Salmonella Agglutination Sera

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: SEE MATERIALS PROVIDED

CE

INTENDED USE

Salmonella Agglutination Sera are prepared for use in serological identification of organisms belonging to the genus *Salmonella* according to Kauffmann-White classification, 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 characterisation and serological identification (serotyping).

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

PRINCIPLE OF THE TEST

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

MATERIALS PROVIDED

Pro-Lab Diagnostics Salmonella 'O' and 'H' monovalent and polyvalent Agglutination Sera are prepared in rabbits using reference strains according to the methods recommended by the World Health Organization (WHO) and absorbed to eliminate cross-reacting antibodies.

Salmonella Agglutination Sera are supplied in dropper bottles containing 3.0 ml of ready-to-use diluted Agglutination Sera with sodium azide or thiomersal as a 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
- 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

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides or test tubes
- Normal Saline (0.85% NaCl solution)
- Disposable or wire loops
- Water bath set to 50°C

STABILITY AND STORAGE

Salmonella Agglutination Sera should be stored at 2-8°C. Do not freeze. Stored under these conditions the Agglutination Sera may be used up to the date of expiry shown on the product label. On storage, some Agglutination Sera may become slightly turbid; this does not necessarily indicate deterioration and the Sera may be clarified by centrifugation or filtration before use. Gross turbidity indicates contamination and such Sera should be discarded.

Allow Sera to reach room temperature before use.

PRECAUTIONS

- Do not use Agglutination Sera after the expiry date shown on the product label.
- Sera assigned a 4-digit numerical lot number prefixed with the letter P contain Sodium Azide as a preservative. Sodium Azide is highly toxic and whilst the amount in the Sera is minimal, safety precautions should be taken in handling, processing and discarding the reagent.
- Sera assigned a 4-digit numerical lot number contain Thiomersal as a preservative. Thiomersal, which is a highly toxic mercury-based compound is present at a concentration of 0.01%. Although the amount used 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.
- Vi antigen can mask the identity of O antigens. In this case, it might be necessary to heat an antigen suspension of the isolate for an hour at 100°C or for 15 minutes at 120°C.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

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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. Emulsify a small part of a suspect *Salmonella* colony from an overnight culture plate into each of the drops of saline. Mix thoroughly to obtain a smooth bacterial suspension. Discard the slide and repeat if auto-agglutination (clumping) occurs.
3. Add one loopful of Sera to one of the bacterial suspension drops. Add one loopful of saline to the other; this will act as a control.
4. Mix the Agglutination Sera with the bacterial suspension using a sterile loop.
5. Gently tilt the slide back and forth for one minute. Under normal lighting conditions, observe for agglutination (clumping) of the suspension with Sera, and clearing of the saline suspension.

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 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. Sera Dilution: In order to use Salmonella Agglutination Sera in a test tube, each Sera must be diluted 1:5 in normal saline before use.
3. Add 150ul of normal saline to a glass test tube and in another tube add an equal volume of diluted Agglutination Sera.
4. Add an equal volume of previously prepared cell suspension to each tube.
5. 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 50ºC is preferable.
6. Stand the tubes on the bench for 30 minutes. Observe for agglutination. Rotation of the tubes to disturb granules in the deposit may be necessary, but do not shake.
7. No agglutination should be visible in the control tube. If auto-agglutination has occurred in the control, discard all tubes and repeat the test.

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.

QUALITY CONTROL PROCEDURE

Refer to lot specific Certificate of Analysis.

INTERPRETATION OF RESULTS

1. For procedure A or B:
   A distinct agglutination (granular clumping) within 60 seconds, without auto-agglutination in the saline control, 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 Salmonella Diagnostic Sera 3	-	+	+	+	+	-	-

LIMITATIONS OF THE PROCEDURE

- The Agglutination Sera should only be used for identification of cultures which have been previously characterised 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 Agglutination Sera 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.

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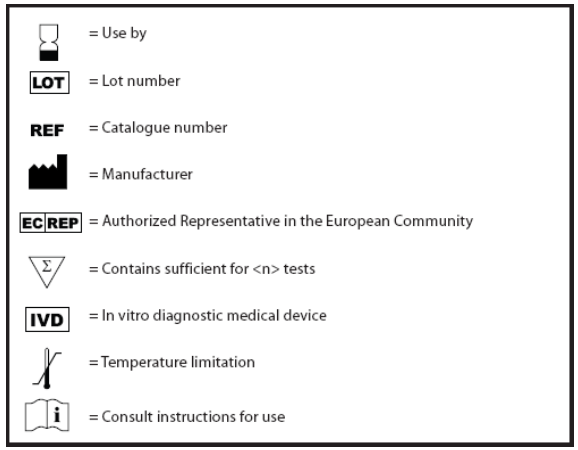

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