

INTENDED USE

Salmonella antisera are prepared for use in serological identification of organisms belonging to the genus *Salmonella* according to Kauffmann-White classification (4), for use by appropriately qualified personnel.

SUMMARY AND EXPLANATION

The genus *Salmonella* contains a wide variety of pathogenic species affecting man and animals worldwide. Complete identification of *Salmonella* requires culture isolation, biochemical characterization and serological identification (serotyping).

Salmonella polyvalent 'O' (somatic) antisera are intended to aid initial serogrouping. Full identification of 'O' antigens can be achieved using monovalent specific 'O' antisera (1). The serotype of *Salmonella* isolates can then be determined by the use of polyvalent and monovalent 'H' (flagella) antisera (1,2).

PRINCIPLE OF THE TEST

The principle of the serological identification of *Salmonella* involves mixing the suspected organism with antiserum containing specific *Salmonella* antibodies. The bacteria will agglutinate (clump) in the presence of homologous antiserum.

MATERIALS PROVIDED

Pro-Lab Diagnostics *Salmonella* 'O' and 'H' polyvalent and monovalent antisera are prepared in rabbits using reference strains according to the methods recommended by the World Health Organization (3,4) and adsorbed to eliminate cross-reacting antibodies.

Pro-Lab Diagnostics antisera are supplied in a dropper bottle containing 3.0 ml of ready-to-use diluted antisera with 0.01% thimerosal as preservative.

Polyvalent Somatic O Antisera

PL.6000	Polyvalent O A - I + Vi
PL.6002	Polyvalent O A-S

Monovalent Somatic O Antisera

PL.6010	Group A, Factor 2
PL.6011	Group B, Factor 4
PL.6012	Group B, Factor 5
PL.6013	Group C, Factor 6,7
PL.6014	Group C2, Factor 8
PL.6015	Group D, Factor 9
PL.6016	Group B/D, Factor 12
PL.6017	Group E, Factor 3,10,15,19,34
PL.6018	Group E1, Factor 10
PL.6019	Group E2, Factor 15
PL.6020	Group E4, Factor 19
PL.6021	Group E3, Factor 34
PL.6022	Group F, Factor 11
PL.6023	Group G, Factor 13,22,23
PL.6024	Group G1, Factor 22
PL.6025	Group G2, Factor 23
PL.6027	Group C3, Factor 20
PL.6029	Group I, Factor 16
PL.6030	Group J, Factor 17
PL.6031	Group K, Factor 18
PL.6032	Group L, Factor 21
PL.6033	Group M, Factor 28
PL.6034	Group N, Factor 30

PL.6035	Group O, Factor 35
PL.6036	Group P, Factor 38
PL.6037	Group Q, Factor 39
PL.6038	Group R, Factor 40
PL.6039	Group S, Factor 41
PL.6040	Vi
PL.6041	Factor 55

Polyvalent Flagella H Antisera

PL.6100	Polyvalent H
PL.6101	Polyvalent H Phase 2, Factors 1,2,5,6,7,z6

Monovalent Flagella H Antisera

PL.6110	Factor a
PL.6111	Factor b
PL.6112	Factor c
PL.6113	Factor d
PL.6114	E Complex eh, enx, enz15
PL.6115	Factor eh
PL.6116	Factor enx
PL.6117	Factor enz15
PL.6118	Factor h
PL.6120	Factor z15
PL.6121	G Complex
PL.6122	Factor gm
PL.6123	Factor gp
PL.6124	Factor p
PL.6125	Factor u
PL.6126	Factor s
PL.6127	Factor m
PL.6128	Factor t
PL.6129	Factor f
PL.6131	Factor q
PL.6133	Factor i
PL.6134	Factor k
PL.6135	L Complex
PL.6136	Factors l, w
PL.6137	Factors l,v
PL.6138	Factor w
PL.6139	Factor v
PL.6140	Factor z13
PL.6141	Factor z28
PL.6142	Factor r
PL.6143	Factor y
PL.6144	Factor z
PL.6145	Z4 Complex
PL.6146	Factor z23
PL.6147	Factor z24
PL.6148	Factor z32
PL.6149	Factor z10
PL.6151	Factor z29
PL.6153	Factor 2
PL.6154	Factor 5
PL.6155	Factor 6
PL.6156	Factor 7

Rapid Salmonella Diagnostic Sera:

PL.6200	Rapid Salmonella Diagnostic Sera 1
PL.6201	Rapid Salmonella Diagnostic Sera 2
PL.6202	Rapid Salmonella Diagnostic Sera 3

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass Slides or Test tubes
- Normal Saline (0.85% sodium chloride solution)
- Disposable or wire loops
- Water bath set to 51°C
- Low power objective.

STABILITY AND STORAGE

Salmonella antisera should be stored at 2-8°C. Do not freeze. Stored under these conditions the antisera may be used up to the date of expiry shown on the product label

PRECAUTIONS

- Do not use antisera after the expiry date shown on the product label.
- The antisera contain thimerosal, which is a highly toxic mercury-based compound. Although the amount of thimerosal in the antisera is minimal safety precautions should be taken in handling, processing and discarding the reagent.
- Avoid contamination of the reagent bottle.
- The test specimen may contain organisms pathogenic to man and should be handled and discarded as infectious material.
- The reagent is intended for in vitro diagnostic use only.
- The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.
- Product contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

SAMPLE STORAGE AND COLLECTION

For specific procedures regarding specimen collection and preparation of primary cultures refer to a standard microbiology textbook. Colonies isolated on enteric differential agar media and suspected of being *Salmonella* should be confirmed with conventional biochemical tests. In general, a low selectivity media e.g. Blood agar or nutrient agar, should be used to grow colonies for 'O' somatic antigen identification. For identification of 'H' flagellar antigen, culture preparation is best made from liquid phase growth.

TEST PROCEDURE

A. Identification of *Salmonella* Somatic and Vi antigen (Slide Test):

1. Place two separate loopfuls of normal saline (0.85% sodium chloride) on a clean glass slide.
2. Take a small part of a suspect *Salmonella* colony from an overnight culture plate and mix thoroughly with both drops of normal saline on the slide to obtain a smooth suspension.
3. Add one loopful of antisera to one of the bacterial suspension drops on the slide, to the other (control) add one loopful of normal saline.
4. Mix the antiserum with the bacterial suspension using a sterile loop.
5. Gently tilt the slide back and forth for one minute and observe for agglutination under normal lighting conditions, preferably using a low power objective.

B. Identification of Salmonella Flagellar (H) Antigen (Slide Test):

1. The procedure is the same as for somatic antigen identification with the exception of using liquid phase growth from semi-solid medium with a Craigie tube (1) or growth in the liquid of an agar slope. If liquid culture is used there is no need to make saline suspensions. Flagellar antigen detection can normally be achieved by slide agglutination tests; however, some strains are poorly flagellated and may only be identified by tube agglutination tests.

C. Identification of Salmonella Somatic, Vi and H Antigen (Tube Test):

- Preparation of Cell Suspensions for Testing: Prepare a dense suspension of the bacteria in normal saline and boil for 10 minutes or use alcohol dehydrated cells resuspended in normal saline to Browns tube 2 for identification of somatic antigens. Prepare formalized killed broth culture for the identification of 'H' antigen. Suspend suspected 'Vi' colonies in 0.5% formal saline to Brown's tube 2 for the identification of 'Vi' antigens.
- Antisera Dilution: In order to use *Salmonella* antisera in a test tube, each antiserum must be diluted 1:5 in normal saline before use.
- Add 150 µl of normal saline to a glass test tube and in another tube add an equal volume of diluted antisera.
- Add an equal volume of previously prepared cell suspension to each tube.
- Incubate in a water bath at 37°C for 2 hours in the case of flagellar antigen identification or for 5 to 18 hours in the case of somatic or 'Vi' identification. For some bacteria, incubation at 50°C is preferable.
- Observe tubes for agglutination.

D. Identification of Salmonella Flagellar (H) Antigen using the rapid Salmonella Diagnostic Sera:

The Rapid *Salmonella* Diagnostic Sera are used in combination to determine flagellar group.

- For the procedure for identification of *Salmonella* flagellar (H) antigen using the slide test refer to procedure B.
- For the procedure for identification of *Salmonella* flagellar (H) antigen using the tube test refer to procedure C.

QUALITY CONTROL PROCEDURE

Refer to lot specific Certificate of Analysis.

INTERPRETATION OF RESULTS

- For procedure A or B:
A distinct agglutination (granular clumping) within 60 seconds, without agglutination in the saline control (auto-agglutination) is regarded as a positive result. Positive results may be confirmed by tube agglutination tests.
- For Procedure C
Granular "clumps" observed in the tube are regarded as a positive result for 'O' antigen identification, whereas a more floccular appearance observed using a bright light against a dark background is regarded as a positive result for 'H' antigen identification.
- For Procedure D
 - Positive results are interpreted for the slide test as in 1.
 - Positive results are interpreted for the tube test as in 2.
 - For interpretation of the results for the Rapid *Salmonella Diagnostic* Sera 1, 2 and 3 as a panel refer to the following chart:










Salmonella flagellar group	
Sera	b d E G k L r
Rapid <i>Salmonella</i> Diagnostic Sera 1	+ + + - - - +
Rapid <i>Salmonella</i> Diagnostic Sera 2	+ - + - + + -
Rapid <i>Salmonella</i> Diagnostic Sera 3	- + + + + - -

LIMITATIONS OF THE PROCEDURE

- The antisera should only be used for identification of cultures which have been previously characterized biochemically as *Salmonella*. The presence of similar antigens on the surface of bacteria other than *Salmonella* has not been tested for and may give false results.
- Rough strains will auto agglutinate, giving false positive results. Therefore, a normal saline control should be included in every test to ensure the specificity of the reaction.
- It is recommended to check the potency of *Salmonella* antisera with stock cultures of known antigenic structure.
- Although the majority of *Salmonella* strains possessing the appropriate antigens will agglutinate with the homologous antiserum, due to slight differences, for example, in the antigenic expression between strains of the same serotype and individual colonies due to form variation (5), agglutination cannot be guaranteed in all cases.
- Sensitivity of the slide test may be reduced if volumes greater than 10 µl are used.

REFERENCES

- Ewing, W. H. 1986 . Edwards and Ewing' s Identification of Enterobacteriaceae, 4th Ed. Elsevier Science Publishing Co., New York.
- Spicer, C.C. 1956. J. Clin. Path. 9:378.
- World Health Organization, Centre for Reference and Research on *Salmonella*. Antigenic formulae of the *salmonella* serovars 1992. WHO International *Salmonella* Centre, Institut Pasteur, Paris.
- Kauffmann, F. 2001. The Bacteriology of Enterobacteriaceae. The Williams & Wilkins Co., Baltimore.
- Bergan T. (Ed) 1984 Methods in Microbiology. Vol 15. Serology Of *Salmonella*. Lindberg A, Minor L-1-141.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use



Advena Ltd. Tower Business Centre, 2nd Floor,
Tower Street, Swatar, BKR 4013, Malta.
T: +44(0) 1926 800 153

