HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Salmonella Serotyping		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To provide a standardized policy and procedure for *Salmonella* serotyping.

2. DEFINITONS:

2.1 Serotypes are groups within a single species of microorganisms, such as bacteria or viruses, which share distinctive surface structures. For e.g. the outermost portion of the bacteria's surface covering, called the O antigen and a slender thread-like structure, called the H antigen, that is part of the flagella.

2.2 Polyvalent anti-sera: Salmonella are distinguished by their antigenic characteristics. Polyvalent anti-sera allow the presumptive identification of Salmonella and can be the first step in full identification.

3. POLICY:

3.1 Serotyping is useful for diagnostic & epidemiological purposes for all positive cultures for *Salmonella*.

3.2 Polyvalent anti-sera allow the presumptive identification of Salmonella and can be the first step in full identification.

3.3 In screening procedures colonies or isolates which show no agglutination in both polyvalent O and polyvalent H sera can be eliminated from further study, but colonies or isolates which agglutinate in either or both sera should be subjected to further identification.

3.4 All positive cultures are reported with identification and appropriate sensitivities.

3.5 Notify physician, ID and Public health departments for any salmonella isolate confirmed by serotyping.

4. PROCEDURE:

4.1 **Principle:**

4.1.1 Salmonella Agglutinating Sera are intended for use in slide and tube agglutination tests for serological identification of Salmonella cultures for epidemiological and diagnostic purposes.

4.1.2 Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will agglutinate with bacteria carrying homologous antigens.

4.2 **Specimen:**

4.2.1 All positive cultures for salmonella.

4.3 **Assay Procedure:**

4.3.1 **Slide Agglutination Test:**

4.3.1.1 Put two separate drops (40 µl each) of saline on a glass slide. Emulsify portions of the culture under test with a loop in each drop of saline to give a smooth, fairly dense suspension.

4.3.1.2 To one suspension, as a control, add one drop (40 µl) of saline and mix. To the other suspension add one drop (40 µl) of undiluted antiserum and mix.

4.3.1.3 Rock slide for one minute and observe for agglutination, which can be more easily seen by viewing against a dark background using indirect lighting.

4.3.1.4 Discard the used slide for safe disinfection and disposal.

4.3.2 **Reading of Results:**

4.3.2.1 Agglutination should be strong and clearly visible within one minute.

4.3.2.2 There should be no visible agglutination in the control suspension; if agglutination is seen in the control, the suspension is not suitable for testing by this method.

4.3.3 **Interpretation of results:**

4.3.3.1 No definite conclusion may be drawn about the serological identity of a strain until biochemical testing confirms that it reacts as Salmonella, but agglutination in polyvalent O as well as polyvalent H sera gives strong presumptive evidence that it is a Salmonella.

4.3.3.2 Further serological tests should then be performed to determine the serotype.

4.3.3.3 The possibility of serological cross reactions due to common antigens has already been mentioned; of particular relevance are relationships between different O groups, some of which are expressed in the Kauffmann-White scheme.

4.3.3.4 Agglutination in polyvalent O serum but not polyvalent H serum, if consistent with Salmonella species, suggests that flagella are not well developed. The strain should be retested after passage on motility-enhancing medium, such as 0.5% nutrient agar.(S. pullorumand & S. gallinarumare non-motile).

4.3.3.5 Agglutination in polyvalent H serum but not polyvalent O serum and biochemical reactivity consistent with Salmonella species, suggests that the culture is outside the groups covered by the polyvalent O serum or the O antigens are masked by Vi antigen.

4.3.3.6 The latter may be checked using Vi antiserum, and if the presence of Vi antigen is confirmed, identification of the O antigens should be possible using a suspension which has been boiled for one hour, washed and re-suspended in saline. If no agglutination is visible with either serum, the organism is unlikely to be a Salmonella.

4.3.4 **Limitation of the procedure:**

4.3.4.1 Reactions may be obtained with species outside the genus Salmonella or with Salmonella serotypes outside the range given on the bottle label.

4.3.4.2 Serological tests used alone provide no more than presumptive identification and biochemical examination must be performed in addition to serological analysis.

4.4 **Quality control (If QC strains are available):**

4.4.1 From time to time it is advisable to test the anti-sera as described with known positive and negative cultures.

4.4.1.1 Positive Control organism: Salmonella typhimurium 4,5,12:i:1, 2 NCTC 3048.

4.4.1.2 Negative Control organism: Hafnia alvei NCTC 8535.

5. MATERIALS & EQUIPMENT:

5.1 **Salmonella Anti-sera (reagent Kit):**

5.1.1 Salmonella Polyvalent Agglutinating Sera (1 dropper bottle 2 ml)

5.1.2 Salmonella Polyvalent O A-G - (ZC01/R30858101)

5.1.3 Salmonella Polyvalent O A-S - (ZC02/R30858201)

5.1.4 Salmonella Polyvalent H phase 1 and 2 - (ZD01/R30858501)

5.2 **Materials required but not provided in the kit:**

5.2.1 0.85% saline.

5.2.2 Glass slides

5.2.3 Microbiological loop (sterile).

5.2.4 Light source over dark background

5.2.5 Timer

5.2.6 Shaker

5.2.7 Disinfectant

6. RESPONSIBILITIES:

6.1 The C. pathology specialist/ consultant assigned for microbiology section

7. APPENDICES:

7.1 N/A

8. REFERENCES:

8.1 Procedure Manual, Toronto Medical laboratories / Mount Sinai Hospital department of microbiology.
8.2 Bailey & Scott's Diagnostic Microbiology. Feingold & Baron;12th. Ed.2007, C.V. Mosby Co. p. 301.
8.3 Isenberg HD (Ed) Clinical Microbiology Procedures handbook. American Society for Microbiology, Washington, DC, Vol 1, Section 1.4, 1992.
8.4 Kit Insert/literature (www.oxoid.com/ifu).

9. APPROVALS:

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Appendix 7.1

Common types of vascular and hemodialysis access catheters •

Vascular access Devices	Comments
Arterial line	Catheter usually inserted into the radial artery for continuous blood pressure monitoring in ICU.
Broviac catheter	A type of tunnelled CVC that is positioned just above the heart but tunnelled under the skin and brought out on the chest and thigh away from the site where it enters the vein. Broviac and Hickman catheters are similar types of CVCs. Commonly used for oncology patients receiving chemotherapy.
CVC	A central venous access catheter placed into a large vein in the neck (internal jugular), chest (subclavian vein), or groin (femoral vein) and used to give transfusions, draw blood for tests, and administer medications, fluids, and TPN solutions.
Hickman catheter	A type of tunnelled CVC that is positioned just above the heart but tunnelled under the skin and brought out on the chest and thigh away from the site where it enters the vein. A small cuff is located around the catheter about 1 in. inside the skin at the catheter exit site; it helps to anchor the catheter during long-term use and prevents infections. Hickman and Broviac catheters are similar types of CVCs. Commonly used for oncology patients receiving chemotherapy.
Cordis	Term may be used interchangeably for (i) a CVC line holder device that sits in the vein and comes out through the skin through which the line is threaded and (ii) Cordis (Johnson & Johnson) (http://www.cordis.com) makes many types of stents, including coronary artery stents.
Double lumen	A type of central venous line with two channels used for blood draws and administration of medications.
Gortex graft	Gortex stretch vascular grafts are used in many vascular surgery procedures to replace parts of an artery or create a prosthetic connection between an artery and vein.
IJ line	A CVC that is inserted into the internal jugular vein.
PICC	A PICC (or PICC line) is a peripherally inserted central catheter used for prolonged intravenous access (e.g., chemotherapy, extended antimicrobial therapy, or TPN).
Swan-Ganz catheter	Pulmonary artery catheters inserted in ICU patients to directly and simultaneously measure the pressures of the right atrium, right ventricle, and pulmonary artery and the filling or "wedge" pressure of the left atrium.