DebenDiagnosticsLtd

CAMPYLOBACTER ANTISERA

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES **RUO**

Campylobacter is a Gram-negative spiral curved rod with bipolar flagella. Eleven species of *Campylobacter* have been identified, of which *Campylobacter jejuni* is reported as the most common species to cause enteric infection in humans.

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

PRODUCT

1. Antisera

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

Set: 25 vials:
Groups A, B, C, D, E, F, G, I, J, K, L, N, O, P, R, S, U, V, Y, Z, Z_2, Z_4, Z_5, Z_6, Z_7.

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 sera and contains 0.08% w/v sodium azide as a preservative. The serum is provided as a 2mL volume in a vial with a dropper attachment and is ready to use.

PACKAGE

Campylobacter Antisera: 2mL serum in a vial with dropper
Set: 26 vials/set
Each serum is also available individually.

INTENDED USE

The *Campylobacter jejuni* Antisera are intended for serogrouping research into prepared *Campylobacter jejuni* bacterial cultures, using plate agglutination to qualitatively detect the presence of bacterial antigens. *Campylobacter jejuni Antisera* are intended for use by trained laboratory personnel.

This product is for research use only and must not be used in diagnostic procedures.

PRINCIPLE OF MEASUREMENT

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

PRECAUTIONS

1. General precautions

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

2. Precautions for test procedure

1) Sufficient stirring during the 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.

3. Precautions for interpretation

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

4. Handling precautions

1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
2) If reagent comes into contact with skin, mucous membranes or eyes, wash immediately with copious amounts of water and seek medical attention if necessary.
3) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
4) Reagents should be allowed to stand at 15-25°C for at least 30 minutes before use.
5) Used containers should not be used for other purposes.
6) Sera with different lot numbers should not be mixed.
7) Special precautions should be taken to ensure that the reagent caps are not exchanged.
8) Avoid microbial contamination of opened reagent bottles. Do not use reagents if they are contaminated or cloudy.

5. Precautions for disposal

1) The reagent contains 0.08% w/v sodium azide. Sodium azide may react with lead or copper pipes to form explosive heavy metal azides so the reagent should be disposed of with a large amount 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.

PROCEDURES

1. Material required but not provided

1.5mL centrifuge tubes, centrifuge, micropipettes, 96-well microplate (V type), microplate mixer, 37°C water bath, moisture box, test tubes, reagent for preparing sensitised blood cells, physiological saline

2. Preparation of reagents

The reagents are ready to use.

3. Specimens

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

4. Method

A) Preparation of antigen suspension for sensitisation.
   1) Pipette 0.25mL of physiological saline into 1.5mL centrifuge tubes.
   2) Suspend a loopful of the organisms in the physiological saline.
   3) Add 0.25mL Extraction reagent-1 and Extraction reagent-2.
   4) Mix with a vortex mixer and allow to react for 10 minutes.
   5) Add 0.25mL Extraction reagent-3 and stir well.
   6) Centrifuge at 7,000rpm or more for 5 minutes and use the supernatant as the antigen solution for sensitisation.

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

C) Preparation of sensitised cells
   1) Add 0.5mL 1.5% Fixed Chick RBCs suspension in 1.5mL centrifuge tubes containing 0.5mL sensitising antigen solution.
   2) Incubate in a water bath at 37°C for 30 minutes, while stirring occasionally.
   3) Centrifuge the mixture at 6,000rpm for 30 seconds and remove the supernatant.
   4) Add 1.0mL 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 2µL sensitised 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.

INTERPRETATION OF THE RESULTS

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

Agglutination description	Determination	Representation
Agglutination of cells in the centre of well	-	
Marked agglutination but not all over the bottom of well	+	
Agglutination of inconsequential number of cells in the centre	++	
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 +, ++, or +++ 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.

PERFORMANCE CHARACTERISTICS

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

STORAGE AND SHELF LIFE

Storage: 2-10°C
Shelf life: Up to the expiry date on the label.

REFERENCES

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)

	Manufacturer
	Consult instruction for use
	Catalogue number
	Batch code
	For research use only – not for use in diagnostic procedures
	Storage temperature limitation
	Use by
	Contains or presence of natural rubber latex

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