DebenDiagnosticsLtd

SALMONELLA ANTISERA

CE UKCA IVD

Salmonella is a gram negative, non-spore forming rod shaped bacteria belonging to family *Enterobacteriaceae* and causes many types of infections, from mild gastroenteritis to life threatening typhoid fever. *Salmonella* can be serotyped according to somatic antigens O (group O: O2-O67 groups), surface Vi, phase I flagellar and phase II flagellar (H types: H-a - H-z81) antigens. The somatic O antigen are heat stable and upon which grouping of the organism is based. The H antigen are heat labile and usually associated with motility. H antigen exists in 2 phases, phase I and phase II.

Salmonella Antisera are liquid products containing specific agglutinins for each group O antigen, Vi antigen and H antigen that are used for the serological identification of various *Salmonella* serotypes according to Kauffman-White classification. The sera are prepared by hyperimmunizing healthy rabbits with reference strains, heating to 56°C for 30 minutes or using formalin treatment, removing analogous agglutinins by suction, before antiseptic filtration. For the preparation of H-sera, reference strains of the organisms killed by formalin treatment are used as an antigen, and for the preparation of Vi serum, *Citrobacter* ballerup, a strain that has Vi antigen, is used. Group O sera are used for the determination test of serotypes of *Salmonella* using slide agglutination, and H-sera for the determination test using either tube agglutination or slide agglutination.

PRODUCT

Salmonella Antisera are produced from rabbits and contain 0.08% w/v sodium azide as a preservative. The following serum types are provided as 2mL or 5mL volumes in vials with a dropper attachment and are ready to use.

1. Group O sera
   1) Polyvalent sera (2mL each)
      Omnivalent Polyvalent A-G Polyvalent A-S
      Polyvalent O Polyvalent O1
   2) Monovalent sera (2mL each)
      group O2 group O4 group O7 group O8
      group O9 group O9, 46 group O3, 10 group O1, 3, 19
      group O11 group O13 group O6, 14 group O16
      group O18 group O21 group O35
2. Vi serum (2mL)
3. H sera
   1) H serum (2mL for slide agglutination, 5mL for tube agglutination).
      H-a H-b H-c H-d H-e,h H-G* H-i H-k
      H-L* H-r H-y H-n* H-1* H-z H-z4* H-z10
      H-z29
      * Antigen group
   2) H factor serum (2mL for slide agglutination, 5mL for tube agglutination)
      H-L factor H-v H-w H-z13 H-z28
      H-1 factor: H-2 H-5 H-6 H-7 H-z6
      H-G factor: H-f H-m H-p H-q H-s H-t H-u
      H-z4 factor: H-z23 H-z24 H-z32
      H-e,n factor: H-x H-z15
   3) Polyvalent serum (2mL each)
      H-E(complex) Rapid 1 Rapid 2 Rapid 3 Phase 1&2

Constitution of Sets

1. Set 1: group O sera, 17 vials; Vi serum, 1 vial
   1) Group O (2mL each)

Polyvalent sera	Monovalent sera
Polyvalent O	group O2 group O4 group O7 group O8 group O9 group O9,46 group O3,10 group O1, 3, 19
Polyvalent O1	group O11 group O13 group O6,14 group O16 group O18 group O21 group O35

   2) Vi serum (2mL)
2. Set 2: H sera (5mL), 17 vials
   H-a H-b H-c H-d H-e,h H-G* H-i H-k H-L
   H-r H-y H-e,n H-1 H-z H-z4 H-z10 H-z29
3. Set 3: H-L factor sera (5mL), 4 vials
   H-v H-w H-z13 H-z28
4. Set 4: H-1 factor sera (5mL), 5 vials
   H-2 H-5 H-6 H-7 H-z6
5. Set 5: H-G factor sera (5mL), 7 vials
   H-f H-m H-p H-q H-s H-t H-u
6. Set 6: H-z4, H-e, n factor sera (5mL), 5 vials
   H-z23 H-z24 H-z32 H-x H-z15
7. Set 7: group O sera 2 vials, H sera 2 vials, Vi serum (for identification of S. typhi and S. Paratyphi-A)
   1) group O sera (2mL each)
      group O2 group O9
   2) H sera (5mL each)
      H-a H-d
   3) Vi serum (2mL)

INTENDED USE

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

PRINCIPLE OF MEASUREMENT

When this reagent is mixed with a *Salmonella* 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**
   Glass slide, glass pencil, small test tubes, pipette and micropipette, microbiological loop, physiological saline, physiological saline containing 1% vol. formalin, water bath (50°C), autoclave (121°C) or water bath (100°C), 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 *Salmonella* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified. For determination test of the H type, motile strains should be used.
4. **Procedures**
   A. Slide agglutination for O antigen and Vi grouping
   1) Suspend an amount of bacterial growth (3-5 times the amount of a match head) in 0.5 mL physiological saline and use antigenic suspension for group O.
   2) Place a drop of polyvalent antiserum 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 for group O (5-10µL) onto the serum and physiological saline on the glass slide.
   4) Mix the reagents with tilting of 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 in 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 showing positive.

      When no agglutination is found with both O and O1 polyvalent sera, repeat steps 2) - 4) above using Vi serum. If a positive reaction is found with the Vi serum, perform steps 6) - 8) below.

   6) Add a 0.2mL antigenic suspension for O group into 2mL physiological saline and heat to 121°C for 15 minutes or 100°C for 1 hour. Centrifuge the heated solution at 900g for 20 minutes, discard the supernatant, suspend the precipitate with 0.2mL physiological saline and use as a heated cell suspension.
   7) Perform steps 2) - 4) using polyvalent sera and Vi serum with the heated cell suspension.
   8) If the live cell shows a negative result with the polyvalent sera and a positive result with the Vi serum, while the heated cell shows a positive result with polyvalent sera and negative result with Vi serum, the specimen is probably regarded as *S*. Typhi or *S*. Paratyphi-C. Repeat the agglutination test using group O7 and group O9 sera with the heated cell suspension.

   B. H antigen serotyping

   a. Tube agglutination test for H antigen serotyping
   1) Using a liquid culture of organisms grown at 37°C for 6-8 hours, make a 1:2 dilution by adding an equal volume of physiological saline containing 1% vol. formalin and use as an antigenic suspension for H types.
   2) Add three drops of the required type of H serum and 100µL physiological saline respectively into a small test tube and add 0.5mL of antigenic suspension for H type to it.
   3) Mix the contents of the test tube by shaking thoroughly and allowed to stand in a water bath at 50°C for 1 hour for observed agglutination. Agglutination is grossly observed under sufficient halation of a fluorescent light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. A cotton wool-like agglutination observed after the reaction with each serum should be regarded as positive. If a homogeneous suspension is still observed, it should be regarded as negative.
   4) If a specimen tests positive with H-L, H-1, H-G, H-z4, or H-e,n perform steps 2) - 3) above using each H factor serum.

   b. Slide agglutination test for H antigen serotyping
   1) Suspend an amount of bacterial growth (3-5 times the amount of a match head) in 0.5mL physiological saline and use as an antigenic suspension for H serotyping.
   2) Place a drop of H-serum 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 for H serotyping (5-10µL) onto the serum and physiological saline on the slide glass.
   4) Mix the reagents by tilting the glass slide back and forth for 1 minute and the agglutination pattern should be observed. Agglutination is grossly observed under transmitted light including fluorescent light. It should be first confirmed that no agglutination is found with 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 H-L, H-l, H-G, H-z4, or H-e,n serum, perform steps 2) - 4) above using each H factor serum. If specimens test positive with the H-polyvalent serum, perform steps 2) - 4) above using each H-serum contained within the polyvalent serum showing positive.

PRECAUTIONS

1. Bacteria culture should be performed using non-selective media e.g., nutrient agar. If selected media are used, antigen production may be insufficient or autoagglutination may occur.
2. When antigenic suspension and serum are mixed as a procedure of slide agglutination, the microbiological loop should be sterilized with a flame for each serum to avoid cross contamination among sera.
3. When an H type of a strain with weak motility is determined, the strain should be passed through a semi-liquid medium inserted into a Craigie's tube before determination to enhance the motility.
4. When agglutination is found upon the reaction of antigenic suspension and physiological saline the determination test is repeated after a colony is reselected.

5. When both O and O1 polyvalent sera give positive results, reconfirm the biochemical properties. If the strain is identified as *Salmonella*, it may possess other O antigens that are not included *in Salmonella* antisera.
6. If the strain is identified as *Salmonella* and tested negative for polyvalent sera and Vi serum, it may possess O antigen that is not included in *Salmonella* antisera.
7. If a heated antigenic suspension after heating twice and centrifugation tests positive for Vi serum and negative for O polyvalent sera, its biochemical properties should be reconfirmed.
8. Some strains of *Citrobacter* and *Escherichia* are known to possess Vi antigen. If a viable organism suspension tests positive for Vi serum, and heated antigenic suspension tests negative for Vi serum and O polyvalent serum, then the biochemical properties should be reconfirmed.
9. As the aggregate from the reaction of flagella is very fragile, the test tubes should not be shaken during the observation. If agglutination is indistinct after an hour of reaction, it should be determined after an additional hour of incubation.
10. If the specimen tests negative with any of the H sera, the strain of specimen may have a serum type other than the types tested or the flagella of the strain may not have grown sufficiently. The determination test of the H type should be repeated for confirmation after mobility enhancement procedures.

PERFORMANCE

1. Sensitivity test

1) O sera: When one drop of the product reacted on a glass slide with a reference strain of a known serotype, granular agglutination was grossly observed.
2) H sera: When 3 drops of the product reacted in a small test tube with a reference strain of a known serotype, cotton wool-like agglutination was grossly observed.
3) Vi serum: When one drop of the product reacted on a glass slide with a reference strain of a known serotype, granular agglutination was grossly observed.

2. Specificity test

In a test performed in a similar manner to the sensitivity test, the antiserum agglutinates only with the reference strain corresponding to the serotype, while in reactions with non-corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTION 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) Antigenic components of S*almonella* are shared widely throughout the *Enterobacteriaceae*. It is important to confirm the organism being used by biochemical test.

2. Handling precautions

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 nor 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 reagents should be used according to the described procedure.
8) The reagents 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

Salmonella Antisera: Each type in a 2mL (group O serum, Vi serum, H serum for slide agglutination), or 5mL (H serum for tube agglutination) vial with pipette.

- Set 1: 18 vials (17 vials of group O sera, 1 vial for Vi serum), 1 package
- Set 2: 17 vials (H sera – 5mL) 1 package
- Set 3: 4 vials (H-L factor sera - 5mL), 1 package
- Set 4: 5 vials (H-1 factor sera - 5mL), 1 package
- Set 5: 7 vials (H-G factor sera - 5mL), 1 package
- Set 6: 5 vials (3 vials of H-z4 factor sera, 2 vials of H-en factor sera – 5mL), 1 package
- Set 7: 5 vials (2 vials of group O sera, 2 vials of H sera - 5mL, 1 vial of Vi serum), 1 package

* Each serum is also separately available.

- group O polyvalent: Omnivalent. A-G, A-S

- H sera - 2mL: H-a, H-b, H-c, H-d, H-e,h, H-G, H-i, H-k, H-L, H-r, H-y, H-e,n, H-E, H-1, H-z, H-z4, H-z10, H-z29, H-v, H-w, H-z13, H-z28, H-2, H-5, H-6, H-7, H-z6, H-f, H-m, H-p, H-q, H-s, H-t, H-u, H-z23, H-z24, H-z32, H-x, H-z15, Rapid 1, Rapid 2, Rapid 3, Phase 1&2, H-E(Complex)

REFERENCES

1) Supervised by the Ministry of Health, Labour and Welfare: *Salmonella,* Microbiological test manual, Bacteria and fungi test, Third edition, Japan Public Health Association, D-43 (1987).
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3) Reeves, M. W., et al.: Clonal nature of *Salmonella "hi* and its genetic relatedness to other *Salmonella* as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori* comb. nov., J. Clin. Microbiol., 27, 313 (1989).

Agglutinins contained in each *Salmonella* O antiserum

Name of serum	Contained O agglutinins	Name of serum	Contained O agglutinins
Omnivalent	2, 3, 4, 5, 7, 8, 9,10, 12, 15, 19, 34, 46, 11, 13, 14, 16, 17, 18, 21, 22, 23, 24, 28, 30, 35, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 63, 65, 66, 67	Group O2	2
		Group O4	4, 5
		Group O7	7
		Group O8	8
		Group O9	9
Polyvalent A-G	2, 3, 4, 5, 7, 8, 9,10, 12 15, 19, 34, 46, 11, 13,	Group O9,46	46
		Group O3, 10	10, 15, 34
Polyvalent A-S	2, 3, 4, 5, 7, 8, 9,10, 12 15, 19, 34, 46, 11, 13, 14, 16, 17, 18, 21, 22, 23, 24, 28, 30, 35, 38, 39, 40, 41	Group O1, 3,19	19
		Group O11	11
		Group O13	13,22,23
		Group O6,14	14,24,25
Polyvalent O	2, 3, 4, 5, 7, 8, 9,10, 12 15, 19, 34, 46	Group O16	16
		Group O18	18
Polyvalent O1	11, 13, 14, 16,18, 21, 22, 23,24,35	Group O21	21
		Group O35	35

Agglutinins contained in each *Salmonella* H antiserum

Name of serum	Contained H agglutinins	Name of serum	Contained H agglutinins
H-a	a	H-5	5
H-b	b	H-6	6
H-c	c	H-7	7
H-d	d	H-z6	Z6
H-e,h	h	H-f	f
H-G	g, f, p, m, t	H-m	m
H-i	i	H-p	p
H-k	k	H-q	q
H-L	l, w	H-s	s
H-r	r	H-t	t
H-y	y	H-u	u
H-e,n	n, x	H-z23	z23
H-1	1,2 5, 6, 7, z6	H-z24	z24
H-z	z	H-z32	z32
H-z4	z4, z23, z24	H-x	x
H-z10	z10	H-z15	z15
H-z29	z29	Rapid 1	b, d, h, n, x, r
H-v	v	Rapid 2	b, h, n, x, k, l, w
H-w	w	Rapid 3	d, n, h, x, k, g, f, p, m, t
H-z13	z13		
H-z28	z28	H-E(Complex)	h, n, x
H-2	2	Phase 1&2	All H-types

	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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