SALMONELLA ANTISERA

(for in vitro diagnostic use)

INTENDED USE

PRO-LAB Vision *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 world-wide. Complete identification of Salmonella requires culture isolation, biochemical characterization and serological identification (serotyping).

PRO-LAB polyvalent 'O' (somatic) antisera are intended to aid initial serogrouping, TABLE 1. 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]

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.

REAGENTS

PRO-LAB *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 absorbed to eliminate cross-reacting antibodies.

PRO-LAB antisera are supplied in a dropper bottle containing 2ml or 3.0 ml of ready-to-use diluted antisera with 0.1% sodium azide as preservative.

PRECAUTIONS

1. Do not use antisera after the expiry date shown on the product label.
2. The antisera contains soidum azide (0.1%) as a preservative. Although it is not considered hazardous at this level, please note that accumulated sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Thorough flushing of plumbing is recommended.
3. Avoid contamination of the reagent bottle.
4. The test specimen may contain organisms pathogenic to man and should be handled and discarded as infectious material.
5. The reagent is intended for in vitro diagnostic use only.
6. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.
7. Product contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

MATERIAL 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
Microscope

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.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

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 eg. 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.

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):

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):

1. 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.
2. Antisera Dilution: In order to use PRO-LAB *Salmonella* antisera in a test tube, each antiserum must be diluted 1:5 in normal saline before use.
3. Add 150 ul of normal saline to a glass test tube and in another tube add an equal volume of diluted antisera.
4. Add an equal volume of previously prepared cell suspension to each tube.
5. Incubate in a water bath at 51°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.
6. Observe tubes for agglutination.

INTERPRETATION OF RESULTS

1. **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.
2. **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.

LIMITATIONS OF THE PROCEDURES

1. 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* have not been tested for and may give false results.
2. Rough strains will autoagglutinate, giving false positive results. Therefore a normal saline control should be included in every test to ensure the specificity of the reaction.
3. It is recommended to check the potency of *Salmonella* antisera with stock cultures of known antigenic structure.
4. 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.
5. Sensitivity of the slide test may be reduced if volumes greater than 10 µl are used.

REFERENCES

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

REAGENTS AVAILABLE

TABLE 1. *Salmonella* Polyvalent Somatic O Antisera

Cat No.	Description	*Salmonella* O group antigen
PLA003	O Polyvalent A-I	A, B, C, D, E, F, G, H, I
PLA004	OMA	A, B, D, E, L
PLA005	OMB	C, F, G, H
PLA006	OMC	I, J, K, M, N, O, P
PLA007	OMD	Q, R, S, T, U, V, W
PLA008	OME	X, Y, Z, 51, 52,53,61
PLA009	OMF	54, 55, 56, 57, 58, 59
PLA010	OMG	60, 62, 63, 65, 66, 67

Table 2. *Salmonella* Polyvalent H Antisera

Cat. No.	Description	Factors Present
PLA121	Polyvalent H (phase 1 & 2)	a b c d i e,h e,n,x e,n,z15 f,g g,m g,p g,q g,s,t g,z51 m,t k l,v l,w l,z13 l,z40 r y z z6 z10 z29 z35 z36 z38 z39 z41 z42 z44 z60 z4,z23 z4,z24 z4,z32 1,2 1,5 1,6 1,7
PLA122	HMA	a b c d i z10 z29
PLA123	HMB	E complex G complex
PLA124	HMC	k y z r L complex Z4 complex
PLA125	HMD	z35 z36 z38 z39 z41 z42 z44 z60
PLA126	HME	Z52 Z53 Z54 Z55 Z57 Z61
PLA127	HMF	1,2 1,5 1,6 1,7 Z6
PLA128	H : 1 Complex	1,2 1,5 1,6 1,7
PLA129	H : E Complex	e,h e,n,x e,n,z15
PLA130	H : G Complex	f,g f,g,s f,g,t g,m g,m,s g,m,s,t g,m,t g,p g,p,s g,p,u g,q g,s,t g,z51 g,t g,m,q m,t
PLA131	H : L Complex	l,v l,w l,z13 l,z28 l,z40
PLA132	H : Z_4 Complex	z4,z23 z4,z24 z4,z32

Table 3. *Salmonella* H-Phase and H-Factor Antisera

Cat. No	Description
PLA141	H : a
PLA142	H : b
PLA143	H : c
PLA144	H : d
PLA145	H : i
PLA146	H : k
PLA147	H : r
PLA148	H : y
PLA149	H : z
PLA150	H : z6
PLA205	H : 5
PLA206	H : 6
PLA207	H : 7
PLA208	H : z13
PLA209	H : z15
PLA210	H : z23
PLA211	H : z24
PLA212	H : z28
PLA213	H : z32
PLA214	H : z51


= Use by


= Lot number


= Catalogue number


= Manufacturer


= In vitro diagnostic medical device


= Temperature limitation


= Consult instructions for use