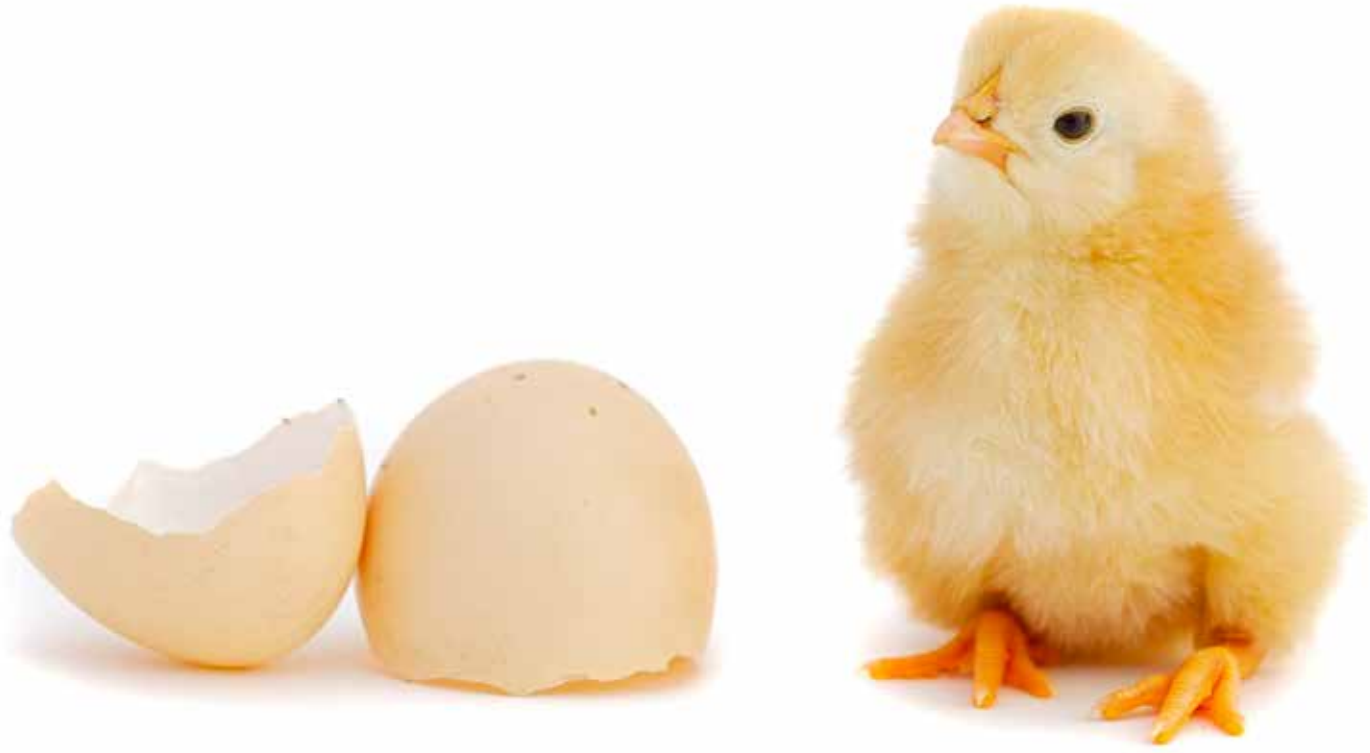
SSI DIAGNOSTICA

SALMONELLA ANTISERA



Comprehensive range for complete serotyping according to the Kauffmann-White scheme

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Improving
Microbiology

CONTENT

INTRODUCTION

BACKGROUND

Diarrhoeal diseases are the most common illnesses resulting from unsafe food, with 550 million people falling ill each year, including 220 million children under the age of 5 years. *Salmonella* is 1 of the 4 key global causes of diarrhoeal diseases[1]. Hence the need for serotyping of *Salmonella* to improve diagnosis, identify sources of infection and improve food safety.

Salmonella is a Gram-negative, rod-shaped bacteria. The genus consists of about 2,600 different serotypes described in the Kauffmann-White scheme[2]. 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 75 years SSI Diagnostica has been engaged in the development of the *Salmonella* typing system and in the production of diagnostic *Salmonella* antisera. We offer the full product range of *Salmonella* antisera to determine all the serotypes described in the Kauffmann-White Scheme.

DESCRIPTION

Our *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 available in 3 mL bottles sufficient for 150 tests. Our antisera for serological confirmation, O group pool and H phase pool antisera are also available in 1 mL bottles sufficient for 50 tests.

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

Advantages of SSI Diagnostica *Salmonella* antisera

- Quick results, read within 10 seconds
- Only 20 µL antiserum is used per test
- Our antisera are quality controlled and free of any known cross-reactions
- High quality with clear reliable results hence no ambiguous interpretation
- IVD certified and CE marked

Our H antisera for phase inversion are high titer and intended for use in swarm agar plates. These antisera are available in 3 mL bottles sufficient for 30 tests.

PRODUCT RANGE

- Poly 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 complete serotyping
- Sero-Quick Group kit for serotyping to the serogroup level
- Sero-Quick ID kit for serotyping of *S.* Enteritidis and *S.* Typhimurium

METHODS

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[3]). After full serotyping of the *Salmonella* culture the nomenclature of the serotype can be determined by using the Kauffmann-White Scheme[2]. Slide agglutination and phase inversion are described in 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. Our *Salmonella* antisera has a quick reaction time and the result is 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).

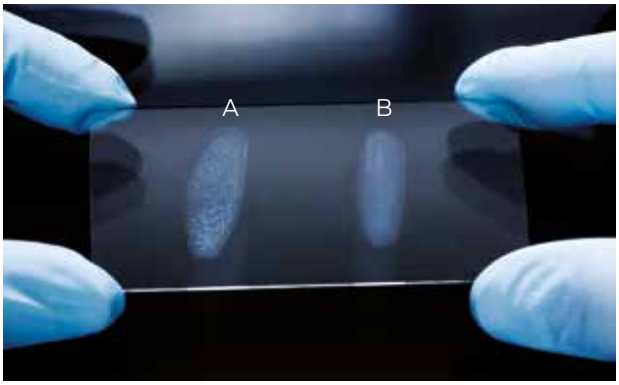


Figure 1. Sample A is a positive reaction and sample B is a negative reaction.

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 adding phase inversion antiserum. Phase 2 is determined by adding the corresponding phase inversion antiserum for phase 1 to the swarm agar. This allows the bacteria to swarm by expressing the phase 2 H antigens.

Swarm agar

Swarm agar (Schwörm agar) is a soft nutrientrich 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[3]). SSI Diagnostica swarm agar products are:

- 200 mL bottles (Schwörm agar art. no. 62411)
- 60 mL bottles (Schwörm agar, 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.

Day 3

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

Figure 2. Procedure for use of Swarm agar

A. Add phase inversion antiserum

B. Add swarm agar

C. Mix well

FULL SEROTYPING

Complete serotyping of a *Salmonella* culture is described in the 2 flowcharts below. The first flowchart shows the standard serotyping procedure. The second flowchart describes all the steps of serotyping the *S.* Enteritidis and *S.* Virchow, respectively.

Note: 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

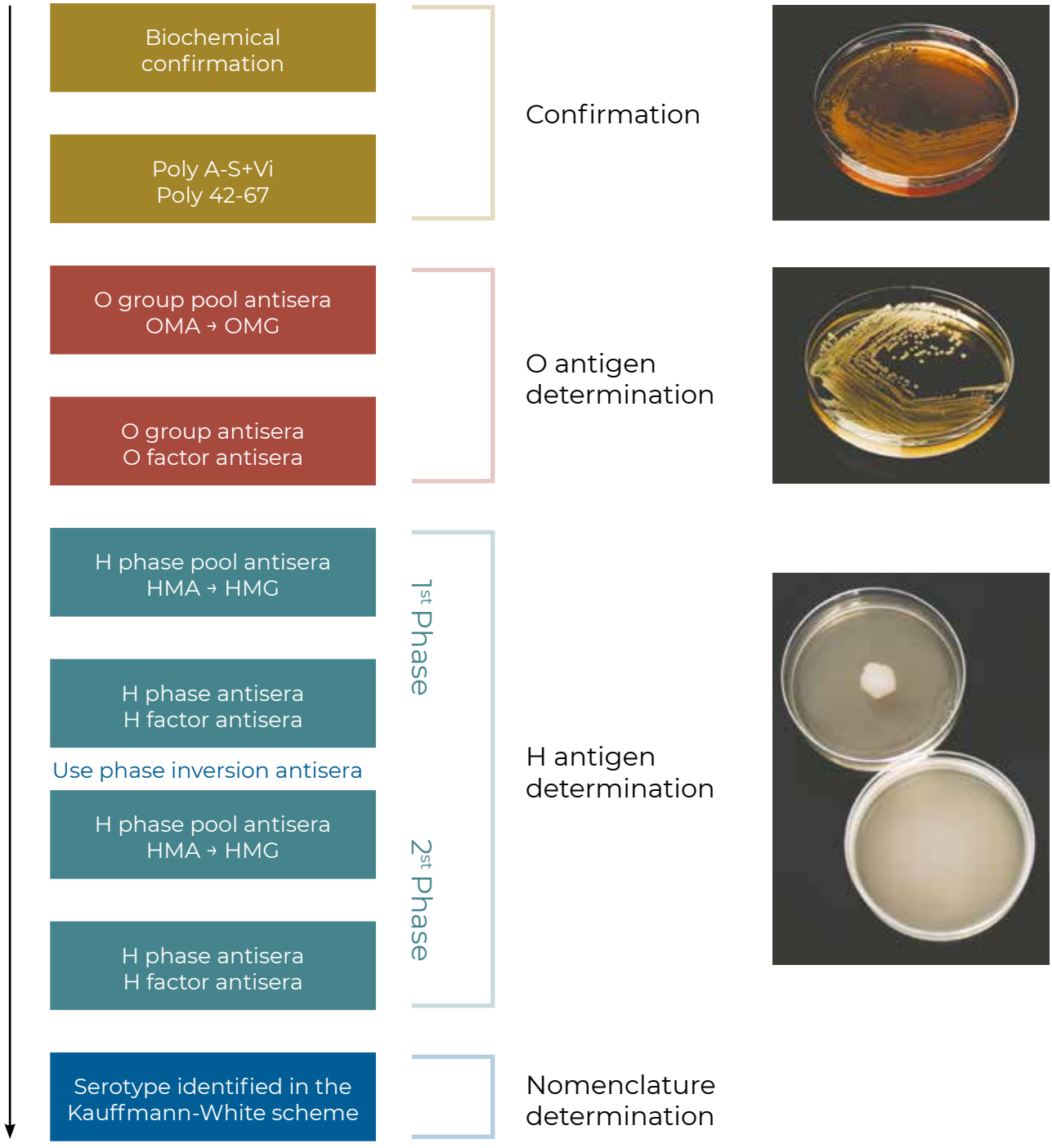


Figure 3. Flowchart for serotyping with *Salmonella* antisera.

FULL SEROTYPING OF *S.* ENTERITIDIS AND *S.* VIRCHOW

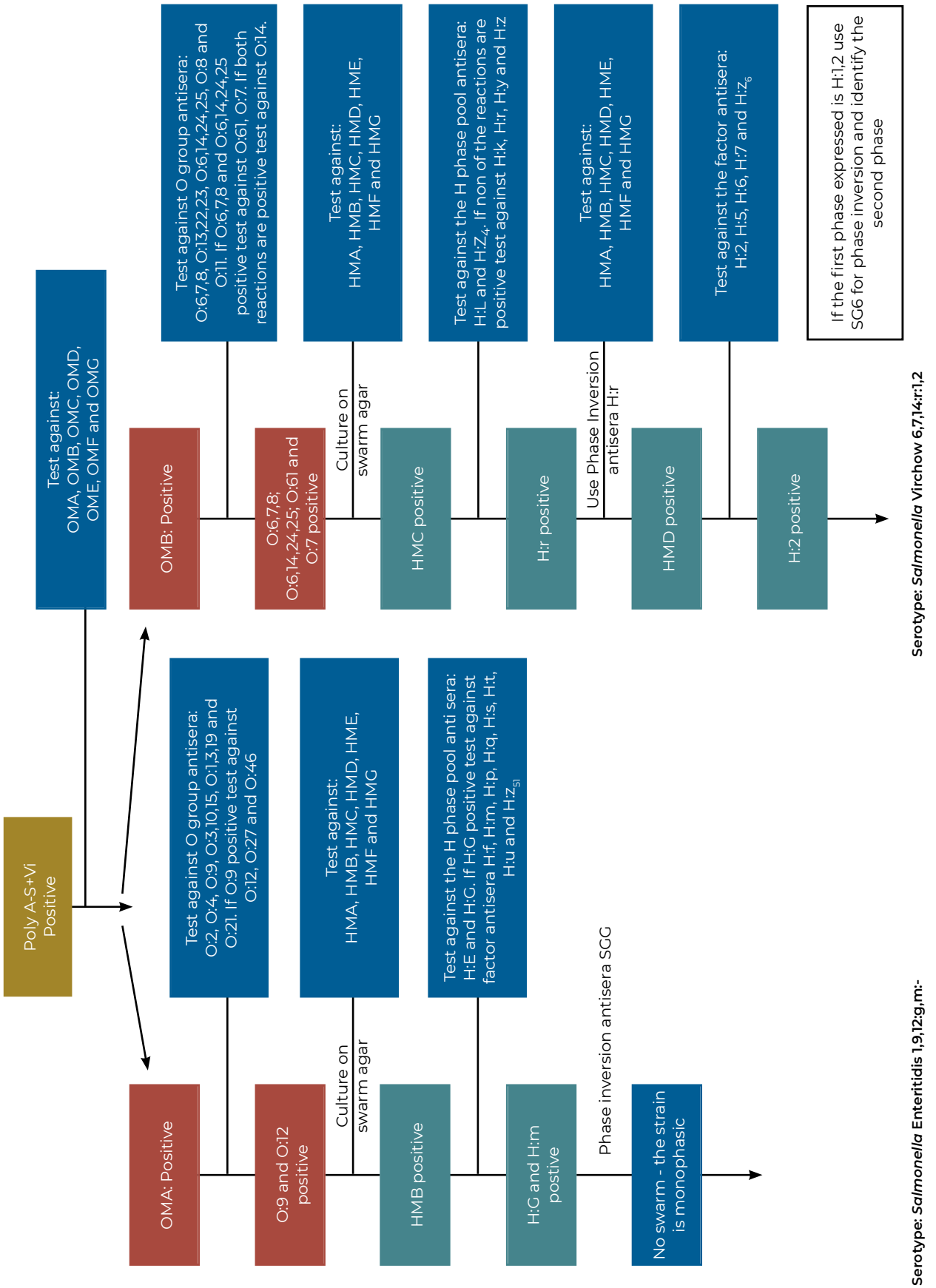


Figure 4. Full serotyping of S. Enteritidis and S. Virchow.

SALMONELLA ANTISERA KITS

We offer 2 kits - *Salmonella* Sero-Quick Group Kit and *Salmonella* sero-quick ID Kit. The *Salmonella* Sero-Quick Group Kit is used to determine the most common *Salmonella* groups and the *Salmonella* sero-quick ID Kit is used to identify the two most commonly occurring *Salmonella* serotypes, *S.* Enteritidis and *S.* Typhimurium.

SERO-QUICK GROUP KIT

FOR QUICK SEROGROUPING OF SALMONELLA

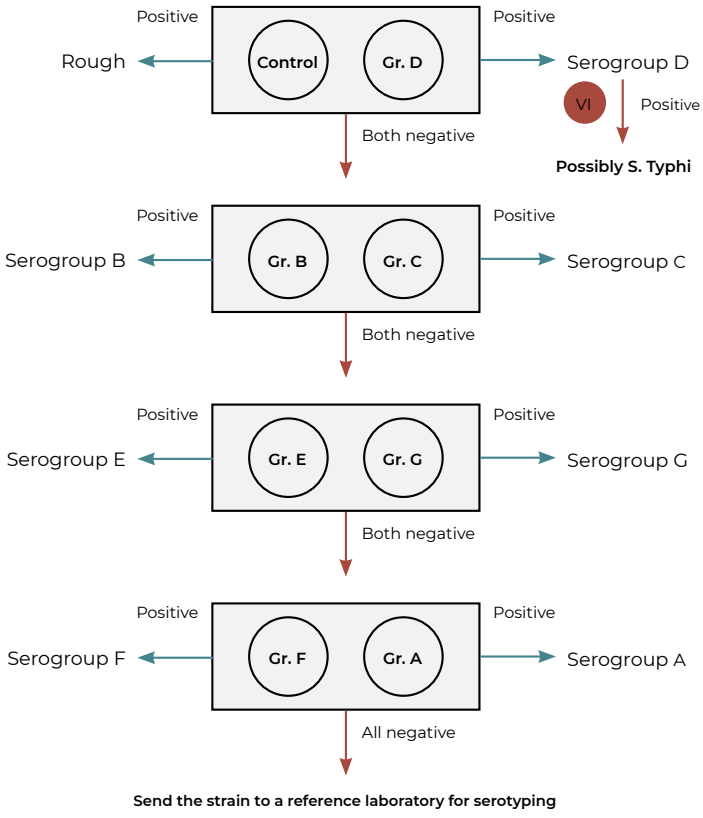
Description and application

The *Salmonella* Sero-Quick Group Kit with 8 different ready-to-use antisera is for identifying *Salmonella* isolates at the serogroup level. Our kit antisera are for the most common serogroups of *Salmonella*, i.e. A-G and the capsular antigen Vi. The *Salmonella* Sero-Quick Group Kit can be used to perform about 150 tests.

This kit can be used as a surveillance tool either for preliminary *Salmonella* sero-grouping or when full serotyping is performed by a Human or Veterinary Reference Centre. In the food industry it can be used for screening of *Salmonella*. The most common serogroups are B (*S.* Typhimurium) and D (*S.* Enteritidis). Vi and serogroup A are clinically important as they are expressed by *S.* Typhi and *S.* Paratyphi respectively.

The 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 5.

Figure 5. Serogrouping with *Salmonella* Sero-Quick



PRODUCT RANGE

- Article No. 18349: *Salmonella* Sero-Quick Group Kit, 1 box
- Article No. 18350: *Salmonella* Sero-Quick ID Kit, 1 box

Table 1: Designation of O groups

Serogroup	Antigen identified
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

SERO-QUICK ID KIT

FOR QUICK IDENTIFICATION OF *S.* ENTERITIDIS AND *S.* TYPHIMURIUM

Description and application

The *Salmonella* Sero-Quick ID Kit is intended for complete serotyping of *S.* Enteritidis (1,9,12:g,m:-) and *S.* Typhimurium (1,4,[5],12:i:1,2). The kit facilitates quick identification of the two most common serovars isolated in humans - *S.* Enteritidis and *S.* Typhimurium constitute 81 % of all *Salmonella* strains isolated from humans[5].

The kit consists of 8 different ready-to-use antisera: O:4, O:9, H:i, H:m, H:2, H:q,s,t,p,u, SG2 and SG6 which are available in 3 ml vials. About 150 tests can be performed with the Sero-Quick ID Kit.

The 8 antisera are used as indicated in figure 6. Begin with the O antisera followed by the H antisera and then 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 which do not combine with antigen combination H:g,m in serogroup O:9 (D1). Additional serotyping to identify additional serotypes can be done.

	REACTION		
	S. Enteritidis	*S.* Thyphimurium	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.	+[a]	swarm agar
SG6	n.p.	+[b]	

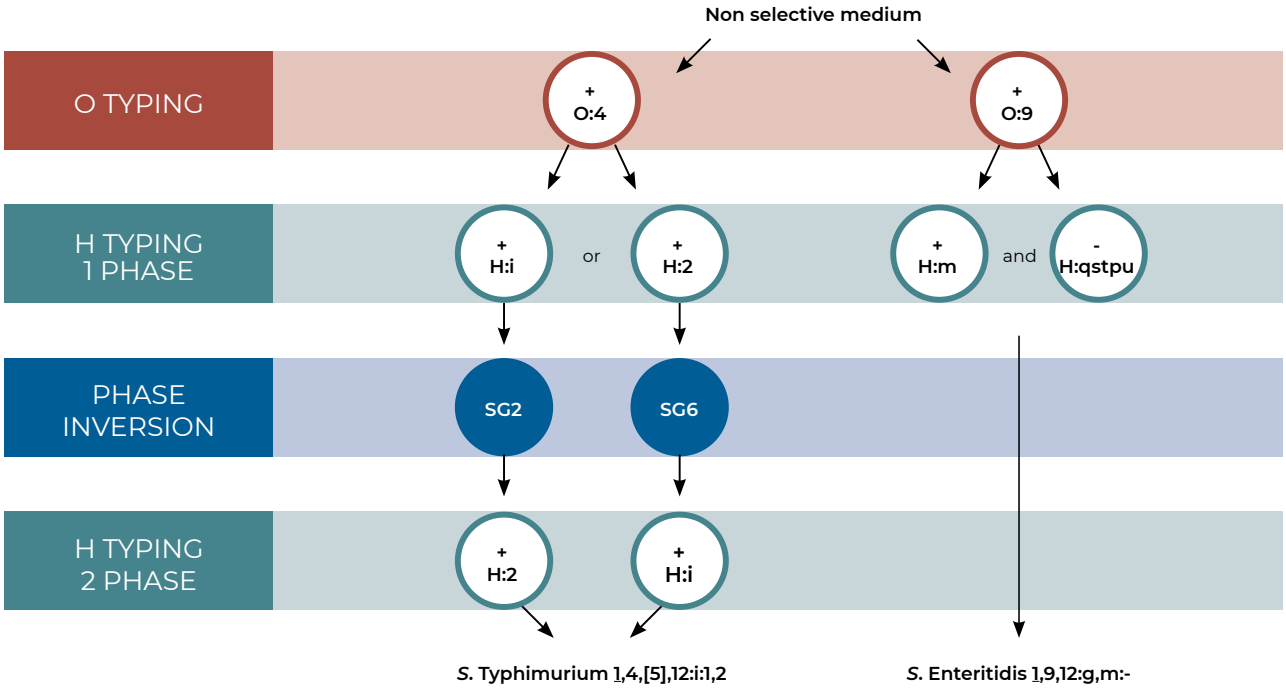
Table 2. Reaction *S.* Enteritidis *S.* Thyphimurium

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. Hillingdon (9,46:g,m:-) cannot be distinguished from *S.* Enteritidis with this kit since both serotypes will be positive in O:9 and H:m and negative in H:q,s,t,p,u. However, the prevalence of *S.* Hillingdon is very low.

Figure 6. Serotyping of *Salmonella* Enteritidis and *Salmonella* Typhimurium.



HOW TO SEROTYPE THE FIVE MOST COMMON SALMONELLA SEROTYPES

Following is the procedure for screening of the five most common *Salmonella* types isolated in human - *S.* Typhimurium, *S.*Enteritidis, *S.* Infantis, *S.* Virchow, *S.*Hadar.

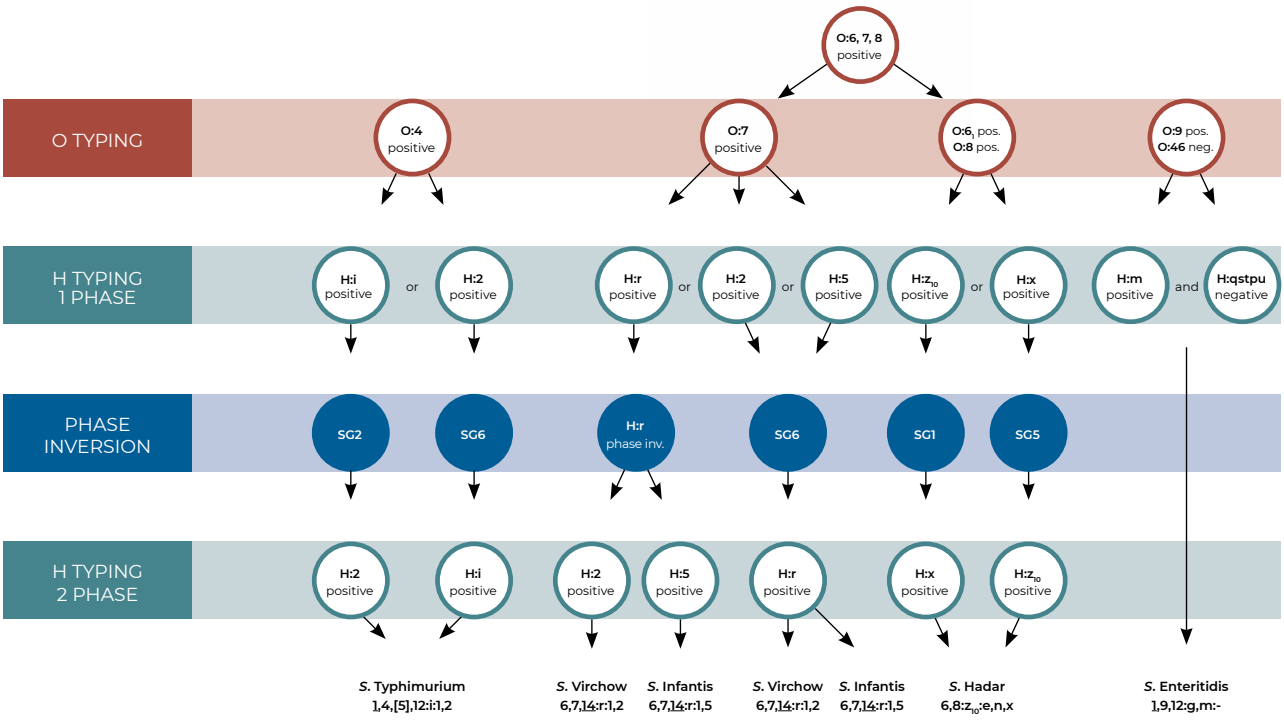


Figure 7. Serotyping of the five most common *Salmonella* serotypes.

GENERAL INFORMATION

Quality and storage
SSI Diagnostica ready-to-use antisera are CE-marked and produced in accordance with DS/EN ISO 13485. The shelf life is 4 years from the date of production. Sodium azide 0.09% is added to all *Salmonella* antisera as a preservative.

The antisera must be stored at 2–8°C

Support
Technical: Please send enquiries concerning use of our products to SSI Diagnostica at info@ssidiagnostica.com

References

1. WHO Fact Sheet, February 2018
2. 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.
3. Gard, S. Das Schwärmphänomen in der *Salmonella*-gruppe und seine praktische Ausnützung. Zeit. f. Hyg. Inf. Krankh. 1938, 120;615-619.
4. ISO/TR 6579-3:2014 Guideline "Microbiology of food and animal feed – Horizontal method for the detection, enumeration and serotyping of *Salmonella*"
5. Herikstad et al. 2002. Epidemiol. infect. 129, 1-8. *Salmonella* Surveillance: a global survey of public health serotyping.

Information and ordering
For ordering please visit our webshop shop.ssidiagnostica.com, or contact one of our distributors listed on ssidiagnostica.com.

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