Vibrio cholerae EN
Agglutinating Sera

1. INTENDED USE
Vibrio cholerae agglutinating sera are intended for the serological identification of V. cholerae for epidemiological and diagnostic purposes. The (ZM05/R30165001) serum is intended for use in slide agglutination screening tests: the subtype (ZM06/R30165101, ZM07/R30165201) sera are suitable for use in slide and tube agglutination tests as described below.

2. SUMMARY AND EXPLANATION OF THE TEST
Formalised suspensions are recommended for the tube test, but some workers prefer to use saline suspensions. It has been suggested that boiling suspensions for two and a half hours prior to testing by slide agglutination will eliminate a number of false positive and false negative reactions. Inaba and Ogawa subtypes of V. cholerae are closely related and therefore cross-reactions may occur. The subtype sera have been absorbed so that no cross-reaction should be seen in slide agglutination tests. Cross-reactions may be observed in tube agglutination tests but will show at least a fourfold reduction in titre compared with the specific reactions may be observed in tube agglutination tests but will show at least a fourfold reduction in titre compared with the specific antigens. Therefore, a titre at or near that stated on the bottle label should indicate homology.

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 visibly agglutinate with bacteria carrying homologous antigens.

4. REAGENTS

<table>
<thead>
<tr>
<th>KIT CONTENTS</th>
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<tbody>
<tr>
<td><strong>Vibrio cholerae Agglutinating Sera</strong> 2 ml</td>
</tr>
<tr>
<td>ZM05/R30165001</td>
</tr>
<tr>
<td>1 dropper bottle (blue cap)</td>
</tr>
<tr>
<td>ZM06/R30165101</td>
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<tr>
<td>1 dropper bottle (blue cap)</td>
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<tr>
<td>ZM07/R30165201</td>
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<td>1 dropper bottle (blue cap)</td>
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5. DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

<table>
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<tr>
<th>ROOM TEMPERATURE</th>
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| **Vibrio cholerae Agglutinating Sera** produced in rabbits and are preserved with 0.5% phenol. Each bottle, fitted with teat and dropper, should contain sufficient sera for 40 to 50 tests and are ready to 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.

6. WARNINGS AND PRECAUTIONS

<table>
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<td><strong>For in vitro diagnostic use only.</strong></td>
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For professional use only.

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

6.1. Health and Safety Information

6.1.1 NOTE: V. cholerae are classified as category 2+ organisms; handle according to appropriate local and statutory guidelines.

6.1.2 Non-disposable apparatus should be sterilised by an 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.

6.1.3 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, alcohol should be disposed of as biohazardous waste.

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

6.1.5 These reagents contain phenol. Although the concentration is low, phenol is 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.

6.1.6 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.

6.2. Analytical Precautions

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

6.2.2 Do not modify the test procedure, incubation time or temperatures.

6.2.3 After use return sera to recommended storage temperature.

7. SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The use of fresh cultures on non-selective media is recommended eg nutrient agar. Do not use TCBS or any selective media. For details on specimen collection and preparation a standard textbook should be consulted.

8. PROCEDURE

MATERIALS PROVIDED
See Kit Contents.

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. 0.5% formalin or phenol saline.

TEST PROCEDURE

Slide Agglutination Test

Step 1 Put two separate drops (40 µl each) of saline on a glass slide. Emulsify portions of the culture under test with a loop in each drop of saline to give a smooth, fairly dense suspension.

Step 2 To one suspension as a control add one drop (40 µl) of saline and mix. To the other suspension add one drop (40 µl) of undiluted antisera and mix.

Step 3 Rock slide for one minute and observe for agglutination, which can be more easily seen by viewing against a dark background using indirect lighting. Discard the used slide for safe disinfection and disposal.

Tube Agglutination Test (ZM06/R30165101, ZM07/R30165201 only)

Step 1 The antigen may be prepared by emulsifying growth from a pure culture in plain, isotonic saline or in saline containing 0.5% formalin or phenol to give a light suspension (about 107 bacteria/ml).

Step 2 Make serial dilutions of antisera in 0.5 ml volumes in saline from 1 in 10 to 1 in 320. Round-bottomed glass tubes approximately 9 mm x 85 mm long. are suitable.

Step 3 To each tube add 0.5 ml of the antigen suspension. This doubles the dilution of antisera.

Step 4 A control tube should be set up containing only saline and diluent.

Step 5 Shake the tubes and incubate overnight (16 to 20 hours) at 50°C.

Step 6 Examine for agglutination.

9. RESULTS

Slide Agglutination

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

Tube Agglutination

In a positive reaction there should be obvious granular agglutination.

In a negative reaction and the saline control the appearance of the suspension should be unchanged. Note that after overnight (16 to 20 hours) incubation the suspension may settle but any sediment should resuspend on flicking.

Agglutination in the control tube indicates a rough suspension, which is unsuitable for testing.

QUALITY CONTROL

It is recommended to test the product, throughout its use, with known positive and negative cultures.

10. INTERPRETATION OF RESULTS

The titre of the serum is the last dilution showing positive agglutination. A titre at or near that stated on the bottle label should indicate homology.

In the majority of cases, titres of 1 in 20 or less are not considered significant. However with Vibrio cholerae Inaba serum, the extensive absorption required to render the serum specific often results in a low homologous activity and in this case 1 in 20 can be a significant titre.

11. LIMITATIONS OF THE PROCEDURE

Serological tests used alone provide no more than presumptive identification and confirmatory biochemical identification tests must be performed.

If inconclusive results are achieved with slide agglutination, cultures may be steamed to reduce non-specific reactions.
12. EXPECTED RESULTS

Visible agglutination in the presence of homologous cultures.

13. SPECIFIC PERFORMANCE CHARACTERISTICS

The 01 Polyvalent Serum (ZM05/R30165001) should show visible agglutination in the slide test with *V. cholerae* subtypes. The Ogawa and Inaba sera should show visible agglutination in the slide and tube agglutination test with *V. cholerae* Ogawa and Inaba subtypes respectively. Differentiation of El Tor Vibrios from *V. cholerae* should be carried out biochemically.

BIBLIOGRAPHY


PACKAGING

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Volume</th>
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<tbody>
<tr>
<td>ZM05/R30165001</td>
<td>2 ml</td>
</tr>
<tr>
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<td>ZM07/R30165201</td>
<td>2 ml</td>
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Symbol legend

- **REF**: Catalog Number
- **IVD**: In vitro diagnostic medical device
- **IFU**: Consult instruction for use (IFU)
- **LOT**: Temperature limitation (Storage Temp.)
- **Titre**: Batch code (Lot Number)
- **Use by**: (Expiration Date)
- **Titre**: Last dilution showing positive agglutination
- **Manufacturer**: Manufacturer

Remel Europe Ltd.
Clipper Boulevard West, Crossways
Dartford, Kent, DA2 6PT
UK

For technical assistance please contact your local distributor.