



Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project
of the World Health Organization

Laboratory Protocols

Level 2 Training Course

Serotyping of *Salmonella enterica* O and H antigen.

6th Ed. January. 2004

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1. Serotyping of *Salmonella enterica* and *Salmonella bongori*

Introduction

Serotyping is a definitive typing method used for epidemiological characterisation of bacteria. Serotyping of *Salmonella* strains is carried out by identification of surface antigens (LPS, O-antigens) and flagella antigens (proteins, H-antigens). Most commonly, strains of *Salmonella* express two phases of H- antigens but aphasic, monophasic and triphasic variants are known. The definition of the serotypes is based on the antigen combination present and is given in the “Kauffmann-White scheme”, Popoff and Le Minor, WHO Centre for Reference and Research on *Salmonella*, Institut Pasteur, France, 1997.

Materials

Equipment

- Inoculation loops (1 µl)
- Slide
- Small petri dishes
- 10 µl pipettes

Media

- Swarm agar
- *Salmonella* O- and H-antisera (from National *Salmonella* Centre – Statens Seruminstitut, Copenhagen, Denmark or other reference centre)

Bacterial strains

- At least 2 *Salmonella* strains on nutrient agar plates

Safety

Carry out all procedures in accordance with the local codes of safe practice.

Procedure

O-typing

Day 1

Place a loop full of saline on a slide and a second loop full next to the first.
From the inoculated nutrient agar plate remove a loop full of growth and mix into the first saline drop on the slide. Repeat into the second drop (negative control test) ensuring a smooth, opaque suspension in both drops.
Add a drop of poly O antisera with or without Vi antiserum to the first drop. Mix antisera and culture (antigen) with a loop or stick for up to 1 minute. Do not tip the slide. Compare appearance of drop 1 with that of drop 2 if unsure. If drop 2 has lumps in it, the culture is autoagglutinating and no further typing is possible.

Mix a loop full of culture from the nutrient agar and a drop of an O-serum on a slide.

Rock the slide gently for a maximum of 2 minutes.

A homogenous suspension is a negative reaction. Lumping is a positive reaction.

First, the strains are tested in the O-sera-pools. Afterwards, the strains are tested in the individual O-sera represented in the positive O-pool.

O-antigens detected are noted. Both positive and negative reactions are noted.

Theory / comments

Agglutination will be seen as particulate matter or “lumps” forming within the drop. Autoagglutinating cultures may be referred to as “rough” strains of *Salmonella*. If a strain autoagglutinates, subculture on blood agar or Mueller-Hinton agar in order to recover the smooth state of the strain, and repeat the agglutination test.

If agglutination does not occur with Poly O antisera it is unlikely to be *Salmonella* and serotyping should not be carried out.

Detection of the O- antigen is performed by slide agglutination. Antibodies in the specific sera agglutinate with the bacteria when the corresponding antigens are present.

Procedure

H-typing

Day 1

Sub-culture from nutrient agar to Swarm agar.

Inoculate in one spot at the centre of a Swarm agar. Incubation overnight at 37 °C.

Day 2

Place two drops of saline with a loop next to each other on a slide. Remove a loop full of growth from the edge of the motility zone on Swarm agar and mix into the first drop of saline. Repeat into the second drop (negative control test) ensuring an opaque suspension in both drops.

Add a drop of Poly H antisera to the first suspension and mix with a loop or stick for up to 1 minute. Do not tip the slide.

A loop full of culture from the edge of the motility zone and a drop of an H-serum is carefully mixed on a slide. Rock the slide gently for a maximum of 2 minutes.

A homogenous suspension is a negative reaction. Lumping is a positive reaction.

The strains are first tested in the H-antisera-pools. Afterwards, the strains are tested in the individual H-antisera represented in the positive H-pool.

H-antigens detected (1. Phase) are noted. Both positive and negative reactions are noted.

10 µl antisera against the detected H-antigen (1. Phase) is added to a petri dish (small size) together with approximately 5 ml Swarm agar (55-60°C).

Theory /comments

The H-antigen is connected to the flagella. *Salmonella* swarms through the medium from the inoculation site and the H-antigen is detected by using material from the motility zone.

Detection of the H- antigen (1. phase) is performed by slide agglutination.

Flagella agglutination is more “floccular” in appearance than somatic agglutination and may form only around the edge of the drop. It is easier to see if the slide is placed against a dark background. If agglutination occurs in the negative control suspension discontinue tests and report as “rough” strain.

Phase inversion is performed before detection of 2. H-antigen phase. Antisera inhibit the present H-antigen phase and if the *Salmonella* strain has a 2. phase it will swarm with this phase.

Procedure

Wait until the agar has solidified.
Inoculate in one spot at the centre of the agar.
Incubation overnight at 37 °C.
(Alternatively U-tubes or Craigie tubes can be used. Ref 2).

Day 3

2. phase H-antigens are detected by the same methods as described for the 1. phase.

H-antigens (2. phase) detected are noted. Both positive and negative reactions are noted.

Serotype identification

Combine the O- and H reactions and identify the specific type (the serovar) in the "Kauffmann-White scheme" (ref. 1).

In some instances the scheme indicates other possibilities than the *S. enterica* subsp. *enterica*. To distinguish between the possible *Salmonella* subspecies perform the necessary biochemical tests according to table 3 (Appendix 5). (E.g. perform malonate test to distinguish between subspecies *enterica* and *salamae*.)

References

1. DS/EN ISO 6579: 2002, Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.
2. Antigenic formulas of the *Salmonella* serovars, Popoff, MY, WHO Centre for Reference and Research on *Salmonella*, Institut Pasteur, France, 2001 .(“Kauffmann-White scheme”)

2. Composition and preparation of culture media and reagents

If no reference is given, it is the procedure used at DVL.

The media and reagents are available from several companies including Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturers description if it differs from the description given here.

Nutrient agar (ref. 2)

Meat extract	3.0 g
Peptone	5.0 g
Agar	12 g to 18 g ¹⁾
Water	1000 ml

¹⁾ Depending on the gel strength of the agar.

Preparation:

Dissolve the dehydrated medium in the water by heating if necessary. Adjust pH to ~7.0 after sterilisation, transfer into bottles and autoclave at 121°C for 20 min. Pour 15 ml of melted medium in each plate.

Swarm agar

Trypto-casein soja Broth	1000 ml
Potassium Nitrate	1 g
Bacto agar (Difco)	2 g

Preparation:

Dissolve medium and chemicals in water by heating if necessary. Adjust pH to 7.4, pour into 250 ml flasks and autoclave at 110°C for 15 min.

(Potassium Nitrate is added to inhibit gas production. The same medium can be used for Craigie tubes, but some authors are advising to add 4g of bacto agar instead of 2g)

References

1. Post D. E. (1997) Food-borne pathogens monograph number I *Salmonella*. Oxoid limited, Hampshire, England.
2. ISO 6579 :1993(E) 3rd ed. Microbiology - General guidance on methods for the detection of *Salmonella*.
3. NMKL method no. 71, 2nd ed., 1999: *Salmonella*. Detection in food. Nordic committee on food analysis.

Serotyping of Salmonella strains

Recordsheet

Date: _____

Work Bench no: _____

Sample:	Antigenicreaction:
	<p data-bbox="347 622 671 651">Nutrient agar (O-antigen):</p> <p data-bbox="347 775 655 804">Swarm agar (H-antigen):</p> <p data-bbox="347 837 448 866">Phase 1:</p> <p data-bbox="347 1021 448 1050">Phase 2:</p>
	<p data-bbox="347 1209 671 1238">Nutrient agar (O-antigen):</p> <p data-bbox="347 1361 655 1391">Swarm agar (H-antigen):</p> <p data-bbox="347 1424 448 1453">Phase 1:</p> <p data-bbox="347 1576 448 1606">Phase 2:</p>

Antigenicformula: _____

Serovar: _____