SSI DIAGNOSTICA

Instructions for use

SALMONELLA ANTISERA

EN

Digitally signed by Lazari Cristina
Date: 2025.02.07 14:27:01 EET
Reason: MoldSign Signature
Location: Moldova

SALMONELLA ANTISERA

For *in vitro* diagnostic use

Intended use

Salmonella antisera are used as an *in vitro* diagnostic aid for qualitative manual complete or partial bacterial serotyping by slide agglutination and H phase inversion. It is important to use pure culture isolates for determination of bacterial antigens.

Description

Salmonella O Group and O Factor and H Phase and H Factor antisera are for screening of live cultures from a non-selective agar plate. *Salmonella* Phase Inversion antisera are for inversion of H phases.

Antisera type	Vial volume	Number of tests
O, H, and monoclonal Vi	1 ml	50
O, H, and monoclonal Vi	3 ml	150
Phase Inversion	3 ml	30

Table 1. Products included in this instruction for use.

All *Salmonella* antisera are absorbed free of cross-reactions except for Poly A-E+Vi, Poly A-I+Vi, Poly A-S+Vi, Poly 42-67, and Poly H.

The antisera are polyclonal, prepared in rabbits using reference strains according to the methods recommended by the Pasteur Institute[1] and absorbed to eliminate cross-reacting antibodies.

SSI Diagnostica antisera are for use by laboratory professionals and/or healthcare professionals only.

Principle

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[4]). After full serotyping of the *Salmonella* culture the nomenclature of the serotype can be determined by using the Kauffmann-White Scheme[3].

Precautions

- Before using SSI Diagnostica *Salmonella* antisera, confirm that the strain is a *Salmonella*, e.g. by using a biochemical method.
- Rough cultures/strains will self-agglutinate and cause false positive reactions.
- Excessive amount of culture compared to antisera might cause false positive reactions.
- For the antisera for slide agglutination, please make sure that result is read within 10 seconds.
- Turbidity due to lipoprotein precipitation can occur after prolonged storage. If you experience precipitation and/or contamination, it can be removed by centrifugation (10,000 g) followed by sterile filtration (0.22 µM).
- The antisera have only been validated for serotyping by the below described methods.
- Antisera that have accidentally been frozen should not be used.
- The strain to be tested must be grown on a non-selective agar plate. Be sure that the strain is a pure culture.
- Do not use the antisera after the expiry date.
- Inspect the vial before use to ensure it is intact. Any damaged vials should be discarded.

Materials provided

SSI Diagnostica *Salmonella* antisera are supplied in dropper bottles containing 1 or 3 mL ready-to-use antisera (see table 1).

Materials required but not provided

- Non-selective agar medium (e.g. beef extract agar)
- Physiological saline pH 7.4
- Inoculating loop or toothpick
- Glass slides
- Incubator (35-37 °C)
- Kauffmann-White Scheme

Additional material for phase inversion

- Sterile petri dishes (diameter 6 cm)
- Water bath (>90 °C)
- Swarm agar
- Pipette

Storage and stability

Expiry date is printed on the labels.

Salmonella antisera must be stored at 2-8 °C in a dark place. Do not freeze. Stored under these conditions the antisera may be used up to the date of expiry shown on the product label.

The in-use stability is not affected by working with the antiserum on the bench throughout the day if it is stored at 2-8 °C when not in-use, for no longer than 4 years from date of production.

Salmonella antisera have been tested after being stored at 37 °C for up to four weeks. The antisera were still fully functional.

Preservative

The *Salmonella* antisera contains less than 0.1% sodium azide (NaN_3) as preservative.

Sample collection and storage

For sample storage please follow your local standard procedure.

Quality control

Before use, check the vials to ensure that there is no damage and/or leak. In case of damage or leak discard the vial.

Saline is used as negative control to confirm that the strain is not self-agglutinating.

Procedure

Slide agglutination with O and H antisera

1. The *Salmonella* strain is grown over night at 35-37 °C on a non-selective agar medium. Swarm agar is the best suited medium for growing cultures for H typing. H antigens cannot be serotyped from a non-selective agar.

2. Apply a small drop of antiserum (approx. 20 µL) on the glass slide.

3. Transfer culture from 3 to 5 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.

4. Tilt the slide for 5-10 seconds.

5. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).

6. A positive reaction is seen as a visible agglutination (see figure 1 reaction A). A negative reaction is persistence of the homogeneous milky turbidity (see figure 1 reaction B). A late or weak agglutination (after 10 seconds) should be considered negative.

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

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.

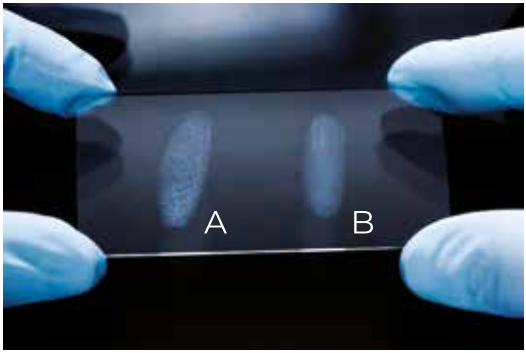

Figure 1. Sample A is a positive reaction and sample B is a negative reaction.

H phase inversion on swarm agar plates (S. Gard method)[4]

1. Melt the swarm agar e.g. in a water bath (>90 °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 center of a small, sterile petri dish (diameter 6 cm).

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 for 10-15 min.

5. Inoculate the plate in the center with a loop full of fresh bacterial culture from an agar plate or broth culture.

6. Incubate overnight at 35-37 °C.

7. Use culture material from the edge of the growth zone for slide agglutination. Make sure that the strain swarm all the way to the edge of the petri dish before testing by slide agglutination. Select the relevant H antisera by using the Kauffmann-White Scheme.

8. If 100µL phase inversion antisera do not completely inhibit the phase in question, redo the procedure from step 2 using 200µL phase inversion antisera. If the second phase is not expressed, it does not exclude the strain having a second phase.

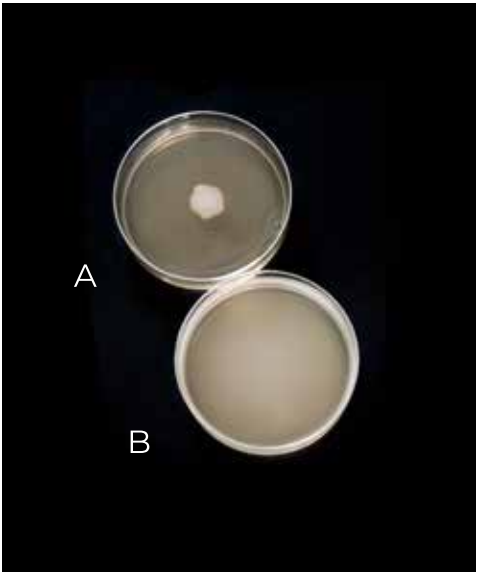

Figure 2. Illustration of phase inversion of a monophasic and a diphasic *Salmonella* culture. The monophasic culture does not swarm (A) and the diphasic culture is swarming to the edge of the petri dish (B).

Interpretation of results

Slide agglutination:

A positive reaction is seen as a visible agglutination, whereas a negative reaction is seen as homogeneous milky turbidity (see figure 1).

Do not interpret the results after 10 seconds as any reaction seen after 10 seconds cannot be considered a true positive result.

Phase inversion:

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 second phase H antigens. *Salmonella* strains can have up to 3 phases. To find the third phase, phase inversion antisera against phase 1 and 2 must be added to the swarm agar. The phase can be serotyped using H antisera for slide agglutination.

Disposal

Follow your local procedures and/or national guidelines for disposal of biological materials.

Limitations

- The culture must be confirmed *Salmonella* before serotyping using antisera from SSI Diagnostica.

- Phase inversion antisera cannot be used for slide agglutination, and slide agglutination antisera cannot be used for phase inversion, even though they are directed against the same antigen.

Performance

Sensitivity, specificity, and repeatability

***Salmonella* antiserum overall results**		
	Percent (number positive/ actual positive)	95% confidence interval
Sensitivity	96% (291/302)	93-98
Specificity	99% (307/310)	97-99
Repeatability	98% (940/958)	97-99

Reproducibility

The reproducibility within the different groups of antisera and all antisera combined is 100% (99%-100%). Therefore, all produced antisera have a high level of reproducibility throughout time and lots.

Incident reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Quality certificate

SSI Diagnostica's development, production and sales of *in vitro* diagnostics are quality assured and certified in accordance with ISO 13485[2]. Certificate of analysis can be downloaded from our website: ssidiagnostica.com

REF IVD CE

For the list of products and composition, see our website:

O and VI antisera

- https://www.ssidiagnostica.com/salmonella-antisera-o-and-vi/

H antisera

– https://www.ssidiagnostica.com/salmonella-antisera-h/

References

1. Grimont, P.A.D. and Weill, F.-X. Antigenic formulae of the *Salmonella* serovars, WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France, 9th ed., 2007.

2. ISO/TR 6579-3:2014 Guideline "Microbiology of food and animal feed – Horizontal method for the detection, enumeration and serotyping of *Salmonella*"

3. 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.

4. Gard, S. Das Schwärmphänomen in der *Salmonella*-Gruppe und seine praktische Ausnützung. Zeit. f. Hyg. Inf. Krankh. 1938, 120;615-619.

Information and ordering

SSI Diagnostica A/S
Herredsvejen 2
3400 Hillerød
Denmark
T +45 4829 9100
info@ssidiagnostica.com
www.ssidiagnostica.com
shop.ssidiagnostica.com

7th edition · June 2023 · 15983

SSI Diagnostica A/S
Herredsvejen 2
3400 Hillerød
Denmark

ssidiagnostica.com

Improving
Microbiology