



SALMONELLA ANTISERA

FASTEST TO RESULTS

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

Improving
Microbiology

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 method³). After full serotyping of the *Salmonella* culture the nomenclature of the serotype can be determined by using the Kauffmann-White Scheme². 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 μ L) 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.

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

A. Add phase inversion antiserum

B. Add swarm agar

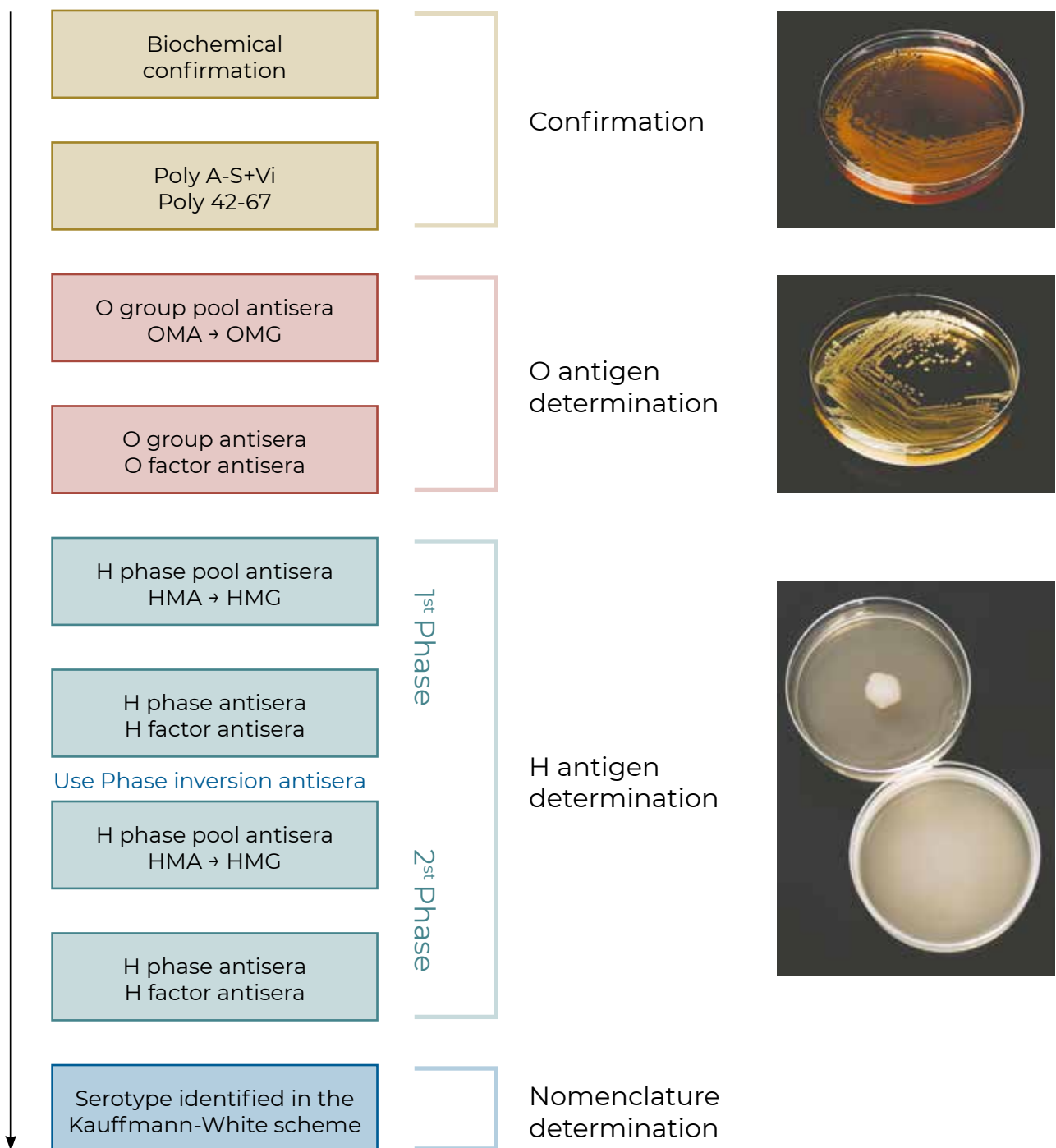
C. Mix well

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



SALMONELLA ANTISERA KITS

We offer 3 kits - *Salmonella* O sero-group kit, *Salmonella* sero-quick ID kit and *Salmonella* Big Five kit. The *Salmonella* sero-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 namely *S. Enteritidis* and *S. Typhimurium*.

Our *Salmonella* Big Five kit facilitates *Salmonella* serotyping according to ISO/TR 6579-3⁴.



SERO-QUICK GROUP KIT FOR QUICK SEROGROUPING OF SALMONELLA

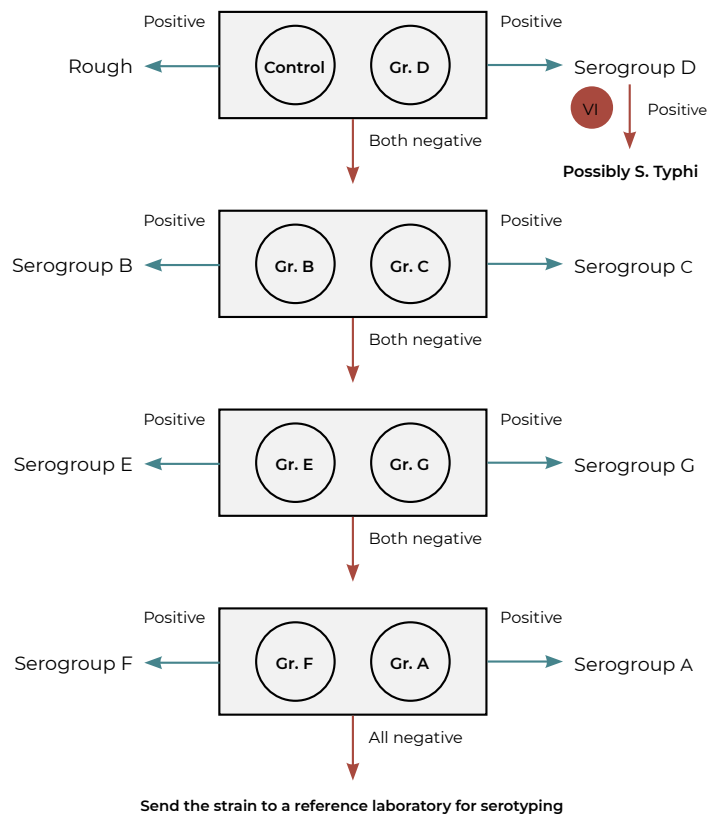
Description and Application

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*, serogroups i.e. A-G and the capsular antigen Vi. The antisera are vials in differentiated volumes according to standard lab use. The *Salmonella* Sero-Quick Group kit can be used to perform about 100 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 3.

Figure 3. Sero grouping with Salmonella Sero-Quick



PRODUCT RANGE

- Article No. 60898: *Salmonella* Sero-Quick Group kit, 1 box
- Article No. 62984: *Salmonella* Sero-Quick ID kit, 1 box
- Article No. 89074: *Salmonella* Big Five kit – ISO 6579-3, 1 box

Table: 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 humans⁵. Therefore, it is very important to serotype these strains quickly.

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 2 ml vials. About 100 tests can be performed with the Sero-Quick ID kit.

The 8 antisera are used as indicated in Figure 4. 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			
	<i>S. Enteritidis</i>	<i>S. Typhimurium</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.	+ ^a	swarm agar
SG6	n.p.	+ ^b	

n.p. = not performed

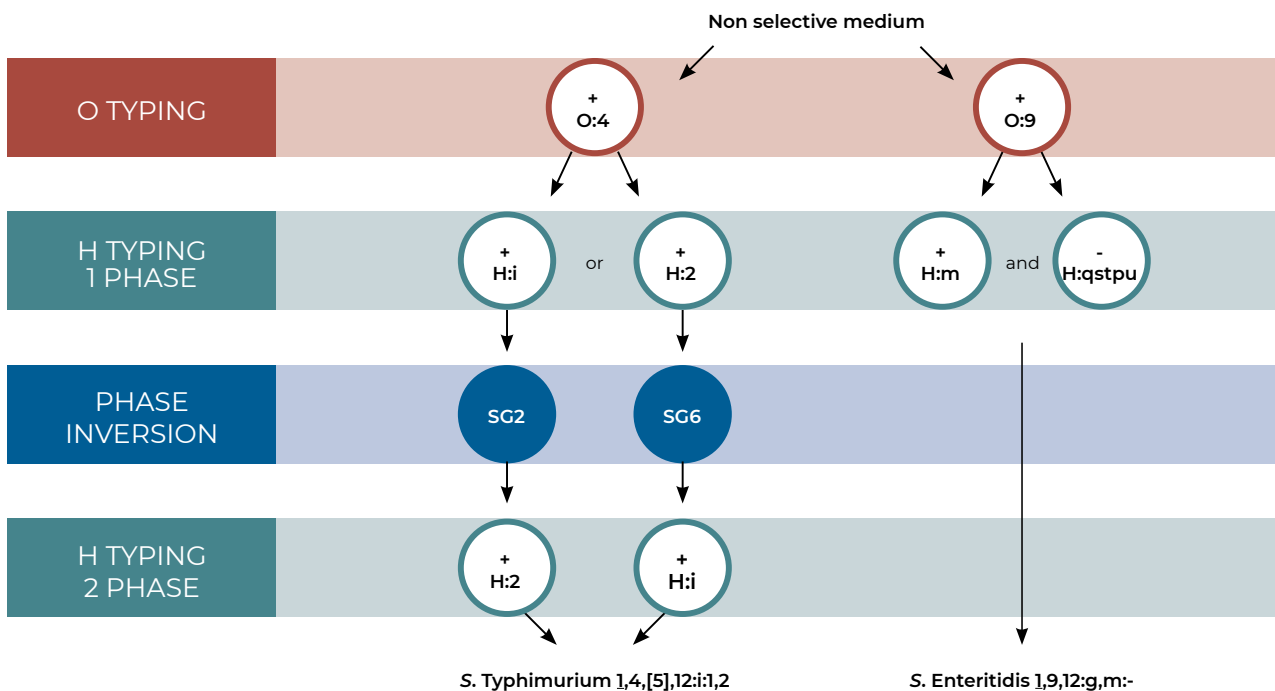
a = SG2 is used for phase inversion of strains initially expressing H:i

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



SALMONELLA BIG FIVE KIT – ISO 6579-3

Description and Application

The *Salmonella* Big Five kit is used for screening of *S. Typhimurium*, *S. Enteritidis*, *S. Infantis*, *S. Virchow*, *S. Hadar* according to ISO 6579-3 (Figure 5). These are the five most common *Salmonella* types isolated in humans. The kit has all the antisera to screen for the Big Five *Salmonella*. It includes O antisera (O:4, O:8, O:6₁, O:7, O:4₆), H antisera (H:q,s,t,p,u, H:i, H:r, H:z₁₀, H:m, H:x, H:2, H:5) and H phase inversion (SG 1, SG 2, SG 5, SG 6, H:r).

The 20 different ready-to-use antisera are marketed as 3 mL (150 tests) vials in a single kit.

The kit facilitates compliance with the ISO 6579-3 Standard.



Figure 5. Flow chart showing how to serotype the five most common *Salmonella* serotypes according to ISO/TR 6579-3

