HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

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

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

2. DEFINITONS:

2.1 **The genus Shigella:** Recent phylogenetic studies indicate that the shigellae are not a unique genus; rather they are an immunologic and biochemically distinct lineage of Escherichia coli. However, for medical purposes and to maintain historical consistency, the shigellae have not been merged with E. coli.

2.2 **Shigella Species:** are four; Shigella dysenteriae (also referred to as Group A), Shigella flexneri (also referred to Group B), Shigella boydii (also referred to Group C), & Shigella sonnei (also referred to as Group D). Included among these organisms is the etiologic agent of epidemic dysentery, S. dysenteriae serotype 1. With the exception of S. sonnei, each species may be further divided into **serotypes** on the basis of reactivity with hyper-immune sera: S. dysenteriae (15 serotypes), S. flexneri (6 serotypes and 2 variants), &S. boydii (20 serotypes).

2.3 **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. Serotyping is useful for epidemiological purposes.

2.4 **Polyvalent antisera:** Shigellae are distinguished by their antigenic characteristics. Polyvalent antisera allow the presumptive identification of the organism and can be the first step in full identification.

3. POLICY:

3.1 Shigella Polyvalent Agglutinating Sera are suitable for use in slide agglutination tests to identify Shigella cultures presumptively for epidemiological and diagnostic purposes.

3.2 Antisera provide serological identification only; full identification of an organism must be made in conjunction with biochemical testing.

3.3 In screening procedures colonies or isolates which show no agglutination in polyvalent anti-sera can be eliminated from further study, but colonies or isolates which agglutinate 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 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 shigella.

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 Non-specific agglutination due to minor antigenic relationships or roughness may occur, the latter particularly with cultures taken from selective media. These non-specific reactions are usually slow to appear and if due to roughness will also be apparent in the control suspension.

4.3.4 Limitation of the procedure:

4.3.4.1 The sera are made specific within the Shigella genus but reactions with organisms outside the Shigella genus may be found.

4.3.4.2 Serological tests used alone provide no more than presumptive identification and confirmatory biochemical identification tests must be performed.

4.3.4.3 Some isolates of Shigella and Alkalescens-Dispar possess K antigens which mask the O antigens. These capsular antigens (K) can be destroyed by heating at 100°C for two hours.

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. The Code / species Positive control is as follows:

4.4.1.1	ZE02/R30163701 *S. dysenteriae*1-10	NCTC 9955*Shigella dysenteriae* 5
4.4.1.2	ZF01/R30163801 *S. flexneri*1-6, x, y	NCTC 8522*Shigella flexneri* 4b
4.4.1.3	ZG01/R30163901 *S. boydii* 1-6	NCTC 9771*Shigella boydii* 6
4.4.1.4	ZG02/R30164001 *S. boydii* 7-11	NCTC 9355 *Shigella boydii* 9
4.4.1.5	ZG03/R30164101 *S. boydii* 12-15	NCTC 9363*Shigella boydii* 13
4.4.1.6	ZH01/R30164201 *S. sonnei*1,2	NCTC 8219*Shigella sonnei*2
4.4.1.7	ZH05/30164301 Alkalescens-Dispar 1-4	NCTC 7925 Alkalescens-dispar 1

4.4.2 If any antiserum shows agglutination with a known negative culture or shows no agglutination with a known positive culture, it should be discarded.

5. MATERIALS & EQUIPMENT:

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

5.1.1	Shigella Polyvalent Agglutinating Sera 1 dropper bottle (2 ml)	
5.1.2	Shigella dysenteriae1-10	ZE02/R30163701
5.1.3	Shigella flexneri1-6, x, y	ZF01/R30163801
5.1.4	Shigella boydii 1-6	ZG01/R30163901
5.1.5	Shigella boydii 7-11	ZG02/R30164001
5.1.6	Shigella boydii 12-15	ZG03/R30164101
5.1.7	Shigella sonnei1 and 2	ZH01/R30164201
5.1.8	Alkalescens-Dispar 1-4	ZH05/30164301

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).

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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 assigned technologist for microbiology section.
6.2 The C. pathology specialist/ consultant.

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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