

Campylobacter Antisera

INTENDED USE

Serogrouping of *Campylobacter jejuni*

SAMMARY AND EXPLANTION

Campylobacter is a gram-negative spirally curved rod that contain a single flagellum. Eleven species *Campylobacter* have been identified, in which *Campylobacter jejuni* is reported as the most common species that cause enteric infection in humans.

There are two internationally accepted serotyping methods for *Campylobacter*, Penners serotyping using heat-stable antigens, and the other using heat-labile antigens. This product is for *Campylobacter jejuni* serogrouping based on Penners scheme by the passive hemagglutination (PHA) method.

PRINCIPLE

Heat-stable specific antigen of *Campylobacter jejuni* extracted by nitric acid is sensitized to the blood cells. When the sensitized cells are mixed with the antiserum, specific reaction occurs and agglutination is observed. The reagent is used for PHA based on the principle above.

PRODUCT

1. Antisera

Campylobacter antisera are produced from rabbits and contain 0.08 w/v% sodium azide as a preservative. The following sera are provided in 2 mL vials with a dropper and are ready to use.

Set: 25 vials/set

Groups A, B, C, D, E, F, G, I, J, K, L, N, O, P, R, S, U, V, Y, Z, Z₂, Z₄, Z₅, Z₆, Z₇

Serum groups: antigens

Group A: 1, 44	Group K: 12	Group Y: 37
Group B: 2	Group L: 15	Group Z: 38
Group C: 3	Group N: 18	Group Z ₂ : 41
Group D: 4, 13, 16, 43, 50	Group O: 19	Group Z ₄ : 45
Group E: 5	Group P: 21	Group Z ₅ : 52
Group F: 6, 7	Group R: 23, 36, 53	Group Z ₆ : 55
Group G: 8	Group S: 27	Group Z ₇ : 57
Group I: 10	Group U: 31	
Group J: 11	Group V: 32	

2. Reference Antiserum

Blood cell control serum is produced from rabbit and contains 0.08 w/v% sodium azide as a preservative. The serum is provided in 2 mL vials with a dropper and are ready to use.

PRECAUTIONS

1. General precautions

- 1) For in vitro diagnostic use only.
- 2) Only bacteriological trained laboratory staff should handle the reagents.
- 3) Reagents should only be used for the intended use.
- 4) Reagents should be used according to the described procedures.

2. Handling precautions

- 1) All specimens, samples and containers coming into contact

with samples should be treated as infectious substance.

- 2) If reagent comes into contact with skin, eyes, or mouth wash immediately with copious amounts of water, seek medical attention if necessary.
- 3) When the antisera are dropped, dropping vessels should be retained vertically to drop them after the tip of vessels is wiped with tissue paper.
- 4) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
- 5) Reagents should be allowed to stand at 15°C-25°C for at least 30 minutes before use.
- 6) Used containers should not be used for other purposes.
- 7) Sera with different lot numbers should not be mixed.
- 8) Special precautions should be taken to ensure that the reagent caps are not exchanged.
- 9) Avoid microbial contamination of opened reagent bottles. Do not use reagents if they are contaminated or cloudy.

3. Precautions for disposal

- 1) The reagent contains 0.08 w/v% sodium azide. Sodium azide may react with lead or copper to form explosive heavy metal azides. The reagent should be disposed with copious amounts of water.
- 2) All specimens, spills, inoculated products and equipment used in this test should be treated by one of the following methods.
 - [1] Soaking in 0.1 w/v% hypochlorite for 1 hour or more.
 - [2] Autoclave at 121°C for 20 minutes or more.

TEST PROCEDURE

1. Material required but not provided

1.5 mL centrifuge tubes, centrifuge, micropipettes, 96-well microplate (V type), microplate mixer, 37°C water bath, moisture box, test tubes, Reagent for Preparing Sensitized Blood cells (Denka product marketed separately), physiological saline

2. Preparation of reagents

Ready to use.

3. Specimens

Campylobacter jejuni cultivated under aerobic conditions on enrichment media including blood agar plate medium at 42°C for 48 hours.

4. Method

A) Preparation of antigen suspension for sensitization

- 1) Pipette 0.25 mL physiological saline into 1.5 mL centrifuge tubes.
- 2) Suspend a loopful of the organisms in the physiological saline.
- 3) Add 0.25 mL Extraction reagent-1 and Extraction reagent-2.
- 4) Mix with a vortex mixer and allow to react for 10 minutes.
- 5) Add 0.25 mL Extraction reagent-3 and stir well.
- 6) Centrifuge at 7000 rpm or more for 5 minutes, and use the supernatant as the antigen solution for sensitization.

B) Preparation of Fixed Chick RBCs suspension

- 1) In test tubes, place 0.5 mL Fixed Chick RBCs suspension per specimen.
- 2) Add the equivalent amount of buffer solution and centrifuge at 3,000 rpm for 10 minutes.
- 3) Remove the supernatant and add 0.5 mL buffer solution per specimen to the residue to suspend the cells.

C) Preparation of sensitized cells

- 1) Add 0.5 mL 1.5% Fixed Chick RBCs suspension in 1.5 mL centrifuge tubes containing 0.5 mL sensitizing antigen solution.
- 2) Incubate in a water bath at 37°C for 30 minutes, while

stirring occasionally.

3) Centrifuge the mixture at 6,000 rpm for 30 seconds and remove the supernatant.

4) Add 1.0 mL buffer solution and suspend.

D) PHA test

1) Place a drop of each antiserum on a microplate.

2) In one well place a drop of the control serum as a control for spontaneous agglutination.

3) Add 25 µL sensitized cell suspension to each well.

4) Mix the contents of the microplate well with a microplate mixer, and then place in a moisture box.

5) Observe for agglutination after 30 minutes.





Precaution on test procedure

1) Sufficient stirring during extraction procedure is required to form a homogenous suspension, as the Fixed Chick RBCs quickly form sediment.

2) Permanent (acrylic) or rigid microplates should be used.

INTERPRETATION OF RESULTS

Interpretation of agglutination is based on general PHA interpretation criteria as follows.

Agglutination	Determination	Scheme
Agglutination of cells in the center of well	-	
Marked agglutination but not all over the bottom of well	+	
Agglutination of inconsequential amount of cells in the center	++	
Uniform agglutination of cells all over the bottom of well	+++	

1) Confirm that the well containing the control blood cell and control serum gives a negative result.

2) A +, ++, +++ is interpreted as positive, and the serotype of the tested organism can be determined.

3) If the organism reacted to multiple serotypes, it is determined as multiple serotypes.

Precaution in interpretation

1) Results should be determined after 30-60 minutes, as agglutinated material may settle to the bottom of the microplate.

PERFORMANCE CHARACTERISTICS

When the reagent is tested using Penner's reference strain of serotype, agglutination is only observed with the corresponding serotype.

STORAGE / SHELF LIFE

Storage: 2°C-10°C.

Shelf life: Up to the expiry date on the label.

PACKAGE

Campylobacter Antisera: 2 mL serum vial with dropper.

Set: 26 vials/set

Each serum is individually available.

Denka Seiken also supplies the following products.

- Reagent for Preparing Sensitized Blood cells (50 tests)

Contents: Fixed Chick RBCs	25 mL × 1
Extraction reagent-1	13 mL × 1
Extraction reagent-2	13 mL × 1
Extracting reagent 3	13 mL × 1
Buffer solution	50 mL × 2

REFERENCE

1) Supervised by the Ministry of Health, Labour and Welfare: Oral infectious diseases, *Campylobacter*, the Microbiological test manual, Bacterial and fungi tests, Third edition, D-118 (1987)

2) Penner, J. L., et al.: Passive Hemagglutination Technique for serotyping *Campylobacter fetus* subsp. *Jejuni* on basis of soluble heat-stable antigens, J. Clin. Micro., 12, 732 (1980)

Please feel free to contact us at the following with your questions or comments :

Tel : +81-3-3669-9421 FAX : +81-3-3669-9390

Symbols

 Batch code

 Use by

 In Vitro Diagnostic Medical Device

 Temperature limitation (Store at)

 Catalogue number

 Consult Instruction for use

 Contents of kit



DENKA SEIKEN CO., LTD.

3-4-2 Nihonbashikayaba-cho, Chuo-ku, Tokyo, Japan

TEL : +81-3-3669-9421 FAX : +81-3-3669-9390

DENKA SEIKEN UK Ltd.

2 Coronation Lane
Oakthorpe, Swadlincote,
Derbyshire DE12 7QY
United Kingdom

TEL : +44 1530 270010 FAX : +44 1530 272009

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