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Salmonella Agglutinating Sera EN

1. INTENDED USE

Salmonella Agglutinating Sera are intended for use in slide agglutination screening procedures to serologically identify Salmonella cultures for epidemiological and diagnostic purposes. The monovalent Salmonella H Agglutinating sera may be used in phase change procedures[1].

IVD For *in vitro* diagnostic use only.

For professional use only.

2. SUMMARY AND EXPLANATION OF THE TEST

The genus Salmonella is classified in the Kauffmann-White Scheme into serotypes according to combinations of somatic (O) and flagellar (H) antigens, which are identified by agglutination tests[2]. Numerous antigenic relationships occur between Salmonella and organisms from other genera so that identification procedures should include cultural and biochemical examination in addition to serology. Agglutinating sera should be used in confirmatory tests but may also be used with appropriate caution in screening tests[7].

The sera are absorbed to remove agglutinins for other Salmonella antigens and the 'paracolon' α agglutinin. Salmonella Somatic Agglutinating Sera (ZC series) are intended for the identification of O antigens.

Salmonella Flagellar Agglutinating Sera (ZD series) are intended for use in the identification of H antigens. The commonly occurring Phase 1 H antigens, with the exception of factor i, may be identified by slide agglutination tests using Rapid Diagnostic Sera (Table 1) Salmonella Agglutinating Sera

Table 1

Pattern of agglutination reactions of Rapid Diagnostic Sera and corresponding H serotypes

Antigen	ZD02	ZD03	ZD04
b	+	+	-
d	+	-	+
E	+	+	+
G	-	-	+
k	-	+	+
L	-	+	-
r	+	-	-

3. PRINCIPLE OF THE PROCEDURE

Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will agglutinate with bacteria carrying homologous antigens.

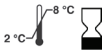
4. REAGENTS

4.1. KIT CONTENTS

Each kit contains one bottle of antisera (2ml) and Instructions For Use. The specificity of the antisera is given on the bottle label.

4.2. Description, Preparation for Use and recommended Storage Conditions

See also **Warnings and Precautions**.



The sera should be stored at 2 to 8°C under which condition they will retain their potency at least until the date shown on the bottle label.

AGGLUTINATING SERUM

The Salmonella antisera are preserved with 0.1% Sodium Azide. The sera are produced in rabbits.

Each bottle, fitted with teat and dropper, contains 2 ml liquid and is supplied ready for use.

On storage, some sera become slightly turbid. This does not necessarily indicate deterioration and normally it will not interfere with the results, but the sera may be clarified by centrifugation or membrane filtration (0.45 µm) before use. Gross turbidity indicates contamination and such sera should be discarded.

5. WARNINGS AND PRECAUTIONS

Please refer to the safety data sheet and the product labelling for information on potentially hazardous components.

5.1. Health and Safety Information

5.1.1 Handle all bacteria according to appropriate local and statutory guidelines.

5.1.2 Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated.

5.1.3 Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant or 70% alcohol. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.

5.1.4 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

5.1.5 The Salmonella antisera are preserved with 0.1% Sodium Azide. Although the concentration is low, toxic by ingestion and skin contact. Avoid ingestion of the reagents. If any come into contact with skin or eyes wash the area extensively by immediately rinsing with plenty of water.

5.1.6 In accordance with the principles of Good Laboratory Practice it is strongly recommended that samples and reagents should be treated as potentially infectious and handled with all necessary precautions

5.2. Analytical Precautions

5.2.1 Do not use antisera beyond the stated expiry date. Microbiological contamination of the antisera must be avoided as this may cause erroneous result and reduce product life.

5.2.2 Do not modify the test procedures, incubation times or temperature. Do not dilute.

5.2.3 After use return sera to recommended storage temperature.

5.2.4 The sera are absorbed, but because other serotypes within the genus as well as some heterologous species possess common antigens and because of some naturally acquired antibodies to heterologous species in rabbit serum, reactions may be obtained with species outside the genus Salmonella or with Salmonella serotypes outside the range given on the bottle label.

5.2.5 Material for examination should be taken from a subculture of the suspected organism on non-selective media. Growth taken from primary, selective media may give unreliable serological results, and although tests may be performed for preliminary screening purposes, reactions must be interpreted with extreme caution[1].

6. SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The use of fresh cultures on non-selective media is recommended e.g. nutrient agar.

For details of specimen collection and preparation a standard text book should be consulted.

7. PROCEDURE

Materials Provided

The antisera are available in bottles fitted with teat and dropper.

Materials Required but not Provided

1. 0.85% saline.
2. Glass slides.
3. Microbiological loop and bunsen burner.
4. Light source over dark background.
5. Timer.
6. Disinfectant

7.1. Test Procedure

Slide Agglutination Test

Step 1	Put two separate drops (40 µl each) of 0.85% saline on a glass slide. Emulsify portions of the culture under test with a loop in each drop of 0.85% saline to give a smooth, fairly dense suspension.
Step 2	To one suspension, as a control, add one drop (40 µl) of 0.85% saline and mix. To the other suspension add one drop (40 µl) of undiluted antiserum and mix
Step 3	Rock the slide gently for one minute and observe for agglutination using indirect lighting over a dark background. Discard the used slide for safe disinfection and disposal.

Phase Reversal

Many Salmonella serotypes possess diphasic H antigens. Frequently both phases will be apparent in a culture, but if only a single phase is detectable it may be necessary to isolate and identify the alternate phase. This may be accomplished by subculture or repeated subcultures onto 0.5% agar in a Petri dish or 0.2% agar in a Craigie tube[1], containing 1% of the agglutinating serum for the identifiable phase, and testing the leading edge of the growth, after incubation, for the alternative phase.

8. RESULTS

Agglutination should be strong and clearly visible within one minute. There should be no visible agglutination in the saline control; if agglutination is seen in the control, the suspension is not suitable for testing by this method.

In some strains O agglutination may be masked by the presence of Vi antigen. This may be identified in a slide test using Salmonella Agglutinating Serum (Vi). If Vi antigen is present, a saline suspension of the organisms should be heated at 100°C for one hour to destroy the Vi antigen and centrifuged, and a fresh saline suspension of the deposit should be retested with O antisera to identify the O antigens. It should be remembered that the Vi antigen is not exclusive to Salmonella.

9. QUALITY CONTROL

It is recommended to test the product, throughout its use, with known positive and negative cultures. Homologous cultures should be used for positive control organisms. For negative control culture testing use *Hafnia alvei*. Strains with the appropriate serotypes may be obtained from a recognised culture collection such as NCTC or ATCC®.

If any antiserum shows agglutination with a known negative culture or shows no agglutination with a known positive culture it should be discarded.

10. INTERPRETATION OF RESULTS

Agglutination in polyvalent O serum but not polyvalent H serum, if the biochemical reactions are consistent with Salmonella species, suggests that flagella are not well developed. The strain should be retested after passage on motility-enhancing medium, such as 0.5% nutrient agar. (*S. pullorum* and *S. gallinarum* are non-motile). Agglutination in polyvalent H serum but not polyvalent O serum, and biochemical reactions consistent with Salmonella species,

suggests that the culture is outside the groups covered by the polyvalent O serum or the O antigens are masked by Vi antigen. The latter may be checked using Vi antiserum, and if the presence of Vi antigen is confirmed, identification of the O antigens should be possible using a suspension which has been boiled for one hour, washed and resuspended in saline.

If no agglutination is visible with either polyvalent serum, the organism is unlikely to be a Salmonella.

The extent of serological examination depends on the purpose of the investigation. To obtain serological evidence for the presence of a Salmonella, cultures should be tested with polyvalent O, polyvalent H and Vi sera. Should more detailed analysis be required, it is usual to determine the O antigen using the appropriate sera, and then test for the most likely H antigen as indicated by the Kauffmann-White scheme[2,4,5,6,9].

No definite conclusion may be drawn about the serological identity of a strain until biochemical testing confirms that it reacts as a Salmonella. The possibility of serological cross reactions due to common antigens has already been mentioned; of particular relevance are relationships between different O groups, some of which are expressed in the Kauffmann-White scheme[2].

11. LIMITATIONS OF THE PROCEDURE

Although it is impracticable to provide single factor sera for all known Salmonella antigens, a wide range of sera are available, sufficient to identify the majority of salmonella types isolated, with a high degree of accuracy.

Antisera provide serological identification only; full identification of an organism must be made in conjunction with biochemical testing.

While the sera have been absorbed to minimise cross-reactions, the possibility of cross-reactions with other Salmonella antigens or related organisms of other species cannot be completely excluded.

Reactions may be obtained with species outside the genus Salmonella or with Salmonella serotypes outside the range given on the bottle label. Serological tests used alone provide no more than presumptive identification and biochemical examination must be performed in addition to serological analysis[1,8].

12. EXPECTED RESULTS/PERFORMANCE CHARACTERISTICS

Visible agglutination in the presence of homologous antigens (refer to bottle label for specificity of the antisera). See limitations of the procedure.

13. BIBLIOGRAPHY

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9. **Kauffman-White Scheme.**

14. PACKAGING

REF
R30858101/ZC01 Salmonella Polyvalent-O A-G 2ml
R30858201/ZC02 Salmonella Polyvalent-O A-S 2ml
R30956701/ZC11 Salmonella 2-O 2ml
R30956801/ZC12 Salmonella 3,10,15,19-O 2ml
R30956901/ZC13 Salmonella 4-O 2ml
R30957001/ZC14 Salmonella 5-O 2ml
R30957101/ZC15 Salmonella 6,7-O 2ml
R30957201/ZC16 Salmonella 8-O 2ml
R30957301/ZC17 Salmonella 9-O 2ml
R30957401/ZC18 Salmonella Vi 2ml
R30957601/ZC20 Salmonella 11-O 2ml
R30959101/ZC35 Salmonella 39-O 2ml
R30858501/ZD01 Salmonella Polyvalent-H Phase 1-2 . ..... 2ml
R30959801/ZD02 Salmonella Rapid 1-H (b, d, E, r) ... 2ml
R30959901/ZD03 Salmonella Rapid 2-H (b, E, k, l) ... 2ml
R30160101/ZD04 Salmonella Rapid 3-H (d, E, G, k) .. 2ml
R30160201/ZD05 Salmonella a-H 2ml
R30160301/ZD06 Salmonella b-H 2ml
R30160401/ZD07 Salmonella c-H 2ml
R30160501/ZD08 Salmonella d-H 2ml
R30161601/ZD19 Salmonella i-H 2ml
R30162201/ZD25 Salmonella r-H 2ml
R30163201/ZD35 Salmonella Poly-H phase 2 (1,2,5,6,7) 2ml

15. SYMBOL LEGEND

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
LATEX	Contains or prescence of natural rubber latex
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufacturer

CE

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For technical assistance please contact your local distributor.

Replacement Instructions for Use (IFUs), and alternate language versions available via www.oxoid.com/IFU