SAFETY 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 selective differential agar plates 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.

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 antisera 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 (i) 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 Brown’s 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. Antiserum Dilution: In order to use PRO-LAB Salmonella antisera in a test tube, each antisera 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.

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:

<table>
<thead>
<tr>
<th>Sera</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>G</th>
<th>K</th>
<th>L</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Salmonella Diagnostic Sera 1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rapid Salmonella Diagnostic Sera 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Rapid Salmonella Diagnostic Sera 3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

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

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 discarding 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.

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.
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 ml are used.

REFERENCES

REAGENTS AVAILABLE

Polyvalent Somatic O Antiseras

- PL.6000 Polyvalent O A - I + Vi
- PL.6002 Polyvalent O A - S
- PL.6003 Polyvalent A = B, D, E
- PL.6004 Polyvalent B = C1, C2, F, G, H.
- PL.6005 Polyvalent C = I - O
- PL.6006 Polyvalent D = F - S

Monovalent Somatic O Antiseras

- 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.6028 Group H2, Factor 25
- 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

Polyvalent Flagella H Antiseras

- PL.6010 Factor a
- PL.6011 Factor b
- PL.6012 Factor c
- PL.6013 Factor d
- PL.6014 E Complex eh, enx, enz15
- PL.6015 Factor eh
- PL.6016 Factor enx
- PL.6017 Factor enz15
- PL.6018 Factor h
- PL.6020 Factor z15
- PL.6021 G Complex
- PL.6022 Factor gm
- PL.6023 Factor gp
- PL.6024 Factor p
- PL.6025 Factor u
- PL.6026 Factor s
- PL.6027 Factor m
- PL.6028 Factor t
- PL.6029 Factor f
- PL.6031 Factor q
- PL.6032 Factor z51
- PL.6033 Factor i
- PL.6034 Factor k
- PL.6035 L Complex
- PL.6036 Factors L, w
- PL.6037 Factors L,v
- PL.6038 Factor w
- PL.6039 Factor v
- PL.6040 Factor z13
- PL.6041 Factor z28
- PL.6042 Factor r
- PL.6043 Factor y
- PL.6044 Factor z
- PL.6045 Z4 Complex
- PL.6046 Factor z23
- PL.6047 Factor z24
- PL.6048 Factor z32
- PL.6049 Factor z10
- PL.6051 Factor z29
- PL.6053 Factor 2
- PL.6054 Factor 5
- PL.6055 Factor 6
- PL.6056 Factor 7
- PL.6057 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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