Bacterial Serotyping Guide for *Salmonella*

BIO-RAD

Minimizing Risk

The monitoring of veterinary diseases and quality control of industrial products are public health issues. Microbial populations which cause infection vary over time depending on manufacturing and transport conditions. Consequently, to prevent such risks, it is important to take an active role in the overall food chain monitoring process. As part of such an approach, studying bacteria from an epidemiological standpoint is essential for monitoring and anticipating dynamic changes in microbial structures. An antiserum is a high performance reagent enabling accurate identification of bacteria. It thus makes classification and evaluation of changes in such microbial populations possible.

Serotyping as an Identification Tool

Serotyping (serological typing) is based on the long-standing observation that microorganisms from the same species can differ in the antigenic determinants expressed on the cell surface. Serotyping is one of the classic tools for epidemiological study and is applied to numerous species that express different serotypes, such as: *Escherichia coli, Pseudomonas aeruginosa, Salmonella* species, *Shigella* species, *Yersinia* and *Vibrio cholerae.*

Expertise in Serotyping

With an extensive range of immune sera for serotyping, Bio-Rad offers a wide range of reagents serving the needs of veterinary, food and service laboratories. The method's ease of use enables every laboratory to perform identification of bacterial strains. Data collected promotes enhanced prevention of veterinary, human, and industrial risks.

Identification of *Salmonella* by Serotyping

Salmonellae are Gram-negative, flagellated, facultative anaerobic bacilli possessing three major antigens: H or flagellar antigen, O or somatic antigen, and Vi (capsular) antigen (possessed by only a few serovars). The different species are serotyped according to these three different antigens.

- **H (flagellar) antigen** may occur in either or both of two forms, phase 1 and phase 2. There are over 1800 known serovars which current classification considers being separate species. The organisms tend to change from one phase to the other.
- **O (somatic) antigens** occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface.
- **Vi (capsular) antigen** is a superficial antigen overlying the O antigen (additional surface antigen). It is present in a few serovars, the most important being *Salmonella* Typhi, but also present in *Salmonella* Paratyphi C and *Salmonella* Dublin.

Once the O, H-phase 1 and H-phase 2 are identified, the antigenic formula can be used to identify the serotype by referring to a **Kauffman-White reference catalog.**

- The formula gives the O antigen(s) first followed by the H antigen(s). O antigens, Vi (when present), H antigens phase 1, H antigens phase 2 (when present).
- Colons separate the major antigens and commas separate the components of the antigens.
- Further conventions:
  - Underlined O factor is encoded by a bacteriophage (lysogenic strain)
  - [ ]: square bracketed factor may or may not be present (not phage-encoded)
  - { }: curly bracketed factor never coexists with others (exclusive)
  - ( ): parenthesis around a factor indicate weakly agglutinable factor

Examples

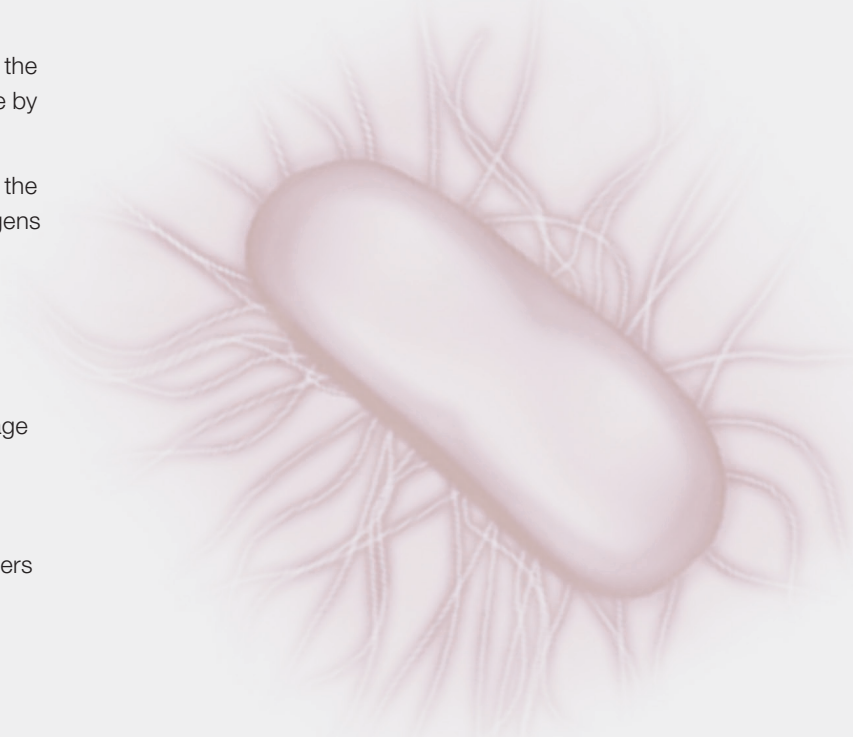
***Salmonella* enterica serotype Typhimurium: 1,4,[5],12:i:1,2**

This strain has the O antigen factors 1, 4, [5], and 12, the flagellar H antigen i (1st phase) and the flagellar H antigens 1 and 2 (2nd phase).

***Salmonella* enterica serotype Lagos: 1,4,[5],12:i:1,5**
This strain has the O antigen factors 1, 4, [5], and 12, the flagellar H antigen i (1st phase) and the flagellar H antigens 1 and 5 (2nd phase).

***Salmonella* enterica serotype Virchow: 6,7,14:r:1,2**
This strain has the O antigen factors 6, 7 and 14; the flagellar H antigen r (1st phase) and the flagellar H antigens 1, 2 (2nd phase).

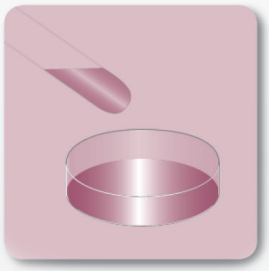
For more examples, please refer to our Quick Guide (#14-0700) including a table with some typical *Salmonella* serotypes isolated in food products.



How to Perform *Salmonella* Serotyping

A Simple Protocol

1

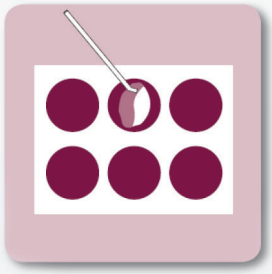


After confirmation, isolate colonies on TSI slant (or recommended agar) for identification by antisera. It is important to use pure cultures.

2

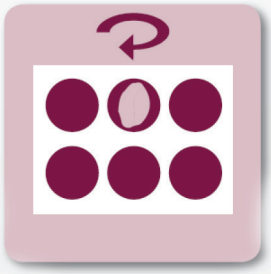
Place 1 free falling drop of antisera onto a slide for agglutination and add a pure colony.

3



Mix reagents thoroughly.

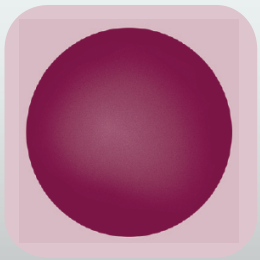
4



Rock the slide in the circular motion for 30 seconds and observe for agglutination.

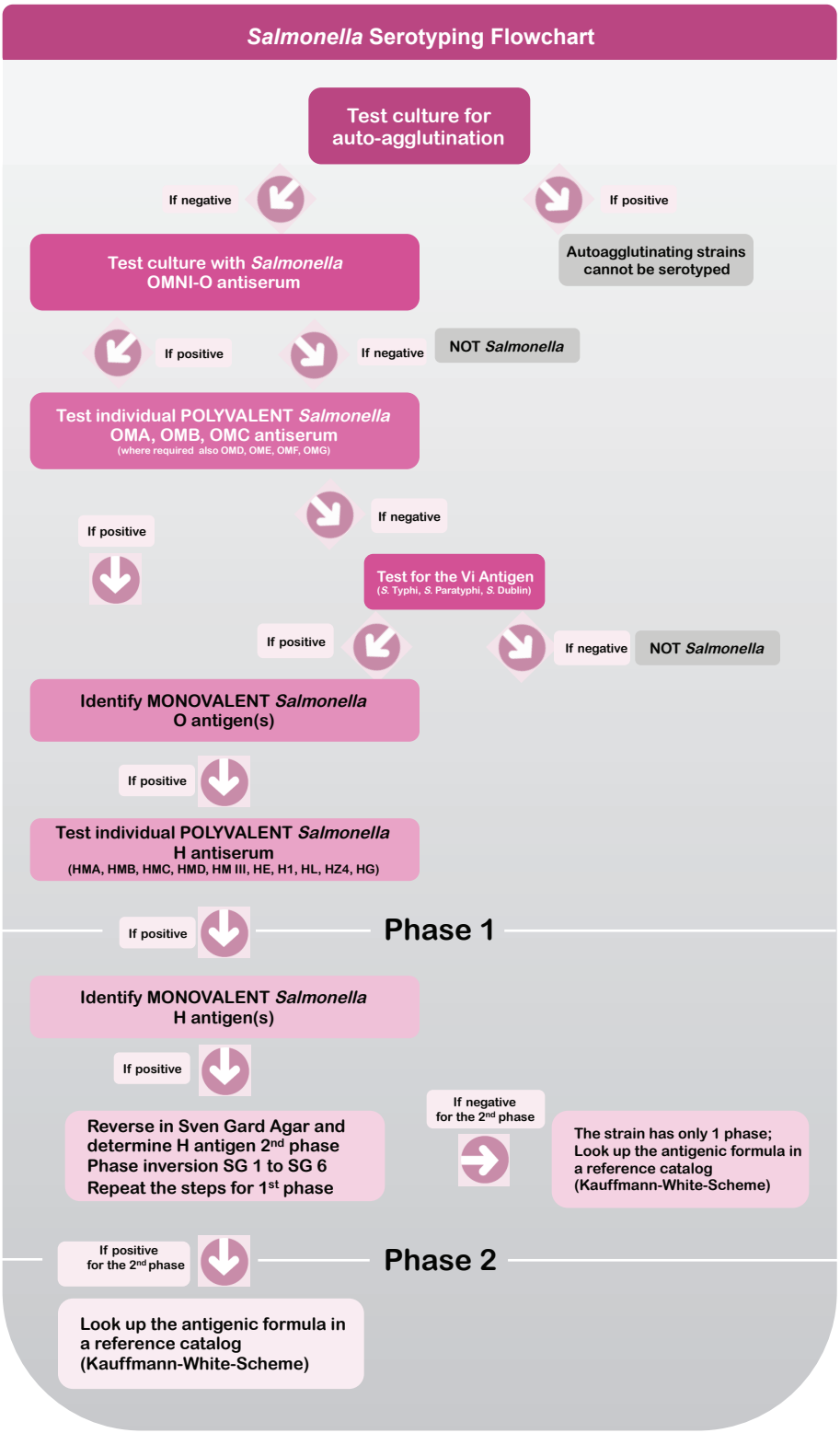
- Serotyping is performed after identification of the species on a fresh, pure culture of *Salmonella* isolated on a non-selective agar medium. There are several media recommended for use, including Müller-Hinton or Nutrient Agar, TSI (Triple Sugar Iron) and/or LIA (Lysine Iron Agar) slants or TSA (Tryptic Soy Agar).
- Where polyvalent and monovalent antisera are available, start by testing agglutination with polyvalent sera, then with the specific monovalent sera corresponding to the mixture giving marked agglutination.
- Agglutination should appear between 1–10 seconds. If agglutination occurs > 60 sec, the antigens can not be identified correctly.

Easy Interpretation



No agglutination = NEGATIVE result

Agglutination = POSITIVE result



1. Test the culture for auto-agglutination

- Test the culture first in Physiological Water/Saline; strains that produce auto-agglutination cannot be serotyped.
- In addition, all strains should be tested with the *Salmonella* Omni-O antiserum which contains antigens A – 60 for the presumptive identification of O-agglutinable strains of Salmonellae.
- Agglutination of a strain of *Salmonella* with Omni-O antiserum indicates that the strain is O-agglutinable and can be serotyped with specific sera.

2. Test for the O antigens

- Begin by testing the isolate with polyvalent O antiserum. The majority (about 98%) of *Salmonella* encountered in warm-blooded animals possess an O antigen corresponding to the agglutinins contained in OMA, OMB and OMC sera.
- When agglutination occurs with one of these 3 groups, the isolate is positive for that group.
- The individual monovalent O antisera are used to identify the O antigen(s).
- Repeat the agglutination step by testing the isolate in each monovalent O antiserum present in the group.

Example: The polyvalent O antiserum OMA shows agglutination therefore the following monovalent O antisera must be tested: O:1,2; O:4,5; O:9, O:46; O:3,10,15; O:1,3,19

- When a strain does not agglutinate the OMA, OMB or OMC polyvalent sera, it is recommended to test this strain with Vi serum and the other polyvalent O sera.
- If a Vi positive reaction is observed, the bacterial suspension must then be heated to 100°C for 30 minutes, before repeating the test with polyvalent OMA, OMB and OMC sera and the corresponding monovalent sera to define the O antigen.

3. Test for the H Antigen — Phase 1

- Begin by testing the isolate with a polyvalent H antiserum (HMA – HG). When agglutination occurs with one of these groups, the isolate is positive for that group.
- The individual monovalent H antiserum is used to identify the H antigen.
- Repeat the agglutination step by testing the isolate in each monovalent H antiserum present in the group.

Example: The polyvalent H Antiserum HMA shows agglutination therefore the following monovalent H antisera must be tested: a; c; d; l; z10.

Once the first H antigen is identified, a phase inversion on the isolate must be performed to force the organism to repress its dominant H phase and grow in the second phase.

4. Phase Inversion – Sven Gard Method

Sven Gard medium is used during serotyping of *Salmonella* to demonstrate the inapparent H antigen phase of biphasic *Salmonella* (Sven Gard method). Sven Gard agar should be used with the following *Salmonella* antisera: SG 1 to SG 6.

Example:

H:1,2 antigens were identified in a culture → SG6 (1,2 + 1,5 + 1,7 + z6) serum is used for phase inversion.

H:r antigen is identified in a culture → SG4 (r + z) antiserum is used for phase inversion.

5. Test for the H Antigen — Phase 2

- A culture at the periphery of the invasion zone of the Sven Gard agar should be taken.
- Start testing again by using the H polyvalent antisera (HMA – HG). If there is no agglutination, this serotype contains only one phase.
- If one of these groups shows agglutination, define the specific H phase by using the relevant H monovalent antisera.

As the antigenic formula with O, H – phase 1 and H – phase 2 are identified, the serotype is now specified by referring to a reference catalog, such as the Kauffman-White scheme.

Serotype	O Antigens	H Antigens Phase 1	H Antigens Phase 2
Agona	1, 4, [5], 12	f, g, s	[1, 2]
Anatum	3, {10} {15} {15, 34}	e, h	1, 6
Bareilly	6, 7, 14	y	1, 5
Blockley	6, 8	k	1, 5
Bovis Morbificans	6, 8 ,20	r, [i]	1, 5
Brandenburg	1, 4, 12	e, h	e, n, z15
Bredeney	1, 4, 12, 27	l, v	1, 7
Chester	1, 4, [5], 12	e, h	e, n, x
Derby	1, 4, [5], 12	f, g	[1, 2]
Dublin	1, 9, 12, [Vi]	g, p	-
Enteritidis	1, 9, 12	[f], g, m, [p]	[1, 7]
Gallinarium	1, 9, 12	-	-
Glouchester	1, 4, 12, 27	i	l, w
Hadar	6, 8	z10	e, n, x
Heidelberg	1, 4, [5], 12	R	1, 2
Indiana	1, 4, 12	z	1, 7
Infantis	6, 7, 14	R	1, 5
Javiana	1, 9, 12	l, z28	1, 5
Kentucky	8, 20	i	z6
Kottbus	6, 8	e, h	1, 5
Lagos	1, 4, [5], 12	i	1, 5
Lille	6, 7, 14	z38	-
Livingstone	6, 7, 14	d	l, w
Mbandaka	6, 7, 14	z10	e, n, z15
Meleagridis	3, {10} {15} {15, 34}	e, h	l, w
Montevideo	6, 7, 14	g, m, [p], s	[1, 2, 7]
Muenchen	6, 8	d	1, 2
Newport	6, 8, 20	e, h	1, 2
Orion	3, {10} {15} {15, 34}	y	1, 5
Paratyphi B	1, 4, [5], 12	b	1, 2
Saintpaul	1, 4, [5], 12	e, h	1, 2
Senftenberg	1, 3, 19	g, [s], t	-
Stanley	1, 4, [5], 12, [27]	d	1, 2
Thomson	6, 7,14	k	1, 5
Typhimurium	1, 4, [5], 12	i	1, 2
Virchow	6, 7	r	1, 2
Weltervreden	3, {10} {15}	r	z6

Bio-Rad provides a complete solution for the detection and confirmation of *Salmonella* spp. and a large panel for the serological identification of a variety of *Salmonella* serotypes.

Detect and Identify *Salmonella* in 4 Simple Steps

Sample enrichment 1

Standard Methods:
EN ISO 6579/A1
FDA BAM
USDA FSIS MLG
MFHPB-20

OR

RAPID´*Salmonella* Agar

OR

iQ-Check Prep, CFX96 Deep Well & and iQ-Check Kits

Detection of *Salmonella* 2

***Salmonella* Latex**
Oxidase Test
ONPG

Biochemical confirmation 3

Omni-O / Vi
Polyvalent / Monovalent O
Polyvalent / Monovalent H
Phase inversion

Serological identification 4

The product range includes the required enrichment media. Detection can be performed by either classical standard reference methods or by one of Bio-Rad´s alternative validated rapid methods, iQ-Check® real-time PCR solution or RAPID´Chromogenic media. Several easy-to-use confirmation and identification tests are also available.