



for *in vitro* diagnostic use

### Application

The *Salmonella* diagnostic antisera from SSI Diagnostica are intended for complete or partial serotyping by slide agglutination and for H phase inversion.

### Description

The *Salmonella* antisera are raised in rabbits and consist of O and H antisera. The Vi antibody is monoclonal produced by culturing a hybridoma cell line *in vitro* followed by concentration of the antibody from the culture supernatant. The antisera may be used separately or in combination depending on the aim of the test. The antisera are supplied in 1 or 3 mL bottles (sodium azide as preservation). All *Salmonella* antisera are specific, achieved by absorption of cross-reactions (with the exception of Poly A-E+Vi, Poly A-I+Vi, Poly A-S+Vi, Poly 42-67 and Poly H).

### Principle

When a bacterial culture is mixed with a specific antiserum directed against bacterial surface components, the cells are bound together through antigen-antibody bonds to form aggregates (agglutination). This is usually visible to the naked eye as clumps in the suspension. By mixing specific antisera with a *Salmonella* culture, the O and H antigens are determined. On the basis of the observed agglutination pattern the serotype is determined using the Kauffmann-White Scheme<sup>1</sup>.

### Material Required but not Provided

- Non-selective agar medium (eg. beef extract agar)
- Inoculating loop or toothpick
- Glass slides
- Physiological saline, pH 7.4
- Kauffmann-White Scheme

### Additional material for phase inversion:

- Sterile petri dishes (diameter 6 cm)
- A microwave oven or a water bath (100 °C)
- Swarm agar
- Thermometer
- Pipette
- Incubator 35-37 °C

## PROCEDURE

### General

Physiological saline is used as a negative control and must be negative. If the negative control is positive (agglutinates), the strain is auto agglutinating, i.e. O rough.

### Slide Agglutination with O and H Antisera

The *Salmonella* is grown over night on a non-selective agar medium. Swarm agar is the best suited medium for growing cultures for H typing, but H antigens can be serotyped from a non-selective agar medium if the H antigens are well expressed.

1. Apply a small drop of antiserum (approximately 20 µL) on the glass slide.
2. Transfer culture from several colonies to the drop of antiserum and mix well. The amount of culture should be sufficient to give a distinct milky turbidity. Use an inoculating loop or a toothpick.
3. Tilt the slide for 5-10 seconds.
4. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).
5. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination should be considered negative.

Absence of reactions may be due to a strain expressing the Vi antigen (see below), a strain not covered by the antisera used, or a strain not being *Salmonella*.

### Demonstration of the Vi Antigen

The presence of the Vi antigen may interfere with or prevent agglutination in O antisera. Negative isolates must therefore be examined for Vi antigen. Due to form variation in the Vi antigen it is important to select single colonies as colony forms expressing the Vi antigen are more opaque than Vi negative colonies.

### H Phase Inversion on Swarm Agar Plates (S. Gard method)<sup>2</sup>

1. Melt the swarm agar in a microwave oven or in a water bath (100 °C) and cool to 45 °C.
2. Apply 100 µL of H antiserum for phase inversion (corresponding to the phase which has already been identified) in the centre of a small, sterile petri dish.
3. Pour 10 mL of the swarm agar onto the antiserum resulting in a final dilution of 1:100.
4. Leave the plate for solidification at the site of pouring at room temperature (22-25 °C) for 10-15 minutes.
5. Inoculate the plate in the centre with a loop full of fresh bacterial culture from an agar plate or a broth culture.
6. Incubate overnight at 35-37 °C.
7. Use culture material from the edge of the growth zone for slide agglutination. Select the relevant H antisera by using the Kauffmann-White Scheme.

If the H phase is not inverted the amount of antiserum in the swarm agar plate should be increased.

### Storage and Shelf Life

Store at 2-8 °C in a dark place. Expiry date is printed on the package. Turbidity due to lipoprotein precipitation is sometimes seen after prolonged storage. Precipitation and/or contamination can be removed by centrifugation (10,000 g) followed by sterile filtration (0.22 µm).

### Typing Support

*Salmonella* strains which cannot be typed, may be sent to The National Reference Laboratory for Enteropathogenic Bacteria, 5 Artillerivej, DK-2300 Copenhagen S, Denmark for further examination.

### References

1. Grimont, P.A.D. and Weill, F.-X. Antigenic formulae of the *Salmonella* serovars, 9th ed., 2007. 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.

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