DebenDiagnosticsLtd

SHIGELLA ANTISERA

CE UKCA IVD

Shigella is a nonmotile gram-negative bacillus belonging to the *Enterobacteriaceae* family. When the organisms are ingested, they invade the epithelial cells of the large intestine to cause shigellosis which most frequently presents symptoms of diarrhoea (often containing mucous or blood), fever and abdominal pain. *Shigella* is classified into the following four species by serological properties of somatic antigens (O antigens), and biochemical properties according to the recommendation of the International Enterobacteriaceae Grouping Subcommittee (1984) based on Ewing's proposal in 1949: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup *D). S. dysenteriae* is classified into 12 serologic types by antigen type, *S. flexneri* into 6 serotypes and 13 subtypes by antigen type and group, *S. boydii* into 18 serotypes by antigen type. There is one serotype as for *S. sonnei,* which is classified into 2 antigens, phase I (smooth: S type) and phase II (rough: R type) according to the S-R mutation of the O antigen.

The *Shigella* Antisera products are polyvalent or monovalent sera used for serotyping of *Shigella* by slide agglutination, each of which contains a specific agglutinin. Each polyvalent serum is prepared by hyperimmunizing healthy pigs with the inactivated organisms, heating the obtained serum at 56°C for 30 minutes, removing analogous agglutinins with suction and aseptically filtering them. Monovalent sera are similarly prepared by hyperimmunizing healthy rabbits.

PRODUCT

Shigella Antisera are liquid products containing specific somatic antibodies (polyvalent sera: pig, monovalent sera: rabbit) to *Shigella* somatic O antigen and 0.08% w/v sodium azide as a preservative.

Polyvalent sera 8 x 2mL vials

	Polyvalent serum	
Subgroup A (*S. dysenteriae)*	Polyvalent A	Mixture of type 1-7 in subgroup A
	Polyvalent A1	Mixture of type 8-12 in subgroup A
Subgroup B (*S. flexneri)*	Polyvalent B	Mixture of each type and group in subgroup B
Subgroup C (*S. boydii)*	Polyvalent C	Mixture of type 1-7 in subgroup C
	Polyvalent C1	Mixture of type 8-11 in subgroup C
	Polyvalent C2	Mixture of type 12-15 in subgroup C
	Polyvalent C3	Mixture of type 16-18 in subgroup C
Subgroup D (*S. sonnei)*	Polyvalent D	Mixture of phase I and phase II

Monovalent sera 41 x 2mL vials

Monovalent Sera							
Subgroup A	Typing sera 12 vials	Type 1 Type 7	Type 2 Type 8	Type 3 Type 9	Type 4 Type 10	Type 5 Type 11	Type 6 Type 12
Subgroup B	Typing sera 6 vials	Type I	Type II	Type III	Type IV	Type V	Type VI
Grouping sera	vials	Group (3) 4		Group 6		Group (7) 8	
Subgroup C	Typing sera 18 vials	Type 1 Type 7 Type 13	Type 2 Type 8 Type 14	Type 3 Type 9 Type 15	Type 4 Type 10 Type 16	Type 5 Type 11 Type 17	Type 6 Type 12 Type 18
Subgroup D	Phase sera 2 vials	Phase I	Phase II				

Constitution of reagents

		Set 1	Set 2	Set 3
Subgroup A	Polyvalent serum 2 vials	○	○	○
	Monovalent serum 12 vials	○	-	-
Subgroup B	Polyvalent serum 1 vial	○	○	○
	Monovalent serum 9 vials	○	○	-
Subgroup C	Polyvalent serum 4 vials	○	○	○
	Monovalent serum 18 vials	○	-	-
Subgroup D	Polyvalent serum 1 vial	○	○	○
	Monovalent serum 2 vials	○	○	-
		49 vials	19 vials	8 vials

INTENDED USE

The *Shigella* Antisera are intended for the qualitative serological identification and screening of prepared *Shigella* serotype bacterial cultures, using slide agglutination to detect the presence of bacterial antigens. *Shigella* Antisera are intended for use by trained laboratory personnel.

PRINCIPLE OF MEASUREMENT

When this reagent is mixed with a *Shigella* strain which has antigens corresponding to the reagent, the antigen-antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype.

PROCEDURES

1. **Material required but not provided**
   Agar media (nutrient agar medium, heart infusion (HI) agar medium: slant or plate medium), physiological saline, glass slides, glass pencils, small test tubes, pipettes, micropipettes and tips, fluorescent light, microbiological loop, incubator (37°C).
   If necessary: autoclave (121°C) or warm water bath, centrifuge.
2. **Preparation of reagents**
   The antisera are ready for use.
3. **Specimen**
   Cultures of organisms, which are derived from a pure culture and identified as *Shigella* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified.
4. **Procedures**
   1) Suspend an amount of bacterial growth (3-5 times the amount of a match head) in 0.5mL physiological saline and use the antigenic suspension.
   2) Place a drop of each of polyvalent and physiological saline (30µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
   3) Place an antigenic suspension (5-10µL) onto the serum and physiological saline on the glass slide.
   4) Mix the reagents with tilting the glass slide back and forth for 1 minute and the agglutination pattern is observed. Agglutination is grossly observed with light through the slide including fluorescent light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within 1 minute of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.
   5) If a specimen tests positive with a polyvalent serum, perform steps 2 - 4 above using each monovalent serum contained within the polyvalent serum.

DETERMINATION OF RESULTS

Agglutination is grossly observed with light through the slide including fluorescent light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within one minute of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.

Results of polyvalent sera	Determination and additional tests.
Some polyvalent sera test positive.	Serotyping is performed using monovalent sera types contained within the polyvalent sera which tested positive
All polyvalent sera test negative	Determination is suspended

When a positive result is obtained using polyvalent sera in subgroups A, C and D

Results of monovalent sera	Determination
One monovalent sera tests positive.	The name of the monovalent serum which tested positive is interpreted as the serotype of the specimen.
Multiple monovalent sera test positive, or all monovalent sera test negative	Determination is suspended

When a positive result is obtained using polyvalent sera in subgroup B (polyvalent B)

Results of monovalent sera	Determination
One monovalent sera tests positive.	The name of the monovalent serum which tested positive is interpreted as the serotype of the specimen.
All monovalent sera test negative	Determination is suspended

PRECAUTIONS

1. Antigenic components of *Shigella* are shared widely throughout the *Enterobacteriaceae,* especially with enteroinvasive *E. coli.* It is important that an organism used as a specimen is *Shigella* by biochemical tests.
2. Bacterial culture should be performed using the indicated media: unselective media. If selective media are used, antigen production may be insufficient or autoagglutination may occur.
3. When an antigenic suspension and serum are mixed in the slide agglutination procedure, the platinum loop should be sterilized with a flame for each serum to avoid cross contamination among sera.
4. When agglutination is found upon the reaction of antigenic suspension and physiological saline, the determination test should be repeated after a colony is reselected.

5. Some *Shigella* strains cannot be serotyped if viable organisms are used. When a strain identified as *Shigella* tests negative with all polyvalent sera, serotyping is repeated after the following heat treatment of an antigenic suspension:
   1) An amount of bacterial growth (3-5 times the amount of a match head) is sampled and placed in 3mL of physiological saline to suspend. The suspension is heated at 121°C for 15 minutes or at 100°C for 60 minutes.
   2) The heated suspension is centrifuged at 900g for 20 minutes. Its supernatant is removed and 0.5mL saline added to the precipitate to suspend equally. This suspension is used as an antigenic suspension.
6. When polyvalent sera test positive and monovalent sera test negative, the strain being tested may be a serotype not contained in this product. After biochemical properties of the specimen are reconfirmed, consultation with a public testing facility is recommended.
7. When multiple polyvalent and monovalent sera give positive results, reconfirm the biochemical properties of the specimen and repeat serotyping using a heated antigenic suspension.
8. Serotyping of *Shigella* should not be determined based solely on the results from polyvalent sera.

PERFORMANCE

1. **Sensitivity**
   When one drop of this antiserum is allowed to react on a slide with a known serotype of the reference strain, granular agglutination is observed macroscopically.
2. **Specificity**
   In a test conducted in a similar manner to the sensitivity test, this antiserum agglutinates only with the reference strain corresponding to the serotype, while in the reaction with non-corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTIONS FOR USE AND HANDLING

1. **General precautions**
   1) This test is for in vitro diagnostic use only.
   2) This kit should only be used by sufficiently trained lab staff.
   3) New serotypes of *Shigella* have been reported and may again in the future; accordingly, strains that do not react or where type cannot be confirmed with the reagent may be *Shigella sp.*
2. **Precautions of handling**
   1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
   2) If reagents come into contact with skin, mucous membranes or eyes, wash immediately with plenty of water.
   3) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
   4) The reagent should be allowed to stand at 15-25°C for at least 30 minutes before use.
   5) Used containers should not be used for other purposes.
   6) Sera with different production numbers should not be mixed.
   7) The reagent should be used according to the described procedures.
   8) The reagent should only be used for the intended use.
   9) Special precautions should be taken to ensure that the reagent caps are not exchanged.

3. **Precautions for disposal**
   1) The reagent contains 0.08% w/v sodium azide. Sodium azide may react with lead or copper to form explosive heavy metal azides. The reagent should be disposed with a large amount of water.
   2) All specimen, spills, inoculated product and equipment used in this test should be treated with one of the following methods.
      [1] Soaking in 0.1% w/v hypochlorite for 1 hour or more.
      [2] Autoclaving at 121°C for 20 minutes or more.

STORAGE AND SHELF LIFE

Storage: 2-10°C
Shelf life: Up to the expiry date on the label.

PACKAGE

Shigella Antisera: Each type in a 2mL vial with pipette

- Set 1: 49 vials (8 vials of polyvalent sera, 41 vials of monovalent sera)
  1 package
- Set 2: 19 vials (8 vials of polyvalent sera. 11 vials of monovalent sera)
  1 package
- Set 3: 8 vials (8 vials of polyvalent sera) 1 package
  *Each serum is separately available.

REFERENCE

1) Supervised by the Ministry of Health, Labour and Welfare: Oral Infectious Diseases, *Shigella,* Microbiological Test Manual. Bacterial and Fungi Tests, Third edition, Japan Public Health Association, D-14 (1987).
2) Brenner, D.J.: Recommendation on recent proposals for the classification of *shigellae,* Int. J. Syst. Bacteriol., 34, 87 (1984)

ANTIGENIC STRUCTURE OF *SHIGELLA FLEXNERI*

Serum / Types	Typing Sera I	II	III	IV	V	VI	Grouping Sera (3)4	6	7(8)	Antigenic Structure
S. flexneri 1a	+	-	-	-	-	-	+	-	-	I : 4
S. flexneri 1b	+	-	-	-	-	-	+	+	-	I : 4, 6
S. flexneri 2a	-	+	-	-	-	-	+	-	-	II : 3, 4
S. flexneri 2b	-	+	-	-	-	-	-	-	+	II : 7, 8
S. flexneri 3a	-	-	+	-	-	-	+/-	+	+	III : (3, 4) 6, 7, 8
S. flexneri 3b	-	-	+	-	-	-	+/-	+	-	III : (3, 4) 6
S. flexneri 4a	-	-	-	+	-	-	+	-	-	IV : 3, 4
S. flexneri 4b	-	-	-	+	-	-	-	+	-	IV : 6
S. flexneri 5a	-	-	-	-	+	-	+	-	-	V : 3, 4
S. flexneri 5b	-	-	-	-	+	-	-	-	+	V : 7, 8
S. flexneri 6	-	-	-	-	-	+	+/-	-	-	VI : (4)
S. flexneri Variant X	-	-	-	-	-	-	-	-	+	- : 7, 8
S. flexneri Variant Y	-	-	-	-	-	-	+	-	-	- : 3, 4

	Manufacturer
EC REP	Authorised representative in the European Community/European Union
	Consult instruction for use
IVD	For in vitro diagnostic use only
REF	Catalogue number
LOT	Batch code
	Storage temperature limitation
	Use by
LATEX	Contains or presence of natural rubber latex

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