

<b>Department:</b>	Laboratory and Blood Bank (Microbiology)		
<b>Document:</b>	Internal Policy and Procedures		
<b>Title:</b>	Shigella Serotyping		
<b>Applies To:</b>	All Laboratory Staff		
<b>Preparation Date:</b>	January 06, 2025	<b>Index No:</b>	LB-IPP-131
<b>Approval Date:</b>	January 20, 2025	<b>Version :</b>	2
<b>Effective Date:</b>	February 20, 2025	<b>Replacement No.:</b>	LB-IPP-131(1)
<b>Review Date:</b>	February 20, 2028	<b>No. of Pages:</b>	03

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

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

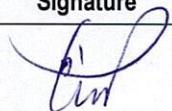
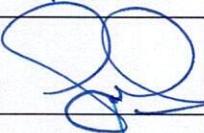
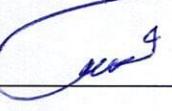
## 7. APPENDICES:

- 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](http://www.oxoid.com/ifu)).

## 9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 06, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 08, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025