonella* strains:

— originally, Kauffmann (Reference [12]) considered each *Salmonella* serovar as a separate species;

— different type species have been used: *S. enterica* vs. *S. choleraesuis*, each having another type strain;

— some "important" *Salmonella* strains (like *Salmonella* Typhi and *Salmonella* Paratyphi) were considered to be species and not being "only" serovars of a species.

The Judicial Commission of the International Committee on Systematics of Procaryotes indicated that many synonyms can be used in *Salmonella* nomenclature (Reference [22]). In this part of ISO 6579, the widely accepted current nomenclature is used, which is also approved by the WHO Collaborating Centre for Reference and Research on *Salmonella* (Reference [9]), the American Society for Microbiology (Reference [20]), the Centers for Disease Control and Prevention (Reference [3]) and *Bergey's manual* (Reference [17]). According to the current nomenclature, the genus *Salmonella* belongs to the family of *Enterobacteriaceae* and consists of only two species: *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica.*

Salmonella serovars belonging to *S. enterica* subsp. *enterica* are isolated most frequently (more than 99,5 % of isolated *Salmonella* strains) and they are designated by a name, usually related to the geographical place where the serovar was first isolated. Serovars belonging to other subspecies of *S. enterica* and those of *S. bongori* are designated by their antigenic formula.

Due to combinations of subspecies and many serovars, the full names are long (e.g. *Salmonella enterica* subsp. *enterica* serovar Typhimurium). It has therefore generally been accepted to use a shorter

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way to indicate the names of the serovars of subspecies *enterica*. The White–Kauffmann–Le Minor scheme suggests the following shortened names: *S. enterica* serovar Typhimurium or *Salmonella* ser. Typhimurium. According to Reference [3], at the first citation of a serovar in a text the genus name should be given followed by the word "serovar" or the abbreviated term "ser". and then the serovar name. Subsequently, the name may be written with the genus followed directly by the serovar name (e.g. *Salmonella* Typhimurium). This way of indicating *Salmonella* serovars is also accepted in the majority of journals [e.g. journals of the American Society for Microbiology (ASM)] and is also used in this part of ISO 6579.

In summary, the nomenclature of *Salmonella*:

family: *Enterobacteriaceae* (first letter capitalized, italicized)

genus: *Salmonella* (first letter capitalized, italicized)

species: *enterica* (not capitalized, italicized)

subspecies: *enterica* (not capitalized, italicized)

serovar (serotype or ser.): e.g. Typhimurium (first letter capitalized, not italicized)

subspecies: *salamae*
arizonae
diarizonae
houtenae
indica

species: *bongori*

In the 47th Supplement to the White–Kauffmann–Le Minor scheme (Reference [10]) more than 2600 *Salmonella* serovars are mentioned and the numbers increase regularly, as summarized in Table 1.

Table 1 — Number of *Salmonella* serovars through the years

Species/subspecies	Supplement		
	1998[a]	2001[b]	2007[c]
	Number of serovars		
Salmonella enterica	2 443	2 502	2 587
subsp. *enterica*	1 454	1 492	1 547
subsp. *salamae*	489	500	513
subsp. *arizonae*	94	95	100
subsp. *diarizonae*	324	331	341
subsp. *houtenae*	70	71	73
subsp. *indica*	12	13	13
Salmonella bongori	20	21	23
Total no. of serovars (genus *Salmonella*)	2 463	2 523	2 610

[a] Reference [18].

[b] Reference [19].

[c] Reference [10], covering 2003–2007.

8.3 Biochemical characteristics

The *Salmonella* species and subspecies are identified based on different biochemical tests. In Table 2, the differential characteristics are listed. See Reference [9] and Annex C for further details.

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Table 2 — Biochemical characteristics of *Salmonella* species and subspecies (Reference [9])

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
Dulcitol	+	+	−	−	−	d	+
ONPG[a] (2 h)	−	−	+	+	−	d	+
Malonate	−	+	+	+	−	−	−
Gelatinase	−	+	+	+	+	+	−
Sorbitol	+	+	+	+	+	−	+
Growth with KCN[b]	−	−	−	−	+	−	+
L(+)-tartrate[c]	+	−	−	−	−	−	−
Galacturonate	−	+	−	+	+	+	+
γ-Glutamyltransferase	+ [e]	+	−	+	+	+	+
β-Glucuronidase	d	d	−	+	−	d	−
Mucate	+	+	+	− (70 %)	−	+	+
Salicin	−	−	−	−	+	−	−
Lactose	−	−	− (75 %)	+ (75 %)+	−	d	−
Lysis by phage O1	+	+	−	+	−	+	d

+ = 90 % or more positive reaction

− = 90 % or more negative reaction

d = different reactions given by different serovars

[a] *o*-Nitrophenyl-β-D-galactopyranoside (test for β-galactosidase).

[b] Potassium cyanide.

[c] = D-Tartrate, Paratyphi B: −, Paratyphi B biovar Java: +

[e] = Typhimurium: d, Dublin: −.

8.4 Antigenic characteristics

8.4.1 General

The important antigenic characteristics of *Salmonella* for serological tests are divided into three main types, being:

— the O-antigen, also called the somatic antigen;

— the H-antigen, also called the flagellar antigen;

— the Vi-antigen, also called the capsular antigen.

The antigenic formula of *Salmonella* spp. exists of these three types of antigens, reported in the following way: O-antigens, Vi-antigen (if present): H-antigens of first phase: H-antigens of second phase. For instance, the antigenic formula of *Salmonella* Paratyphi C is: 6,7,[Vi]:c:1,5; with O-antigens O:6 and O:7; with the Vi-antigen, which can be present or absent (indicated by the square brackets); with H-antigen H:c for the first phase; with H-antigens H:1 and H:5 for the second phase.

8.4.2 The O-antigen (somatic antigen)

This antigen consists of a cell wall component and the main substances are polysaccharide, protein, and phospholipid. The O-antigen is very robust and can resist temperatures up to 100 °C for 150 min, treatment with 95 % volume fraction ethanol or dilute acid (Reference [16]).

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The reaction of the O-antigen with antisera results in granular agglutination. Historically, the O-antigens were classified in individual O-antigen groups in the Kauffmann–White scheme (Reference [9]). The groups were named with Roman letters beginning with group A, which include antigen O:2, up to group Z which contain antigen O:50. As there were more O-antigens than letters, the remaining antigens were not given as group, but were named by the O-antigens O:51 to O:67. Nowadays it is preferred to designate each O-group using the characteristic O-factor. The letters have been kept and are shown inside brackets, e.g. O:4 (B) (Reference [9]). In Table 3, the old and new designations are summarized.

Table 3 — *Salmonella* serogroups (old designation) and relevant O-antigens (new designation)

Group	O-antigen	Group	O-antigen	Group	O-antigen
A	2	G_1-G_2	13	Q	39
B	4	H	6,14	R	40
C_1(, C_4)[a]	6,7	I	16	S	41
C_2, C_3	8	J	17	T	42
D_1	9	K	18	U	43
D_2	9,46	L	21	V	44
D_3	9,46,27	M	28	W	45
E_1(, E_2, E_3)[b]	3,10	N	30	X	47
E_4	1,3,19	O	35	Y	48
F	11	P	38	Z	50

[a] C_4 has been merged into C_1.

[b] E_2 and E_3 have been merged into E_1.

8.4.3 The H-antigen (flagellar antigen)

This antigen is located on the flagellum and the main component is protein. It is less robust than O-antigens. It can easily be decomposed by alcohol, acid, and temperature above 60 °C, but it is resistant to a formalin solution with a volume fraction of 0,5 % (Reference [16]).

The reaction of the H-antigen with antisera results in floccular agglutination. Many *Salmonella* spp. possess two phases of the H-antigen, but monophasic and triphasic variants are also known. The first phase is called the specific phase and the second phase is called the non-specific phase. The first phase is indicated by a lower case letter, a to z. However, since the identification of the z-antigen, many new H-antigens have been detected and are named z_1, z_2, z_3 ... z_{91}.

Examples of monophasic serovars are:

— *Salmonella* Paratyphi A: 1,2,12:a:[1,5]; with H:a for the first phase and where the square brackets indicate that the second phase (H:1,5) can be present or absent;

— *Salmonella* Typhi: 9,12,[Vi]:d:-; with H:d for the first phase;

— *Salmonella* Derby: 1,4,[5],12:f,g:[1,2]; with H:f,g for the first phase and where the second phase (H:1,2) can be present or absent;

— *Salmonella* Enteritidis: 1,9,12: g,m:-; with H:g,m for the first phase. In addition to factors H:g,m, some strains may have factor H:p, or H:f, or H:t. Exceptional strains can have antigen H:1,7 as second phase;

— *Salmonella* Dublin: 1,9,12,[Vi]:g,p:-; with H:g,p for the first phase.

NOTE 1 Underlined O-factors are determined by phage conversion. They are only present if the culture is lysogenized by the corresponding converting phage (Reference [9]).

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NOTE 2 O- or H-factors indicated in square brackets can be present or absent, without relation to phage conversion (Reference [9]).

NOTE 3 Diphasic strains of *Salmonella* Derby and *Salmonella* Enteritidis are very rare. It is possible that phase inversion is required to detect these rare strains. However, this is only necessary for certain (special) cases (e.g. in case of deviating sources and/or in the case of (special) travel-related cases).

8.4.4 Vi-antigen (capsular antigen)

This antigen is a surface (capsular) antigen and can mask the O-antigens so that the bacteria are not agglutinated with O-antisera. The main component of the Vi-antigen is polysaccharide. The *Salmonella* strains which possess a Vi-antigen are more virulent than the strains without Vi-antigen. The Vi-antigen can be present in only three *Salmonella* serovars:

— *Salmonella* Typhi : 9,12,[Vi]:d:-;

— *Salmonella* Paratyphi C : 6,7,[Vi]:c:1,5;

— *Salmonella* Dublin: 1,9,12,[Vi]:g,p:-.

The square brackets indicate that the Vi-antigen can be present or absent.

NOTE The presence of Vi-antigens in *Salmonella* isolates from food or veterinary samples is very rare. If Vi is present, it masks the detection of O-antigens. To detect the O-antigens, it can prove necessary to heat a suspension of the isolate (e.g. in physiological saline solution) at 100 °C for 60 min, or at 120 °C for 15 min.

9 Procedure for *Salmonella* serotyping

9.1 General

Before starting the serotyping, it is important to confirm biochemically that the isolate belongs to the genus *Salmonella* (as specified in ISO 6579-1). Although the H-antigens are specific for *Salmonella,* several O-antigens are common in different genera of the *Enterobacteriaceae* (e.g. *Salmonella, Citrobacter, Hafnia*).

NOTE Alternative procedures can be used to confirm that the isolate belongs to the genus *Salmonella*. provided the suitability of the alternative procedure is verified (see ISO 7218).

Each supplier of antisera produces its own sets of antisera, with its own unique instructions for use. It is therefore not possible to provide here one general set of instructions for serotyping, as it is always important to follow the instructions of the supplier to obtain optimal results. Some manufacturers supply pools of antisera (mixtures of several O-antisera or H-antisera), which are very useful at the beginning of serotyping an unknown type. When the strain agglutinates with an antisera pool, it can be further tested with group antisera and/or single factor antisera relevant to the positive pool. When the focus is on typing only certain serovars and it is sufficient to indicate the other serovars as *Salmonella* spp., the agglutination can immediately be performed with only the specific monofactor antisera of the relevant serovars.

In Annex B, the general procedure is given for serotyping an unknown *Salmonella* isolate.

9.2 Example procedure for serotyping five public health-related *Salmonella* serovars

9.2.1 General

In the following example, the procedure is described for serotyping five important public health-related *Salmonella* serovars (see Annex D). In Table 4 these strains are shown with their antigenic formula.

In the following sections, slide agglutination of *Salmonella* isolates is described, which is the procedure most often performed. However, others also exist, such as the microtitre plate method (see Annex E).

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Table 4 — Antigenic formula of five important public health-related *Salmonella* serovars

Name	O-antigens[b]	H-antigens	
		phase I	phase II
Salmonella Typhimurium	1,4,[5]12	i	1,2
Salmonella Enteritidis[a]	1,9,12	g,m	-
Salmonella Infantis	6,7,14	r	1,5
Salmonella Virchow	6,7,14	r	1,2
Salmonella Hadar	6,8	z_{10}	e,n,x

[a] In addition to factors H:g,m, some strains may have factor H:p or H:f or H:t. Exceptional strains can have antigen H:1,7 as second phase (Reference [9]).

[b] Underlined O factors are determined by phage conversion. They are present only if the culture is lysogenized by the corresponding converting phage (Reference [9]).

9.2.2 Selection of a colony suspected for *Salmonella*

Culture a colony from a pure culture which is suspected to be *Salmonella* according to the biochemical characterization. Use the culture media and methods as prescribed by the manufacturer of the antisera used. If no information is given, a non-selective agar medium like nutrient agar (e.g. see A.2) can be used. Incubate the inoculated nutrient agar plate(s) between 34 °C and 38 °C (6.1) "overnight" (approximately 18 h).

9.2.3 Investigation for auto-agglutination

An example of the test for auto-agglutination is described in the following. Other methods may also be used. Follow, in that respect, the manufacturer's instructions.

— Add one drop of saline (this may vary from 8,5 g/l NaCl to 35 g/l NaCl, where the higher concentration may be more sensitive) on a glass slide (6.4).

— Transfer a small amount of bacterial culture (e.g. the amount which can be taken with a 1 µl disposable loop) on to the glass slide and mix with the drop of saline.

— Gently tilt the slide back and forth. Depending on the manufacturer and/or on the saline concentration, this should take 5 s to 60 s.

— Assess the suspension. The presence of granules in the suspension indicates auto-agglutination. Strains with a positive reaction in the auto-agglutination test are hard to investigate further for serotyping. For auto-agglutinating strains, it is not possible to test for the O-antigens. However, it may sometimes still be possible to investigate for the H-antigens.

If a strain shows auto-agglutination, either one or both of the following can be tried on the same colony and/or on additional colonies.

— Suspend the colony in sterile water instead of in a saline solution and follow the procedure for auto-agglutination as described in the foregoing.

— Grow the strain on a semi-solid agar medium like modified semi-solid Rappaport Vassiliadis (MSRV) agar (as specified in ISO 6579-1) and use colony material from the semi-solid agar to perform the procedure for auto-agglutination as described in the foregoing.

NOTE Auto-agglutinating strains are also called "rough" strains.

9.2.4 Agglutination with O-antisera

The instructions for the use of antisera may differ per manufacturer. Therefore it is important to always follow the manufacturer's instructions.

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Most manufacturers use a slide agglutination method for the detection of O-antigens. In this method, one drop of antiserum is mixed with a bacterial suspension (directly from a plate, tube or broth) on a slide. Gently tilt the slide back and forth. Subsequently observe the slide for agglutination. The presence of granules indicates a positive reaction.

For different manufacturers, the following variations in the procedure can be found:

— the size of the drop on the slide (e.g. 25 µl or a "drop");

— the way the antiserum and bacterial material are mixed on the slide (bacterial material directly from the agar or via a suspension, antiserum directly on the slide or added to a bacterial suspension);

— time of tilting the slide back and forth (can vary from 5 s to 60 s);

— the way of observing agglutination (magnification or not, dark background or normal lighting);

— interpretation of the results (read the footnotes with regard to the limitations of the procedure).

9.2.5 Agglutination with H-antisera

After agglutination with the O-antisera, perform the agglutination with H-antisera. *Salmonella* often possesses two types of H-antigens (phase 1 and phase 2). If one H-phase is found negative for biphasic strains, a phase inversion method is required. The dominant H-phase is repressed with a phase inversion method. By repressing the dominant H-phase the second H-phase can be expressed and identified.

A frequently used method for phase inversion is that of Sven Gard, see 9.2.7. For this, specific phase inversion antiserum is added to a swarming agar medium and the *Salmonella* strain is spot inoculated on the plate. The agar medium shall be sufficiently soft for motile *Salmonella* to swarm over the medium. The agar concentration in the medium may vary from a mass fraction of 0,5 % to 1 % (depending on the gel strength of the agar). Examples of "swarming" media are given in A.3. Other examples for phase inversion are given in Annex F.

For the investigation for agglutination, follow the manufacturer's instructions.

9.2.6 Agglutination tests for serotyping five important public health-related *Salmonella* serovars

9.2.6.1 General

For the detection of the five *Salmonella* serovars mentioned in Table 4, the following O-antisera and H-antisera are needed:

O:4, O:5, O:6 (O:6_1 or O:6,7 or O:6,14,24), O:7, O:8, O:9 and O:46.

H:i, H:2, H:G or H:g (monovalent), H:m, H:q, H:s, H:t, H:r, H:5, H:z_{10} and H:x.

For the serotyping of the *Salmonella* strains in the order shown in Table 4, follow the procedure specified in 9.2.6.2 to 9.2.6.5 (also see the scheme in Annex D).

For more information on sequential tests of the different antisera, see Annex B.

9.2.6.2 If O:4 is positive

Agglutinate with O:5. The result can be either positive or negative for *Salmonella* Typhimurium, still the information may be relevant for epidemiological purposes.

Agglutinate with H:i and H:2 antisera (phase inversion may be necessary).

The serovar of the strain is Typhimurium if both reactions are positive.

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Agglutination with O:12 is not necessary to indicate the isolate to be *Salmonella* Typhimurium, as the positivity of O:4 implicates the presence of O:12. Likewise, the detection of antigen H:1 has no discriminatory power and is not needed to be tested additionally.

9.2.6.3 If O:9 is positive

Agglutinate with H:G(complex) or with H:g (monovalent antibody) antiserum.

If this reaction is positive, subsequently agglutinate with H:m antiserum.

If this is also positive, agglutinate with H:q and H:s antisera as negative controls.

If these latter reactions are negative, agglutinate subsequently with O:46 and, if wanted, with O:12. If O:46 is negative (and O:12 is positive), the serovar of the strain is Enteritidis.

NOTE In addition to factors H:g,m, some *Salmonella* Enteritidis strains can give a positive reaction with H:p, or H:f, or H:t antiserum. However, these strains are very rare. Exceptional strains can have antigen H:1,7 as a second phase.

9.2.6.4 If O:7 is positive

Agglutinate with H:r, H:2 and H:5 antisera (phase inversion may be necessary).

If H:r and H:2 are positive, the serovar of the strain is: Virchow.

If H:r and H:5 are positive, the serovar of the strain is: Infantis.

Detection of antigen H:1 has no discriminatory power and is not needed to be tested additionally.

9.2.6.5 If O:8 is positive

Agglutinate with H:z_{10} and H:x antisera.

If both are positive, subsequently agglutinate with O:6_1 or O:6,7 or O: 6,14,24 antiserum.

If O:6_1 or O:6,7 or O:6,14,24 is positive, the serovar of the strain is: Hadar.

Some batches of O:6,7 antiserum do not react with O:6,8 strains. When purchasing this antiserum, make sure it also reacts with O:6,8 strains (ask the manufacturer).

NOTE Colonial form variation can occur with the expression of the O:6_1 antigen by some serogroup C_2 serovars (Reference [11]). For that reason, it is not always possible to indicate, e.g. *Salmonella* Hadar and *Salmonella* Istanbul as distinct serovars.

9.2.7 Example of phase inversion using the Sven Gard method

The example described here for phase inversion is intended for the serotyping of *Salmonella* Typhimurium. In general, the method is also applicable to other *Salmonella* serovars.

When O:4 and H:i are positive, but H:2 is negative, prepare a swarm agar plate (see A.3 or F.1), with anti-H:i (e.g. phase inversion serum-mix containing H:i). Inoculate the strain by one spot on the centre of the plate. Incubate the plate between 34 °C and 38 °C (6.1) overnight (approximately 18 h).

After incubation, agglutinate the strain again with H:2 antisera.

If H:2 is again negative, subsequently agglutinate with H:i again.

If H:i gives a negative or weak reaction, the strain is not *Salmonella* Typhimurium.

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If H:i gives a (strong) positive reaction, repeat the phase inversion. Again prepare an agar plate with anti-H:i and inoculate this plate with bacterial material from the furthest point of spread of the opaque growth of the first phase inversion agar plate. Repeat the phase inversion procedure as described above.

NOTE 1 Sometimes it is necessary to add more H:i antiserum into the swarm agar (of the repeated phase inversion plate) to get a better reaction.

NOTE 2 Monophasic variants of *Salmonella* Typhimurium also exist, e.g. lacking, or not expressing, the second H phase: 1,4,[5]12:i:-. While serotyping this variant, phase inversion can be repeated once or more, to exclude the presence of the second phase. Alternatively, a molecular method can be used to confirm whether the strain is a variant of *Salmonella* Typhimurium.

10 Quality control

The sera used for agglutination should be clear (unless the antisera are used for latex tests). Always inspect the sera before use. In case of turbidity, follow the instructions of the manufacturer.

Possible procedures for quality control of the serotyping are given in the following.

— Two strains are selected (or the number of strains that represents approximately 2 % of the workload) from the incoming work per week. Each of the selected strains is cultured twice. The duplicate strains are then treated as two new isolates received for serotyping. On completion of the work, the results for the two strains and their duplicates are compared for any discrepancies. If there are any discrepancies, these are then further investigated.

— The laboratory keeps fully characterized *Salmonella* serovars in stock (e.g. from a culture collection or from interlaboratory comparison studies). From this stock, regularly (e.g. weekly) one or two serovars of the 10 serovars most frequently identified in the laboratory are selected to check the serotyping procedure. The serovar(s) used to perform the quality control may vary weekly to ensure that different antisera are tested over the course of time.

— It is important to test the ability of serotyping against other laboratories. For this it is advisable to regularly participate in interlaboratory comparison studies, whenever available.

11 Reporting

For isolates belonging to *S. enterica* subsp. *enterica*, report the (full) name and, whenever possible/needed, also the antigenic formula. For the other (sub)species report the antigenic formula as found.

The notation of the antigenic formula is as follows:

O-antigens (separated by commas), Vi-antigen (if present): H-antigens of first phase (separated by commas): H-antigens of second phase (if present; separated by commas); H-antigens of third phase (if present).

For instance, according to the White-Kauffmann-Le Minor scheme (Reference [9]), the full antigenic formula of *Salmonella* Typhimurium is: 1,4,[5],12:i:1,2.

With O-antigens O:1,4,[5],12; where the underlined O factor is determined by phage conversion and is only present if the culture is lysogenized by the corresponding converting phage and where the square brackets indicate that the factor may be present or absent without a relation to phage conversion (Reference [9]);With H-antigens H:i for the first phase and H:1,2 for the second phase.

For the final reporting, the antigenic formula of an isolate is preferably given without square brackets or underlining. Report the antigenic formula that has been determined, e.g. 4,12:i:1,2.

For other subspecies of *S. enterica*, the subspecies to which the serovar belongs is indicated by the following symbol (Reference [9]):

II for serovar of *S. enterica* subsp. *salamae;*

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IIIa for serovar of *S. enterica* subsp. *arizonae;*

IIIb for serovar of *S. enterica* subsp. *diarizonae;*

IV for serovar of *S. enterica* subsp. *houtenae;*

VI for serovar of *S. enterica* subsp. *indica.*

An example of reporting for a subspecies other than *S. enterica* subsp. *enterica* is: *S.* II 30:z_{10}:e,n,x,z_{15}.

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Annex A
(informative)

Composition and preparation of culture media and reagents

A.1 General

The culture media as described in this annex are related to biochemical tests (see Annex C). The composition of the media as described here can be considered as examples. Media with same names may be described in literature or are commercially available in slightly different compositions. Therefore, it is important for each biochemical test to verify the reactions of the media with well characterized positive and negative control strains.

IMPORTANT — If media or reagents are prepared from dehydrated complete media or reagents, or if ready-to-use media and reagents are used, follow the manufacturer's instructions regarding preparation, storage conditions, expiry date, and use.

The shelf-lives of the media indicated in this annex have been shown in some studies. The user should verify this under their own storage conditions (see ISO 11133).

A.2 Nutrient agar

A.2.1 Composition

Meat extract	3,0 g
Peptone[a]	5,0 g
Sodium chloride (NaCl) (optional)	5,0 g
Agar	9 g to 18 g[b]
Water	1 000 ml

[a] For example enzymatic digest of casein.

[b] Depending on the gel strength of the agar.

A.2.2 Preparation

Dissolve the components in the water by heating.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,0 ± 0,2 at 25 °C.

Transfer the culture medium into flasks (6.6) of appropriate capacity.

Sterilize in the autoclave (6.2) maintained at 121 °C for 15 min.

Transfer approximately 20 ml of the melted medium into sterile Petri dishes with a diameter of 90 mm (6.7). Allow to solidify.

Store the poured plates (upside down), protected for desiccation and in the dark, at 5 °C (6.3) for up to 2 months.

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If necessary, dry the plates before use.

A.3 Swarm agar media — Sven Gard (examples)

A.3.1 Example 1 (Reference [16])

Use of nutrient agar with amended agar concentration

It is necessary to determine the agar concentration of the nutrient agar intended for H-phase inversion in a Petri dish. It can vary from a mass fraction of 0,5 % to 1 % depending on the batch of agar used. The agar medium is required to be sufficiently soft for a spot-inoculated motile *Salmonella* to swarm over the medium after overnight incubation between 34 °C and 38 °C (6.1). Preliminary trials, without addition of antiserum, are therefore necessary, using either a nutrient agar (pH 7,4) prepared for that purpose or ordinary laboratory nutrient agar made semi-solid by the addition of broth in a proportion allowing overnight swarming. The volume required for a 10 cm Petri dish is 30 ml.

Swarming on the surface is easier if sodium desoxycholate (0,3 g/l) is added to the medium (sodium desoxycholate stimulates the swarming), see A.3.2.

A.3.2 Example 2

A.3.2.1 Composition

Meat extract	5,0 g
Yeast extract	1,0 g
Trypto-casein soya broth	30,0 g
Glucose	1,0 g
Sodium desoxycholate	0,35 g
Agar	5 g to 9 g[a]
Water	1 000 ml

[a] Depending on the gel strength of the agar. It can prove necessary to experimentally determine the concentration of agar needed for the optimal swarming of *Salmonella*.

A.3.2.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,6 ± 0,2 at 25 °C.

Transfer the culture medium into flasks (6.6) of appropriate capacity.

Sterilize in the autoclave (6.2) maintained at 110 °C for 20 min.

Cool the agar to 47 °C to 50 °C (6.8), or store in closed bottles at 5 °C (6.3) for up to 2 months.

Immediately before use:

— add a drop of a relevant antiserum (SG1 to SG6) to the molten medium and mix carefully;

— pour approximately 10 ml into Petri dishes with a diameter of 55 mm (6.7), or pour approximately 20 ml into Petri dishes with a diameter of 90 mm.

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A.4 Malonate broth

A.4.1 Composition (Reference [14])

Ammonium sulfate	2,0 g
Dipotassium phosphate	0,6 g
Monopotassium phosphate	0,4 g
Sodium chloride	2,0 g
Sodium malonate	3,0 g
Bromthymol blue	0,025 g
Water	1 000 ml

A.4.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,7 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into tubes which allow sufficient oxygen for the malonate reaction. For example, use tubes with approximate sizes 22 mm x 220 mm.

Sterilize in the autoclave (6.2) maintained at 121 °C for 15 min.

Store in the dark at 5 °C (6.3) for up to 2 months.

A.5 Dulcitol broth (Reference [7])

A.5.1 Complete medium

A.5.1.1 Composition

Meat extract	3,0 g
Peptone[a]	10,0 g
Sodium chloride	5,6 g
Andrade's indicator (A.5.2)	10 ml
Dulcitol solution (A.5.3)	50 ml
Water	1 000 ml

[a] For example enzymatic digest of casein.

A.5.1.2 Preparation

Dissolve the components in the water.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,0 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into tubes with a nominal capacity of 15 ml.

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Sterilize in the autoclave (6.2) maintained at 121 °C for 15 min.

Store in the dark at 5 °C (6.3) for up to 2 months.

A.5.2 Andrade's indicator

A.5.2.1 Composition (after Andrade-Penny, 1895; cited in Reference [7])

Acid fuchsine	0,5 g
Sodium hydroxide (1,0 mol/l)	0,5 g
Water	100 ml

A.5.2.2 Preparation

Dissolve the fuchsine in the water, and add the sodium hydroxide. If, after several hours, the fuchsine is not sufficiently decolourized, add an additional 1 ml or 2 ml sodium hydroxide. The reagent improves somewhat on aging and should be prepared in a sufficiently large amount to last for several years.

A.5.3 Dulcitol solution

A.5.3.1 Composition

Dulcitol	10 g
Water	100 ml

A.5.3.2 Preparation

Dissolve the dulcitol in the water.

Sterilize by filtration through a membrane filter with a 0,22 µm pore size.

Store at 5 °C (6.3) for up to 2 months.

A.6 ONPG solution (0,013 3 mol/l) (Reference [2])

A.6.1 Complete medium

A.6.1.1 Composition

o-Nitrophenyl-D-galactoside (ONPG)	0,08 g
Monosodium phosphate solution ($NaH_2PO_4 \cdot H_2O$; A.6.2)	5 ml
Water	15 ml

A.6.1.2 Preparation

Dissolve the ONPG in the water at approximate 37 °C. Add the 1,0 mol/l NaH_2PO_4 solution (A.6.2.1). The solution should be colourless. Store the solution at 5 °C (6.3).

Before use, warm an appropriate portion (sufficient for the number of tests) of ONPG solution to approximate 37 °C.

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A.6.2 Monosodium phosphate solution (1,0 mol/l)

A.6.2.1 Composition

$NaH_2PO_4 \cdot H_2O$	6,9 g
NaOH solution, 300 g/l	3 ml
Water	45 ml

A.6.2.2 Preparation

Dissolve the $NaH_2PO_4 \cdot H_2O$ in the water. Add 300 g/l NaOH solution and adjust to pH 7,0 ± 0,2 (at 25 °C). Bring the volume to 50 ml with water and store the solution at 5 °C (6.3) for up to 2 months.

A.7 D-Tartrate broth (organic acid tartrate)

A.7.1 Composition

Peptone[a]	10,0 g
Potassium sodium (+) tartrate	10,0 g
10 g/l Aqueous bromothymol blue	2,4 ml
Water	1 000 ml

a For example enzymatic digest of casein.

A.7.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,4 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into tubes with a nominal capacity of 15 ml.

Sterilize in the autoclave (6.2) maintained at 115 °C for 10 min.

Store in the dark at 5 °C (6.3) for up to 2 months.

A.8 Lead acetate solution

A.8.1 Composition

Lead acetate	Approx. 50 g
Water	10 ml

A.8.2 Preparation

Add the water to a suitable screw-capped bottle. Add sufficient lead acetate to make a saturated solution (i.e. undissolved lead acetate is apparent on the bottom of the bottle).

Store at room temperature for up to 6 months.

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A.9 Brain–heart infusion broth (BHI)

A.9.1 Composition

Brain infusion solids	12,5 g
Beef heart infusion solids	5,0 g
Proteose peptone	10,0 g
Glucose	2,0 g
Sodium chloride	5,0 g
Disodium phosphate	2,5 g
Water	1 000 ml

A.9.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,4 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into tubes with a nominal capacity of 15 ml.

Sterilize in the autoclave (6.2) maintained at 121 °C for 15 min.

Store in the dark at 5 °C (6.3) for up to 2 months.

A.10 Formal saline (1 % volume fraction)

A.10.1 Composition

Formaldehyde solution, 370 g/l	40,0 ml
Sodium chloride solution 170 g/l	200 ml
Water	Up to 4 000 ml

A.10.2 Preparation

Add the formaldehyde solution to the sodium chloride solution. Make the total volume up to 4 l with water.

A.11 Craigie tubes

A.11.1 Composition

Nutrient broth[a]	25,0 g
Agar	2,75 g
Water	1 000 ml

[a] For ingredients, see A.2 (without agar).

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A.11.2 Preparation

Dissolve the components in the water by heating.

Adjust the pH, if necessary, so that after sterilization it corresponds to 7,2 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into wide screw-capped tubes or bottles with a nominal capacity of approximately 15 ml and containing Craigie tubes (short narrow tubes, open at both ends). The Craigie tube should extend above the surface of the agar.

Sterilize in the autoclave (6.2) maintained at 115 °C for 10 min.

Store at room temperature for up to 2 months.

A.12 Nutrient gelatine

A.12.1 Composition

Meat extract	3,0 g
Peptone[a]	5,0 g
Gelatine	120,0 g
Water	1 000 ml

a For example enzymatic digest of casein.

A.12.2 Preparation

Dissolve the components in the water by heating.

Adjust the pH, if necessary, so that after sterilization it corresponds to 6,8 ± 0,2 at 25 °C.

Transfer the culture medium in volumes of 5 ml into tubes with a nominal capacity of 15 ml.

Sterilize in the autoclave (6.2) maintained at 121 °C for 15 min.

Allow the medium to cool in an upright position and store at 5 °C (6.3) for up to 2 months.

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Annex B
(informative)

Examples of procedures for serotyping an unknown *Salmonella* isolate

In Figure B.1, the steps to be taken before serotyping *Salmonella* isolates are indicated. For suggestions on how to treat auto-agglutinating strains (rough strains), see 9.2.3.

Examples of procedures that can be used for serotyping an unknown *Salmonella* isolate are summarized in Table B.1 and in Figure B.2. In Table B.2, information is given on the possible composition of polyvalent antisera (which can differ according to the supplier). Figure B.2 and Table B.2 are derived from Reference [6].

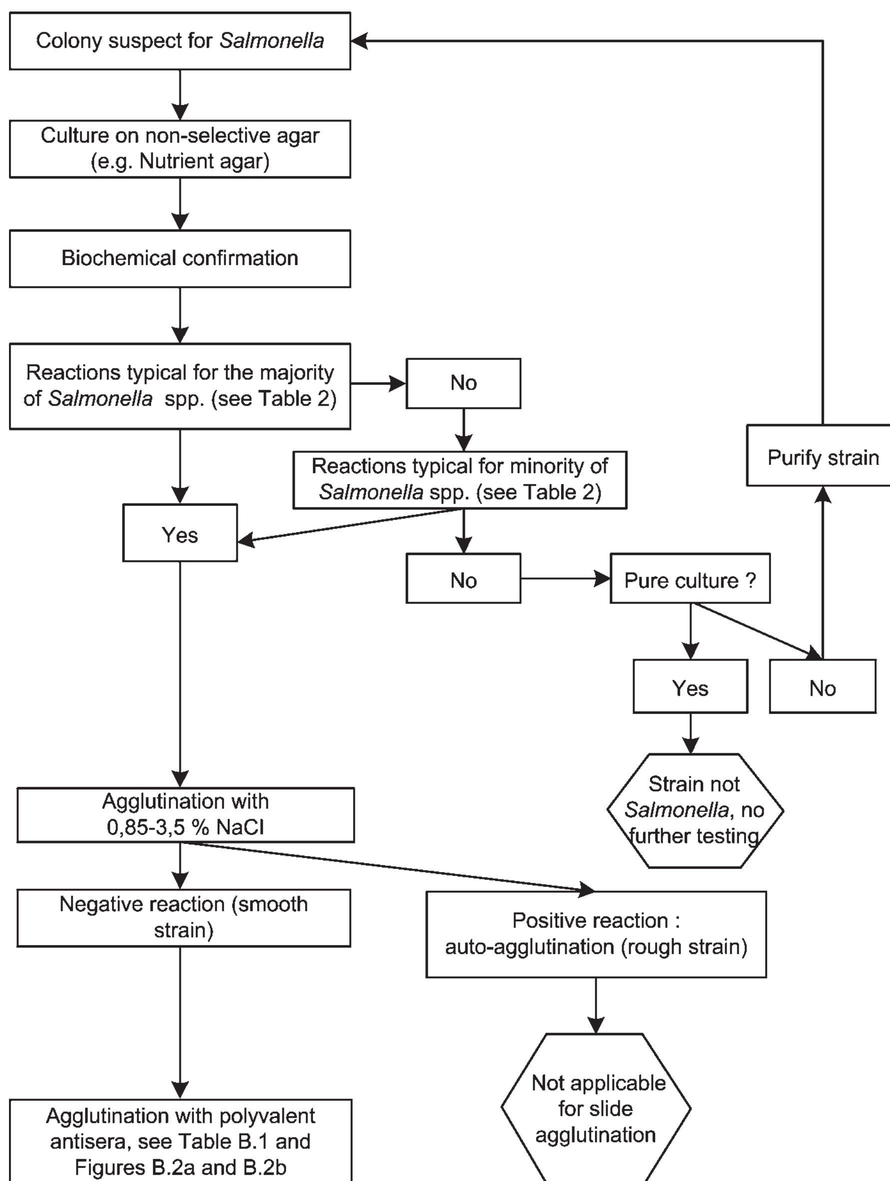

Figure B.1 — Steps to be taken before serotyping *Salmonella* spp.

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Table B.1 — Possible procedure for serotyping unknown *Salmonella* isolates by agglutination with OMA and OMB polyvalent antisera

Step 1 O-antigen analysis	Use of polyvalent antisera	1.1 OMA if positive: Step 2.1.1 if negative: go to 1.2	1.2 OMB if positive: Step 2.2.1 if negative: see footnote a
Step 2 O-antigen analysis	Use of group-specific antisera — allocation of an isolate to a serogroup [b]	2.1.1 O:4,5 or group B if positive: step 3.1.1 if negative: step 2.1.2 2.1.2 O:9 or group D if positive: Step 3.1.2 if negative: Step 2.1.3 2.1.3 O:3,10,15 or group E if positive: step 3.1.3 if negative: step 2.1.4 2.1.4 O:21 or group L if negative: step 2.1.5 2.1.5 O:1,2 or group A	2.2.1 O:6,7,8 or group C if positive: step 3.2.1 if negative: step 2.2.2 2.2.2 O:13,22,23 or O:13 if positive: step 3.2.2 if negative: step 2.2.3 2.2.3 O:11
Step 3 O-antigen analysis	Use of monovalent antisera — identification of serovar specific O-antigens	3.1.1 Isolate of group B: O:5, O:27 3.1.2 Isolate of group D: O:46 for group D_2 O:27 for group D_3 3.1.3 Isolate of group E: O:10, O:15, O:34 for group E_1 O:19 for group E_4	3.2.1 Isolate of group C: O:7 for group C_1 O:8, O:6_1, O:20 for group C_{2-3} O:14, O:24, O:25 for group H 3.2.2 Isolate of group G O:22, O:23
Step 4 Flagellar antigen analysis	Use of polyvalent antisera[c]	Poly H (phase 1 and 2) Poly H (phase 2)	
	Use of monovalent antisera	H:1(1,2) → (H:2, H:5, H:6, H:7) [d] H:a, H:b, H:c, H:d, H:E → (H:h, H:n, H:x, H:z_{15}) [d] H:G(g,m) → (H:f, H:g, H:m; H:p, H:q H:s, H:t, H:u)[d] H:i, H:k, H:L → (H:v, H:w, H:l,z_{13}, H:l,z_{28}) [d] H:y, H:z, H:Z_4 → (H:z_{23}, H:z_{24}, H:z_{32}) [d] H:z_6, H:z_{10}, H:z_{29}, H:z_{35}, etc.	

[a] In the case of a negative reaction with both polyvalent antisera, test the isolate with further polyvalent or group-specific antisera allowing the identification until up to group O:67, or send the isolate to a reference laboratory.

[b] If in step 1 polyvalent antisera of another antibody composition than OMA and OMB are used, the group-specific antisera in step 2 have to be adapted correspondingly.

[c] It is possible to omit the use of polyvalent H-antigen antisera and to choose monovalent antisera corresponding to the incidence of the isolation of serovars of the estimated serogroup.

[d] The antisera within brackets are necessary to identify the antigens of the corresponding H-antigen complex.

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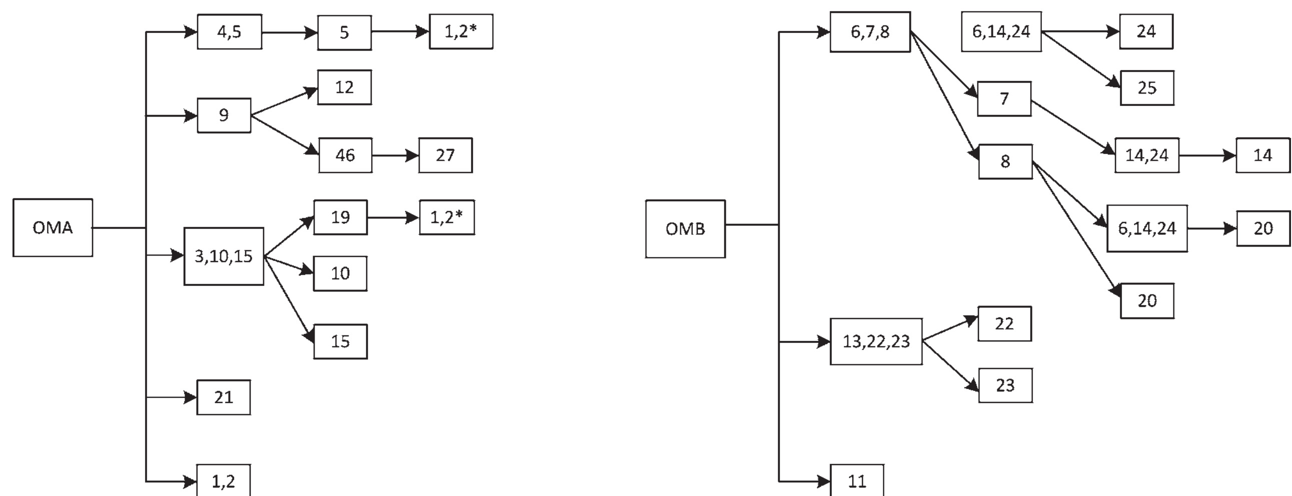

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OMF →	54 →	55 →	56 →	57 →	58 →	59	
OMG →	60 →	62 →	63 →	65 →	66 →	67	

* O:1,2 serum can be used to test for the presence of the O:1 antigen

a) Somatic antigens — Possible sequential tests on polyvalent and monovalent antisera to detect somatic Salmonella antigens (derived from Reference [6]) [The next step in the horizontal line is taken when the previous test shows a positive reaction (e.g. if O:4,5 is positive, then test for O:5, etc.). The composition of the polyvalent antisera as used in this scheme is given in Table B.2.]

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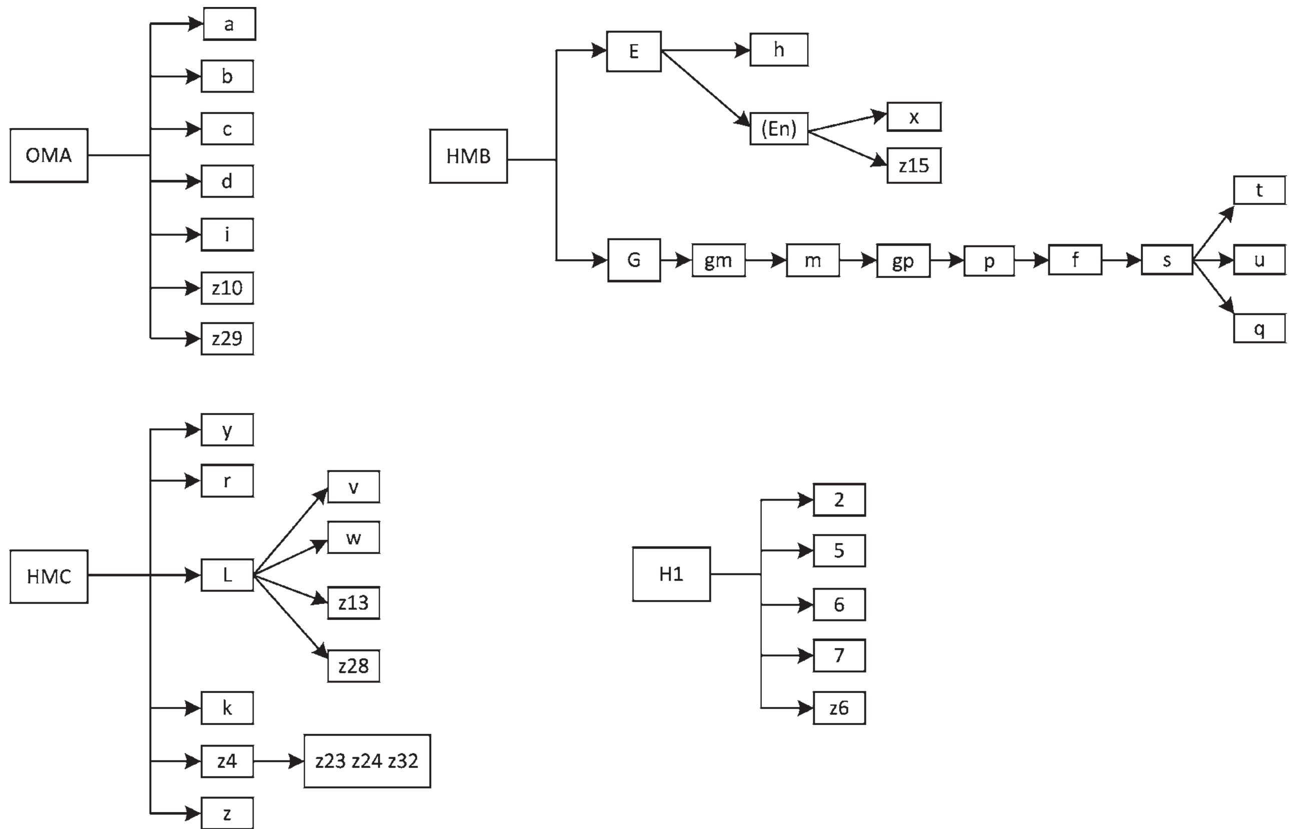

b) Flagellar antigens — Possible sequential tests on polyvalent and monovalent antisera to detect flagellar *Salmonella* antigens (derived from Reference [6]) [The next step in the horizontal line is taken when the previous test shows a positive reaction. The composition of the polyvalent antisera as used in this scheme is given in Table B.2.]

Figure B.2 — Detection of antigens

Table B.2 — Composition of polyvalent antisera referred to in Figure B.2 (Reference [6])

Factor	Polyvalent antisera	Corresponding antigens
O	OMA	1,2,12 + 4,5,12 + 9,12 + 9,46 + 3,10 + 3,15 + 1,3,19 + 21
	OMB	6,7 + 6,8 + 11 + 13,22 + 13,23 + 6,14,24 + 8,20
H	H1	1,2 + 1,5 + 1,6 + 1,7 + z_6
	HE	e,h + e,n,x + e,n,z_{15}
	HG	f,g + g,p + g,m,s + g,m + m,t
	HMA	a + b + c + d + i + z_{10} + z_{29}
	HMB	e,h + e,n,x + e,n,z_{15} + G
	HMC	k + y + L + z_4 + r

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Annex C
(informative)

Biochemical tests

C.1 Distinction between *Salmonella* subspecies

C.1.1 General

To distinguish between *Salmonella* subspecies, some biochemical tests need to be performed (see Table 2). Some examples of tests are given in C.1.2 to C.1.5.

C.1.2 Malonate test

Inoculate malonate broth (see A.4) from a fresh agar slant or broth culture (a 3 mm loopful of broth culture is preferred).

Incubate the inoculated broth between 34 °C and 38 °C (6.1) and observe the reaction after 24 h ± 3 h and after 48 h ± 3h.

In the case of a positive reaction, the colour of the medium changes from green to Prussian blue.

C.1.3 Dulcitol test

Inoculate dulcitol broth (see A.5) with 1 ml of a broth culture or from a fresh agar slant.

Incubate the inoculated broth between 34 °C and 38 °C (6.1) and observe the reaction after 24 h ± 3 h and after 48 h ± 3 h.

In case of a positive reaction (acidification), the colour of the medium becomes pink.

C.1.4 ONPG test

Culture the isolate to be tested on nutrient (or other) agar slants (see A.2) containing 1,0 % mass fraction lactose. Emulsify a large loopful of culture growth into 0,25 ml physiological saline solution to obtain a "heavy" suspension. Add 1 drop of toluene to each tube and shake well to liberate the enzyme.

Place the tubes between 34 °C and 38 °C (6.1) for 5 min.

Add 0,25 ml 0,013 3 mol/l ONPG solution (A.6) to each suspension to be tested. Incubate tubes between 34 °C and 38 °C (6.1) and examine the tubes at intervals up to 24 h. A yellow colour indicates a positive result.

C.1.5 Gelatinase test

Stab-inoculate nutrient gelatine medium (see A.12) with an inoculum from an "overnight" pure culture.

Incubate the tube between 34 °C and 38 °C (6.1) for 24 h ± 3 h.

Place the tube in melting ice or in the refrigerator for approximately 30 min. If the gelatine has been digested, the medium in the tube fails to solidify after refrigeration, which indicates the presence of gelatinase.

Alternatively the tube can be incubated at 25 °C for up to 1 week, checking every day for gelatine liquefaction. As gelatine can already liquefy at 28 °C, it is necessary to cool the incubated tube to be sure

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that the liquefaction is caused by gelatinase and not by the incubation temperature. It can be helpful to use an uninoculated tube as control.

C.2 Distinction between *Salmonella* Paratyphi B and *Salmonella* Paratyphi B biovar Java

To distinguish between *Salmonella* Paratyphi B and *Salmonella* Paratyphi B biovar Java, the reaction on D-tartrate should be tested. Alternatively a PCR test can be performed, as described in Reference [15].

For the D-tartrate reaction, the following procedure can be followed (Reference [1]).

— Culture the strain to be tested on a non-selective agar medium.

— Suspend colony material in an 8,5 g/l saline solution to a density of approximately 10^9 cfu/ml.

— inoculate 50 µl aliquots of each strain in two tubes with D-tartrate broth (see A.6). Inoculate also two control strains into two D-tartrate broths each. Controls: Positive = *Salmonella* Paratyphi B biovar Java, Negative = *Salmonella* Paratyphi B.

— Incubate the tubes aerobically (without shaking) between 34 °C and 38 °C (6.1) for 18 h ± 2 h.

— After incubation, examine the tubes for colour change. In the case of a positive reaction, the colour of the medium changes from blue to yellow.

— Add to one set of broths 0,5 ml of saturated lead acetate solution (see A.8) and leave the tubes to stand for a minimum of 30 min to allow the precipitate to settle.

— Then examine the tubes for the precipitate (typically after 1 h to 2 h). In the case of a positive reaction, the precipitate settles at the bottom of the tube and in the case of a negative reaction, the precipitate remains suspended in the broth. The test tubes are compared to the positive and negative control tubes. If the test is positive the strain is considered to be *Salmonella* Paratyphi B biovar Java and the second D-tartrate tube set up is discarded.

— If the test is negative, the second tube and its controls are incubated for a further 6 days (total incubation = 7 days).

— After the prolonged incubation, lead acetate is added to the tubes as above.

— Strains that are positive after 7 days are considered *Salmonella* Paratyphi B biovar Java and negative strains are considered as *Salmonella* Paratyphi B.

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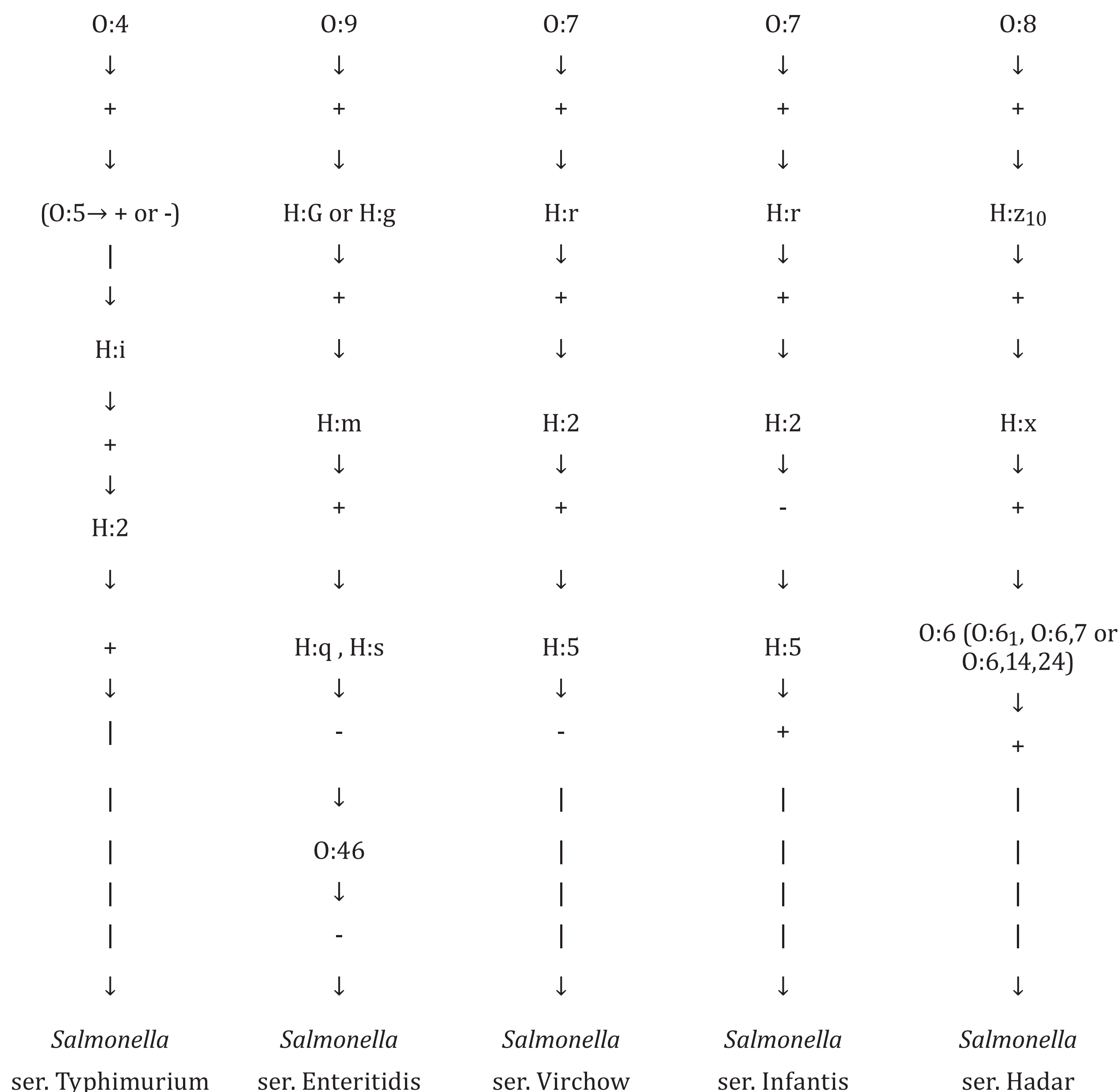
Annex D
(informative)

Schematic overview for serotyping five important public-health related *Salmonella* serovars



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Annex E
(informative)

Microtitre plate method for serotyping *Salmonella* spp.

E.1 General

The procedure described in this annex is based on Reference [21].

In laboratories that serotype a large number of *Salmonella* strains, a technique using microtitre plates combined with a semi-automated system of dispensing the antisera into the plates can be advantageous.

Overview of the method:

— Bacterial suspensions are prepared for the detection of O-antigens (O-suspensions) and H-antigens (H-suspensions). These are prepared by inoculating two brain–heart infusion broths (see A.9) for each strain being tested: one in a universal bottle for the O-suspension and one in a test tube for the H-suspension. The broths are incubated between 34 °C and 38 °C (6.1) for 4 h to 5 h.

— After incubation, the O-suspensions are steamed for 30 min and then diluted with an equal volume of 8,5 g/l saline solution.

— After incubation, the H-suspensions are killed by adding an equal volume of 1 % volume fraction formal saline (see A.10) and left standing at room temperature overnight before testing.

— Antisera are dispensed into 96 well round-bottomed microtitre plates by using a multipipette or a robotic system. Prepared plates can be sealed and stored at 5 °C (6.3) for a maximum of 2 weeks.

— The prepared O- and H-suspensions are manually dispensed into the prepared microtitre plates using a disposable pastette (plastic pipette) or stepper pipette with disposable tips.

— O-agglutination plates are sealed and incubated at 50 °C (6.9) for 18 h ± 3 h.

— H-agglutination plates are incubated for 2 h in a water-bath at 50 °C (6.9). Preferably the water-bath has a shelf without holes at the same level as the water.

— After incubation, the agglutinations are examined. The agglutinations settle in the bottom of the wells and are best viewed from underneath the plate using a microplate mirror.

Before use, the antisera are tested in microtitre plates to determine the titre for use.

Suggested antisera to use in the microtitre plate method are 23 O-antisera arranged in two microtitre plates with a saline control in the second plate. The first plate contains polyvalent O-antiserum (PSO), seven antisera for the groups 4,5,12; 6,7; 6,8; 9,12; 3,10; 13,22; 6,14,24 and four absorbed antisera 1, 20, 27 and 46. The second plate contains absorbed antisera 4, 5, 7, 8, 9, 10, 14, 15, 19, 22 and 23.

E.2 Microtitre plate 1 for O-antigens

Column of plate	1	2	3	4	5	6	7	8	9	10	11	12
Sera in each well of column	PSO	4,5,12	6,7	6,8	9,12	3,10	13,22	6,14,24	1	20	27	46

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E.3 Microtitre plate 2 for O-antigens

Column of plate	1	2	3	4	5	6	7	8	9	10	11	12
Sera in each well of column	4	5	7	8	9	10	14	15	19	22	23	Control

Each strain is tested against all the sera in both microtitre plates. Eight strains can be tested in each set of two microtitre plates.

To identify the H-antigens, each strain is tested against a set of 11 antisera and a saline control. The suggested antisera are polyvalent *Salmonella* H (PSH 1+2) containing antibodies for H = a through to H = z_{29} to cover phase 1 and antibodies for the phase 2 complex H = 1,2,5,6,7 (PSH 2). Four antisera for the complexes H = E;G;L;1,2,5,6,7 and six absorbed antisera for H = b,d,i,r,z,z_{10}.

E.4 Microtitre plate for H-antigens

Column of plate	1	2	3	4	5	6	7	8	9	10	11	12
Sera in each well of column	PSH 1+2	E	G	L	PSH2	b	d	i	r	z	Z_{10}	Control

The identification of phase 1 is complete for those strains that agglutinate with PSH 1+2 and one of the six absorbed antisera and no further testing for phase 1 is required. For strains that agglutinate with PSH and one of the four complexes, further testing is required to identify the single factor antigens. Strains that only agglutinate with PSH are tested against H = a,c,k,y,z_4,z_6 and z_{29} antisera.

After phase inversion, the H-antigen testing procedure is repeated to determine the antigens of the other phase.

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Annex F
(informative)

Examples of procedures for phase inversion

F.1 Simplified Sven Gard method

This method is derived from References [8][13].

Prepare a 55 mm diameter Petri dish (6.7) with 10 ml solidified swarm agar (A.3). Add 0,1 ml anti-*Salmonella* H drop by drop and spread over the surface with a sterile (glass) spatula. Inoculate the strain at a single point in the centre of the dish.

Incubate the inoculated plate between 34 °C and 38 °C (6.1) for 18 h to 21 h. The swarmed strain on the plate can be used to identify the second, uninhibited H-phase.

This simplified method usually enables the second phase to be identified at the first attempt. It also has the advantage of permitting swarm agar plates to be prepared and stored in advance and to be used as required for improving the expression of the H-antigens or for the phase induction.

F.2 Craigie tube method

F.2.1 General

The Craigie tube is used to demonstrate motility. This method consists of a wide screw-capped tube or bottle containing semi-solid agar (a mass fraction of 0,2 % to 0,4 % agar) in the centre of which is embedded a short, narrow tube open at both ends, in such a way that it projects above the agar (see Figure F.1). The strain to be tested is inoculated carefully into the inner tube. After incubation, sub-cultures withdrawn from the top of the agar outside the central tube yield a population of motile cells (Reference [5]).

F.2.2 Preparation of Craigie tube for phase inversion

— Place the Craigie tube medium (see A.11) in a steamer (or other appropriate means) for 30 min to melt the agar.

— Place the Craigie tube with molten agar in a water-bath at 47-50 °C (6.8) to equilibrate.

— Add 200 µl of the required antiserum to the Craigie tube.

— Mix well by shaking gently.

— Place the Craigie tube at 5 °C (6.3) to set.

F.2.3 Procedure for phase inversion using a Craigie tube

— Inoculate the Craigie tube with the *Salmonella* culture using a disposable plastic needle or straight wire (the culture is inoculated inside the inner tube).

— Incubate the Craigie tube between 34 °C and 38 °C (6.1).

— Examine the Craigie tube for growth after 24 h, 48 h, and 72 h.

— When growth appears on the surface of the agar (outside the central tube), wash the growth off into brain-heart infusion broth (see A.9).

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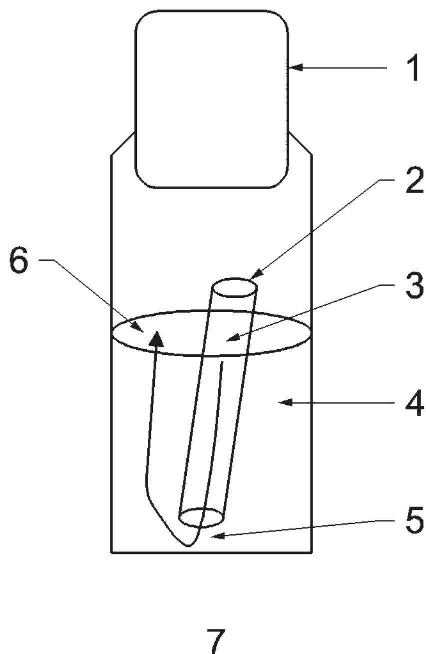
— The broth suspension is then tested for its H-antigen.



Key

1 cap
2 open end tube
3 point of inoculation
4 semi-solid medium
5 route of *Salmonella* after phase invasion
6 area to be subcultured
7 Craigie tube

Figure F.1 — Craigie tube method

F.3 Paper-bridge method

The procedure described in this subclause is taken from Reference [4], which is a modified procedure originally described in Reference [23].

See Figure F.2. A tryptone soya agar strip (1 cm wide) is removed to make a groove across the centre of a plate. Filter paper strips (3 mm × 3 cm) are arranged across this groove. Antiserum against the identified phase is spotted on to the middle of the paper-bridge (indicated by white circles). Bacterial strains are inoculated on the left end of the strips (indicated by white arrows). After incubation, phase variants, which express an alternative flagellar antigen, swarm toward the right end of the strip. After 16 h of incubation, bacterial growth can be seen around the edge of the filter paper (indicated by white triangular points). The strains inoculated in the plate shown in Figure F.2 are (A) *S.* Choleraesuis var. Kunzendorf (6,7:c:-); (B) *S.* Typhimurium (1,4,5,12:i:1,2); and (C) *S.* Stanley (4,5,12:d:1;2)

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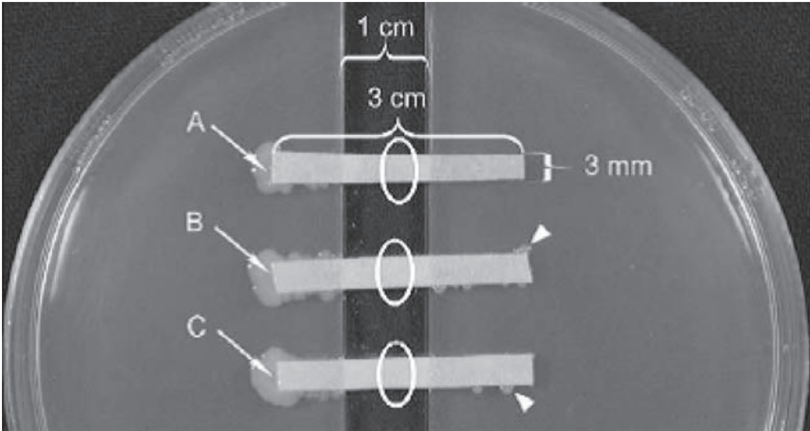

Figure F.2 — Paper-bridged method for *Salmonella* phase reversal

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Bibliography

[1] Alfredsson G.A., Barker R.M., Old D.C., Duguid J.P. Use of tartaric acid isomers and citric acid in the biotyping of *Salmonella* typhimurium. *J. Hyg. (Lond.)*. 1972, **70** pp. 651–666

[2] US Food and Drug officials. 2001. Bacteriological Analytical Manual. BAM R53: ONPG test. Available (viewed 2013-06-04) at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm062262.htm

[3] Brenner F.W., Villar R.G., Angulo F.J., Tauxe R., Swaminathan B. *Salmonella* nomenclature. *J. Clin. Microbiol.* 2000, **38** pp. 2465–2467

[4] Chiou C.S., Huang J.F., Tsai L.H., Hsu K.M., Liao C.S., Chang H.L. A simple and low-cost paper-bridged method for *Salmonella* phase reversal. *Diagn. Microbiol. Infect. Dis.* 2006, **54** pp. 315–317

[5] Craigie J. Studies on the serological reactions of flagella of *B. typhosus. J. Immun.* 1931, **21** p. 417

[6] Danan C., Fremy S., Moury F., Bohnert M., Brisabois A. 2009. Determining the serotype of isolated *Salmonella* strains in the veterinary sector using the rapid slide agglutination test. Cahiers de la Référence, N°.2, CR2-09M01, December 2009. Available (viewed 2013-06-04) at: http://www.afssa.fr/euroreference/numero2/index.htm

[7] Ewing W.H. *Edwards and Ewing's identification of Enterobacteriaceae*. New York: Elsevier, Fourth edition, 1986

[8] Gard S. Das Schwärmphänomen in der *Salmonella*-Gruppe und seine praktische Ausnützung. [The swarming phenomenon in the *Salmonella* group and its practical use]. *Z. Hyg. Infektionskr.* 1938, **120** pp. 615–619

[9] Grimont P.A.D., & Weill F.-X. 2007. Antigenic formulae of the *Salmonella* serovars, 9th edition.[3] WHO Collaborating Centre for Reference and Research on *Salmonella*. Paris: Institut Pasteur. Available (viewed 2013-06-04) at: http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089

[10] Guibourdenche M., Roggentin P., Mikoleit M., Fields P.I., Bockemühl J., Grimont P.A.D. et al. Supplement 2003–2007 (No. 47) to the White–Kauffmann–Le Minor scheme. *Res. Microbiol.* 2010, **161** pp. 26–29

[11] Hendriksen R.S., Mikoleit M., Carlson V.P., Karlsmose S., Vieira A.R., Jensen A.B., et al. WHO Global Salm-Surv External Quality Assurance System for Serotyping of *Salmonella* Isolates from 2000 to 2007. *J. Clin. Microbiol.* 2009, **47** pp. 2729–2736

[12] Kauffmann F. On the principles of classification and nomenclature of *Enterobacteriaceae. Int. Bull. Bacteriol. Nomencl. Taxon*. 1959, **9** pp. 1–6

[13] Koehn A. Technical modification of the swarming plate method according to Sven Gard in *Salmonella* diagnosis. *Zentralbl. Bakteriol.* 1970, **215** pp. 449–455

[14] Leifson E. Fermentation of sodium malonate as a means of differentiating *Aerobacter* and *Escherichia coli. J. Bacteriol.* 1933, **26** pp. 329–330

[15] Malorny B., Bunge C., Helmuth R. Discrimination of D-tartrate-fermenting and non-fermenting *Salmonella enterica* subsp. *enterica* isolates by genotypic and phenotypic methods. *J. Clin. Microbiol.* 2003, **41** pp. 4292–4297

[16] Popoff M.Y. *Guidelines for the preparation of Salmonella antisera. WHO Collaborating Centre for Reference and Research on Salmonella*. Paris: Institut Pasteur, 2001

3) Supplements to the White-Kauffmann-Le Minor scheme are published in *Res. Microbiol.*, a publication of the Institut Pasteur (formerly *Ann. Inst. Pasteur/Microbiol.*), e.g. Reference [10].

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[17] POPOFF M.Y., & LE MINOR L.E. Genus XXXIII. *Salmonella* Lignières 1900, 389. InBRENNER D.J., KRIEG N.R., STALEY J.T. editors. *Bergey's manual of systematic bacteriology*, 2nd edition, Vol. 2, *The Proteobacteria*, Part B The *Gammaproteobacteria*. New York, NY: Springer, 2005, pp. 764–799

[18] POPOFF M.Y., BOCKEMÜHL J., BRENNER F.W. Supplement 1998 (No. 42) to the Kauffmann-White scheme. *Res. Microbiol.* 2000, **154** pp. 63–65

[19] POPOFF M.Y., BOCKEMÜHL J., GHEESLING L.L. Supplement 2001 (No. 45) to the Kauffmann-White scheme. *Res. Microbiol.* 2003, **151** pp. 173–174

[20] American Society for Microbiology. Publications Board meeting minutes. *Salmonella* nomenclature. *ASM News*. 1999, **65** p. 769

[21] SHIPP C.R., & ROWE B. A mechanized microtechnique for *Salmonella* serotyping. *J. Clin. Pathol.* 1980, **33** pp. 595–597

[22] TINDALL B.J., GRIMONT P.A.D., GARRITY G.M., EUZÉBY J.P. Nomenclature and taxonomy of the genus *Salmonella. Int. J. Syst. Evol. Microbiol.* 2005, **55** pp. 521–524

[23] WANG T.K., TSENG T.C., LEE J.H., WANG W.T., TSAI J.L., HO S.I., PAN T.M. Analysis of Salmonella serovars in Taiwan by the phase induction method] [Article in Chinese]. *Zhanghua Min Guo Wei Shang Wu Ji Mian Yi Xue Za Zhi* [Chin. J. Microbiol. Immunol.]. 1994, **27**, pp. 13–24

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