Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

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Serotypning of *Salmonella enterica* O and H antigen

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1. INTRODUCTION

Serotyping of *Salmonella enterica* and *Salmonella bongori*

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.

2. MATERIALS

Equipment
- Inoculation loops (1 µl)
- Glass slides
- Small petri dishes
- 10 µl pipettes
- Incubator 37°C

Media

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

Bacterial strains

Salmonella strains on nutrient agar plates and svarm agar incubated overnight at 37°C

Safety

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

Publications Office

3. METHODS

O-typing

Therory/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.

Day 1

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 1 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

H-typing

Therory/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.

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

Day 1

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 1 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).

Wait until the agar has solidfied.
Inoculate in one spot at the centre of the agar.
Incubation overnight at 37°C

(Alternatively U-tubes or Craigie tubes can be used).

Day 2

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"

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 “Kauffmann-White scheme” (E.g. perform malonate test to distinguish between subspecies enterica and salamae.)

4. REFERENCE

1. DS/EN ISO 6579: 2002, Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.
2. ISO/TR 6579-3:2014 Mocrobiology of the food chain – Horizontal method for the detection, enumeration and serotyping af Salmonella – Part 3: Guidelines for serotyping af Salmonella spp.
3. Antigenic formulas of the Salmonella serovars, Popoff, MY, WHO Centre for Reference and Research on Salmonella serovars, Institut Pasteur, France, 2007 .(“Kauffmann-White scheme”)

Serotyping of Salmonella strains

Recordsheet

Date: _____

Work Bench no: _____

Sample:	Antigenicreaction:
	Nutrient agar (O-antigen): **Swarm agar (H-antigen):** **Phase 1:** **Phase 2:**
	Nutrient agar (O-antigen): **Swarm agar (H-antigen):** **Phase 1:** **Phase 2:**

Antigenicformula:_____

Serovar:_____