SALMONELLA ANTISERA

(for *in vitro* diagnostic use)

PRO-LAB DIAGNOSTICS

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INTENDED USE

PRO-LAB Vision 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 sero-grouping. Full identification of 'O' antigens can be achieved using mon-ovalent 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* anti-bodies. 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 recom-mended by the World Health Organization(3,4) and absorbed to eliminate cross-reacting antibodies.

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

PRECAUTIONS

1. Do not use antisera after the expiry date shown on the product label.
2. The antisera contains 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 discard-ing the reagent.
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 selectiv-ity 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 agglu-tination 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 detec-tion can normally be achieved by slide agglutination tests, however, some strains are poorly flagellated and may only be identified by tube agglutina-tion tests.

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

1. Preparation of Cell Suspensions for Testing: Prepare a dense suspen-sion 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 iden-tification 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' identifica-tion.
6. 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.

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

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.
3. **For procedure D:**
   (i) Positive results are interpreted for the slide test as in 1.
   (ii) Positive results are interpreted for the tube test as in 2.
   (iii) 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 *Salmonella* Diagnostic Sera 1	+	+	+	-	-	-	+
Rapid *Salmonella* Diagnostic Sera 2	+	-	+	-	+	+	-
Rapid *Salmonell*a Diagnostic Sera 3	-	+	+	+	+	-	-

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 speci-ficity 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), agglutina-tion cannot be guaranteed in all cases.
5. Sensitivity of the slide test may be reduced if volumes greater than 10 µl are used.

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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

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

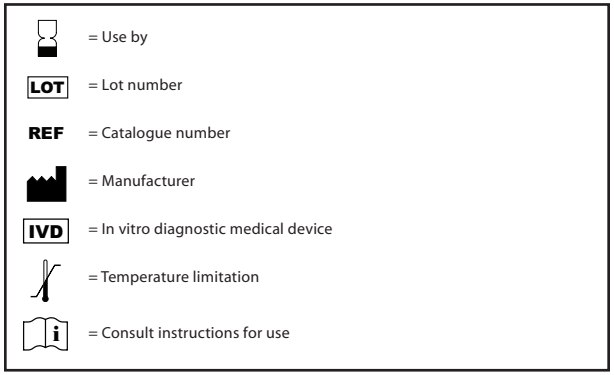
PL.6100 Polyvalent H
PL.6101 Polyvalent H Phase 2, Factors 1,2,5,6,7,z6

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
PL.6157 Factor z6

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



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