

# SALMONELLA ANTISERA

STATENS  
SERUM  
INSTITUT



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# INTRODUCTION

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## Background

*Salmonella* is a leading cause of food poisoning and typhoid fever throughout the world. The increased occurrence of *Salmonella* infections within the last decade has accentuated the need for serotyping of *Salmonella* as a base for proper diagnosis, identification of sources of infection and food control.

*Salmonella* is a Gram-negative, rod-shaped bacteria. The genus consists of more than 2500 different serotypes described in the Kauffmann-White scheme<sup>1</sup>. Determination of O (somatic) and H (flagellar) antigens by using specific antisera enables identification of the antigen combinations and thus differentiation of the many serotypes.

For more than 70 years Statens Serum Institut (SSI) has been engaged in the development of the *Salmonella* typing system and in the production of diagnostic *Salmonella* antisera. SSI Diagnostica offers the full product range of *Salmonella* antisera for determination of all serotypes described in the Kauffmann-White Scheme.

## Description

The SSI *Salmonella* antisera are intended for serological confirmation and serotyping of *Salmonella*. Our product range includes more than 200 different *Salmonella* antisera, which are divided into O group pool, O group, O factor, H phase pool, H phase, H factor, H:R-phase and H phase inversion antisera. All antisera for slide agglutination are delivered in 3 mL bottles sufficient for 150 tests. Our antisera for serological confirmation and our O group pool and H phase pool antisera are also delivered in 1 mL bottles sufficient for 50 tests.

The diagnostic O and H antisera are polyclonal and raised by immunisation of rabbits. The Vi antibody is monoclonal and concentrated from a hybridoma culture supernatant.

Advantages of SSI® *Salmonella* antisera:

- Intended for slide agglutination
- Only 20 µL antiserum is used per test
- The result is read within 10 seconds
- Our antisera are carefully controlled and free of any known cross-reactions
- High titer antiserum is available on request

SSI Diagnostica also offers you the H antisera for phase inversion which are high titered and intended for use in swarm agar plates. These antisera are delivered in 3 mL bottles sufficient for 30 tests.

## Assortment

- Antisera for serological confirmation
- More than 200 different antisera (O group pool, O group, O factor, H phase pool, H phase, H factor, H:R-phase and H phase inversion sera) for partial or full serotyping
- Sero-Quick Group kit for serotyping to the serogroup level
- Sero-Quick ID kit for serotyping of *S. Enteritidis* and *S. Typhimurium*

# METHODS

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## Principle of Serotyping

Antigen-antibody complexes are formed (agglutination) when a bacterial culture is mixed with a specific antiserum directed against bacterial surface components. The complexes are usually visible to the naked eye which allows for easy determination of O and H antigens by slide agglutination. Some cultures are monophasic and may be directly H typed, whereas the second phase in a diphasic culture is determined after phase inversion (the Svend Gard method<sup>2</sup>). After full serotyping of the *Salmonella* culture the name of the serotype can be determined by using the Kauffmann-White Scheme<sup>4</sup>. Slide agglutination and phase inversion is described on the next two pages.

## Slide Agglutination

The slide agglutination test is done on a glass slide and read with the naked eye in front of a light source against a black background. *Salmonella* antisera from SSI Diagnostica have a quick reaction time and reaction can be read within 10 seconds.

### Procedure

1. Add a small drop of antiserum (approx. 20 µL) on a glass slide and mix it with the *Salmonella* culture.
2. Tilt the slide for 5 - 10 seconds.
3. A positive reaction is seen as visible agglutination, whereas a negative reaction is seen as homogeneous milky turbidity (Figure 1).

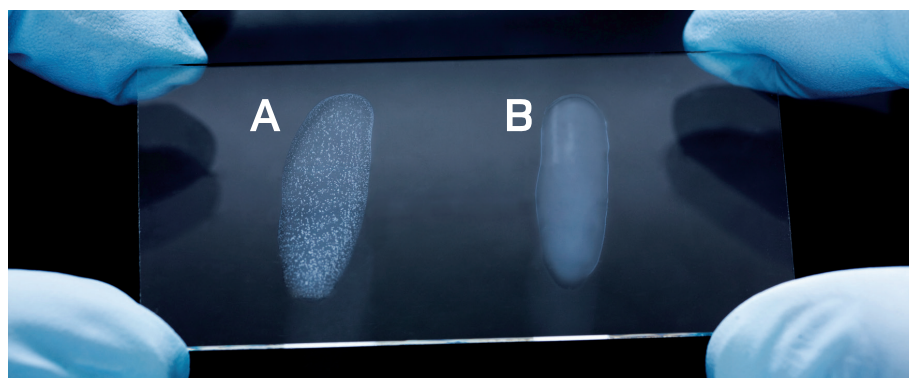


Figure 1. Sample A is a positive reaction and sample B is a negative reaction.

## Phase Inversion

### Description

Many *Salmonella* serotypes are diphasic which means that they can appear in two phases. However, a *Salmonella* bacterium expresses only one phase at a time, e.g. *S. Typhimurium* [1,4,[5],12:i:1,2] either expresses the phase 1 antigen H:i or the phase 2 antigens H:1,2. In a *Salmonella* culture there is usually only one dominating phase which is called phase 1 and this phase can be determined on swarm agar without antiserum. Phase 2 is determined by adding the phase 1 corresponding phase inversion antiserum to the swarm agar which allows the bacteria that express the phase 2 H antigens to swarm.



# METHODS

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## Swarm agar

Swarm agar (Schwörmagar) is a soft nutrient-rich medium that allows motile *Salmonella* to swarm. The medium is used with or without phase inversion antiserum in order to determine the H-phases of *Salmonella* (the Sven Gard method<sup>2</sup>). SSI Diagnostica swarm agar can be purchased in:

- 200 mL bottles (Schwörmagar art. no. 29565)
- 60 mL bottles (Schwörmagar, art. no. 82491)

## Procedure

### Day 1

- Pour 10 mL swarm agar into a petri dish (6 cm in diameter).
- Leave the petri dish on a levelled table to solidify.
- Inoculate the plate in the centre with a loop full of *Salmonella* culture without breaking the surface of the medium, and incubate the plate on a levelled table at 37 °C overnight.

### Day 2

- The phase 1 H antigens are determined by doing slide agglutinations with culture from the periphery of the swarm.
- Apply 2 drops of phase inversion antiserum against the phase 1 H antigens to a petri dish and mix with 10 mL swarm agar (see Figure 2).
- When the swarm agar has solidified, inoculate the plate and leave it at 37 °C overnight as described above.

### Day 3

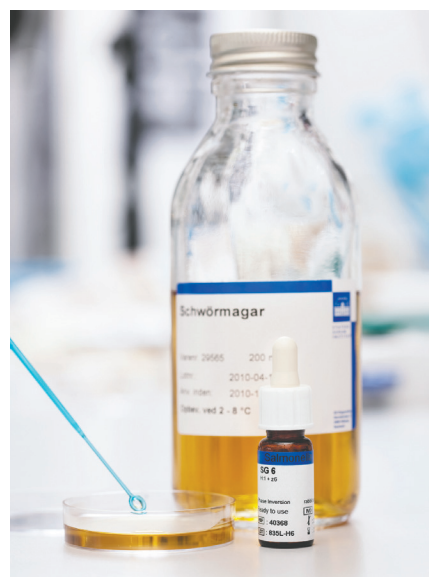
- The phase 2 H antigens are determined by doing slide agglutination with culture from the periphery of the swarm.



Figure 2 A. Add phase inversion anti-serum



B. Add swarm agar



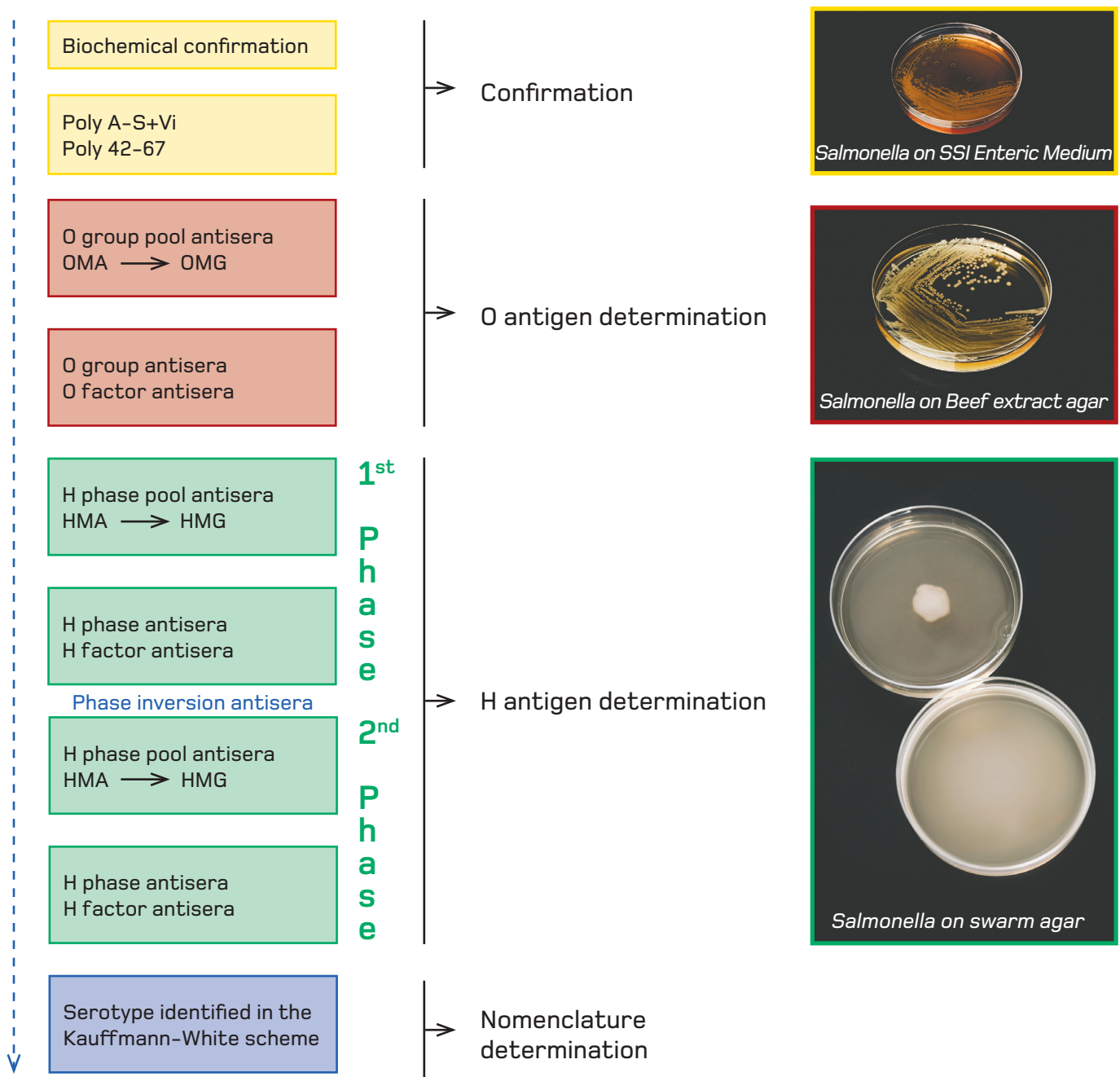
C. Mix well

# FULL SEROTYPING

The following two flowcharts show the steps in a full serotyping of a *Salmonella* culture. The first flowchart shows the procedure in general whereas the second flowchart describes all steps in the serotyping of *S. Enteritidis* and *S. Virchow*, respectively.

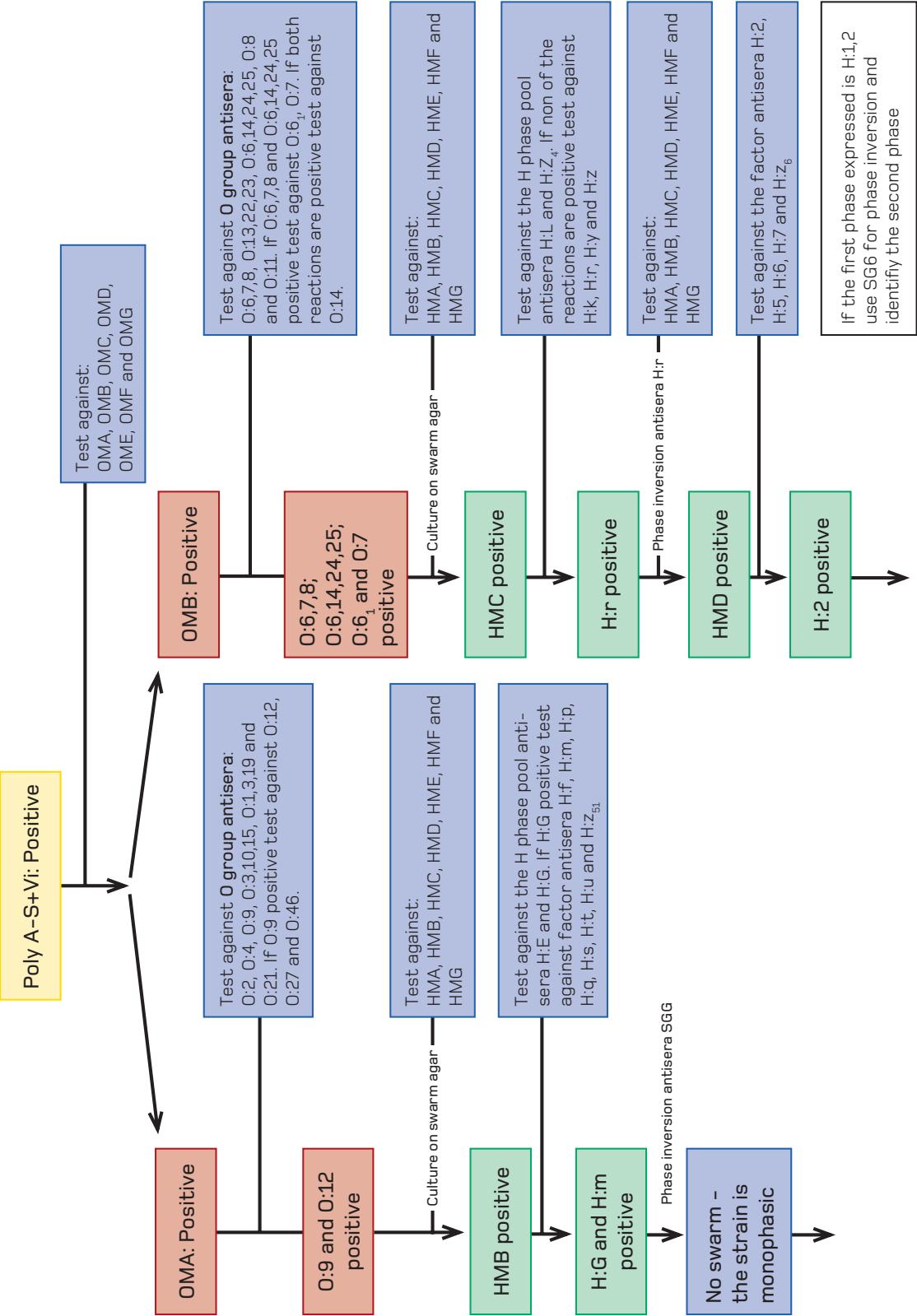
Some *Salmonella* O antisera will agglutinate with other bacteria, e.g. *Citrobacter* spp., *Proteus* spp. or *Shigella* spp. as their O antigens can be very similar to the *Salmonella* O antigens. It is therefore crucial to confirm the identification of the *Salmonella* genus with for example a biochemical test, before the serotyping is initiated.

## Salmonella Serotyping Flowchart



# FULL SEROTYPING

## Full Serotyping of *S. Enteritidis* and *S. Virchow*



Serotype: *Salmonella* Enteritidis 1,9,12:g,m:-

Serotype: *Salmonella* Virchow 6,7,14:r:1,2

# SALMONELLA ANTISERA KITS

SSI Diagnostica offers a *Salmonella* O grouping kit and a *Salmonella* ID kit. The *Salmonella* group kit is used to determine the most common *Salmonella* groups whereas the *Salmonella* ID kit is used to identify the two most commonly occurring *Salmonella* serotypes, *S. Enteritidis* and *S. Typhimurium*. Both kits are mainly used in routine laboratories.



## Available products

- Article No. 60898: *Salmonella* Sero-Quick Group kit, 1 box
- Article No. 62984: *Salmonella* Sero-Quick ID kit, 1 box



# SALMONELLA ANTISERA KITS

## Sero-Quick Group Kit

For quick serotyping of *Salmonella*

### Description

The *Salmonella* Sero-Quick Group kit consists of eight antisera and is a screening kit for identifying *Salmonella* isolates to the serogroup level. The kit includes antisera against the most common serogroups of *Salmonella*, serogroup A-G and the capsule antigen Vi. Antisera are delivered in bottles containing ready to use dilutions and the volumes of the antisera are differentiated according to common use. The antisera can be renewed separately. Around 100 tests can be performed with a *Salmonella* Sero-Quick Group kit.

### Background

*Salmonella* serotyping can be used as a surveillance tool to identify *Salmonella* serogroups, either as preliminary grouping, when full serotyping can be performed by a Human or Veterinary Reference Centre or in the food industry as a screening technique for *Salmonella*. The most common serogroups are B (*S. Typhimurium*) and D, (*S. Enteritidis*). Vi and serogroup A are not common, but clinically important because they are expressed by *S. Typhi* and *S. Paratyphi* respectively.

### Application

The *Salmonella* Sero-Quick Group kit is intended for slide agglutination of *Salmonella* cultures after overnight growth on suitable culture medium, e.g. beef extract agar. We recommend the procedure described in Figure 3.

Table: Designation of O groups

Serogroup	Antigen present
A	O:2
B	O:4
C	O:7 or O:8
D	O:9 or O:9,46 or O:9,46,27
E	O:3,10 or O:1,3,19
F	O:11
G	O:13

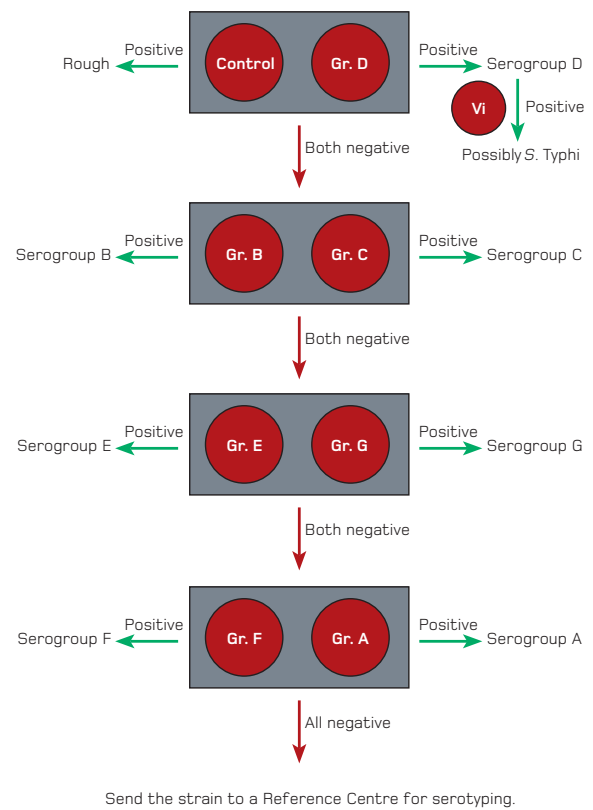


Figure 3. Serotyping with *Salmonella* Sero-Quick

# SALMONELLA ANTISERA KITS

## Sero-Quick ID Kit

For quick identification of *S. Enteritidis* and *S. Typhimurium*

### Description

The *Salmonella* Sero-Quick ID kit consists of eight antisera, and is the minimum of antisera needed for identification of *S. Enteritidis* (1,9,12:g,m:-) and *S. Typhimurium* (1,4,[5],12:i:1,2).

The antisera are delivered in bottles containing ready to use dilutions and can be renewed separately. Around 100 tests can be performed with the Sero-Quick ID kit.

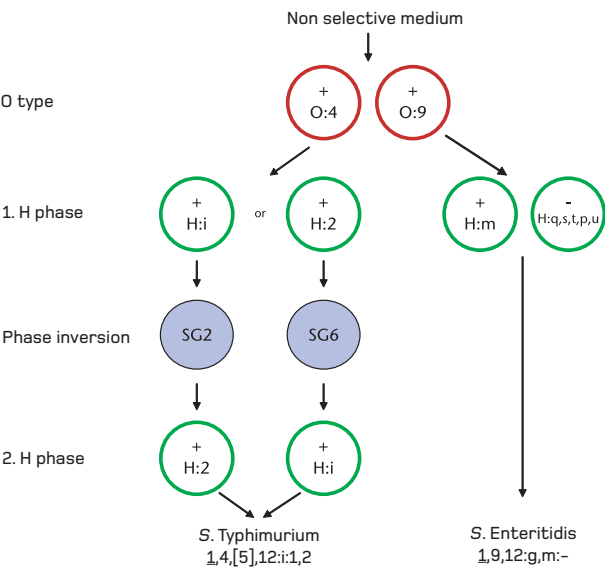
### Background

*S. Enteritidis* and *S. Typhimurium* are the most common serotypes and constitute 81 % of all *Salmonella* strains isolated from humans<sup>3</sup>. It is therefore highly important to be able to serotype these strains quickly.

### Application

The eight antisera are used as indicated in Figure 4. Start using the O antisera and subsequently the H antisera and the phase inversion antisera. The H:q,s,t,p,u antiserum is used to exclude that the antigens H:s, H:t and H:q do not combine with antigen combination H:g,m in serogroup O:9 (D1).

The serotyping may be expanded to identify additional serotypes, and SSI Diagnostica offers assistance in composing a panel covering the specific needs of our customers.



Reaction			
	<i>S. Enteritidis</i>	<i>S. Thyphimurium</i>	Culture medium
O antisera			
O:4	-	+	beef extract agar
O:9	+	-	
H antisera			
H:i	n.p.	+	beef extract agar or swarm agar
H:m	+	n.p.	
H:2	n.p.	+	
H:q,s,t,p,u	-	n.p.	
Phase inversion antisera			
SG2	n.p.	+ <sup>a</sup>	swarm agar
SG6	n.p.	+ <sup>b</sup>	

**Figure 4.** Serotyping of *Salmonella Enteritidis* and *Salmonella Typhimurium*.

n.p. = not performed

a = SG2 is used for phase inversion of strains initially expressing H:i

b = SG6 is used for phase inversion of strains initially expressing H:1,2

### Limitations

*S. Hilligdon* (9,46:g,m:-) can not be distinguished from *S. Enteritidis* using this kit since both serotypes will be positive in O:9 and H:m and negative in H:q,s,t,p,u. The prevalence of *S. Hilligdon* is however very low.

# GENERAL INFORMATION

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## Quality

The quality system of antisera production is certified according to DS/EN ISO 13485 and DS/EN 9001. The antisera are CE labelled.

## Support

The Danish *Salmonella* Reference Centre provides professional assistance with serotyping of *Salmonella* strains. Please enquire about the charges related to this service.

## Storage and Shelf Life

The antisera should be kept in a dark place at 2 - 8 °C. Expiry date is minimum 2 years from date of delivery.

## References

- 1 Michel Y. Popoff and L. Le Minor. Antigenic formulas of the *Salmonella* serovars, 8. Ed. (2001 with supplements). WHO Collaborating Centre for Reference and Research on *Salmonella*. Institut Pasteur, Paris, France.
- 2 Gard, S. Das Schwärmphänomen in der *Salmonella*-gruppe und seine praktische Ausnützung. Zeit. f. Hyg. Inf. Krankh. 1938, 120;615-619.
3. Herikstad et al. 2002. Epidemiol. infect. 129, 1-8. *Salmonella* Surveillance: a global survey of public health serotyping.

## Ordering and Information

For ordering please visit our web shop at [shop.ssi.dk](http://shop.ssi.dk) or contact one of our distributors listed on [www.ssi.dk/ssidiagnostica](http://www.ssi.dk/ssidiagnostica).

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