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Salmonella Agglutinating Sera EN

1. INTENDED USE

Salmonella Agglutinating Sera are intended for use in slide and tube agglutination tests for serological identification of Salmonella cultures for epidemiological and diagnostic purposes. Appropriate sera may also be used as control antisera for Stained Salmonella Suspensions[8], and the Salmonella H antisera 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 in slide agglutination tests, although they may also be used in confirmatory tube tests.

Salmonella Flagellar Agglutinating Sera (ZD series) are intended for use in the identification of H antigens. The polyvalent sera should be used in slide agglutination tests. The monovalent sera may also be used in preliminary slide tests but results obtained in this way should be confirmed by tube agglutination.

In the case of H serotypes possessing common determinants (for example, the G group: fg, gm, gp, gq, gst), the polyvalent sera designated by capital letters should react to approximately the same titre in tube agglutination tests with organisms possessing that factor. The sera designated by small letters should react with organisms possessing homologous antigens to a dilution at least two tubes greater than with those possessing heterologous antigens with common factors (for an example, see Table 1).

Table 1

Typical pattern of tube agglutination reactions of three related H antigens and their corresponding antisera

Antigen	Antiserum			
	G	fg	gm	gp
fg	1:800	1:800	<1:200	<1:200
gm	1:800	<1:200	1:800	<1:200
gp	1:800	<1:200	<1:200	1:800

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.

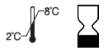
4. REAGENTS

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 either with 0.5% phenol or 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

This product may contain up to 0.02% thiomersal.

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 either with 0.5% phenol or 0.1% Sodium Azide. Although the concentration is low, both are known to be 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.

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. Test tubes and rack.
6. Adjustable waterbath with thermometer.
7. Timer.
8. Pipettes.
9. Stained suspensions (SS05 / R30952901 & SS07 / R30953101) are available as controls.
10. Centrifuge.
11. 0.5% formal saline or formalinised broth.

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.

Tube Agglutination Test

Living suspensions may be used as antigens, but care must be taken to avoid laboratory infections. The antigen for H tube agglutination tests may be prepared by suspending organisms in 0.5% formal saline, or by the addition of formalin to broth cultures[3]. Colonies taken from primary isolation media may be unsatisfactory for use in determining the H serotype, due to the poor motility of the organisms. This may be improved by subculture on a moist agar slope, on 0.5% agar in a Petri dish or in 0.2% agar in a Craigie tube and picking the leading edge of the culture after incubation.

Step 1	Prepare a fairly light suspension of bacteria (approximately 109 organisms per ml).
Step 2	Make serial dilutions of antiserum in 0.5 ml volumes, from 1 in 10 to 1 in 320 for O and Vi factor determinations and from 1 in 25 to 1 in 800 for H factors
Step 3	To each tube add 0.5 ml of the antigen suspension. This doubles the dilution of antiserum.
Step 4	A control tube should also be included containing only suspension and saline.
Step 5	Flick the tubes to mix contents. Incubate the O factor tubes at 50°C for four hours, Vi factor tubes at 37°C for two hours followed by 18 hours at 2 to 8°C (allow these to warm to room temperature (18 to 30°C) before reading), and H factor tubes at 50°C for two hours.
Step 6	Flick tubes and then examine for agglutination using strong back-lighting.

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

Slide Agglutination

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.

Tube Agglutination

In a positive Vi reaction there should be obvious granular agglutination: H agglutination has a characteristic floccular appearance. There may be some clearing of the fluid, and a sediment that rises as a granular mass, and then sinks again to the bottom of the tube, when the tube is flicked with the finger. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, and show a typical swirl on agitation. If there is agglutination in the saline control, the suspension is rough and not suitable for tube agglutination. The titre is the dilution of serum in the last tube showing agglutination. Titres at or near that stated on the bottle label indicate that the antigen is of the same serotype as the antiserum.

When the sera are used as controls for the activity of Stained Suspensions, the procedure is described in the Instructions for Use accompanying the Stained Salmonella O and H suspensions must be followed[8]. A titre within two serial dilutions of that stated on the bottle label should be obtained.

9. QUALITY CONTROL

It is recommended to test the product, throughout its use, with known positive and negative cultures. Examples of positive cultures are shown in Table 2.

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

Table 2
Salmonella Positive Control Cultures

Code	NCTC REF	Salmonella species	Antigenic Structure[9]
ZD19/R30161601	3048	*Salmonella typhimurium*	4,5:i:1,2

Negative control for all codes: Hafnia alvei NCTC 8535

NOTE: all cultures are category 2 unless otherwise stated as denoted by ACDP guidelines.

10. INTERPRETATION OF RESULTS

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].

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.

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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14. PACKAGING

REF R30161601/ZD19 Salmonella i-H.....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

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