Version 11-04/24

User's Manual

Campylobacter IgG ELISA Kit

REF **DEIA-NS2401-18**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Campylobacter IgG kit is a qualitative or quantitative in vitro test for the detection and safe identification of IgG antibodies against Campylobacter jejuni and Campylobacter coli in human serum or plasma. The Campylobacter IgG kit is a screening test based on the principle of an indirect sandwich ELISA.

General Description

The genus Campylobacter comprises gram-negative, spiral-shaped, microaerophilic, mesophilic to thermophilic bacteria with bipolar flagella. In 1963, Sebald and Vernon named a bacterium Escherich

had described as early as 1889 Campylobacter jejuni. Isolation from stool samples was achieved in 1972 by Dekeyser et al. Jones et al. made out a food association in 1981/1982. In taxonomic terms, Campylobacter is classified with the epsilon subdivision of the Proteobacteria.

The pathogen reservoir is mainly the intestinal tract of warm-blooded wild, domestic and pet animals (birds and mammals). Intestinal Campylobacter infections are the second most frequent enteric bacterial infections reported in Germany after enteric salmonellosis (Robert Koch Institute 2004: RKI Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten 2006: Salmonella 52.319, Campylobacter 51.764), whereby Campylobacter jejuni is much more frequent, accounting for over 90% of cases as compared to Campylobacter coli at approx. 9%.

Contaminated foods (mainly poultry) and drinking water (tropical countries) constitute the main sources of infection. Unreported cases not reflected in the statistics probably outnumber reported cases many times over. Annual incidence rates vary seasonally (higher rates in the summer months) as well as regionally, with diagnoses following travel to warm countries throughout the year due to considerably higher incidence in Third World countries.

Acute disease

The course of infection with Campylobacter is almost exclusively oral, the pathogen is highly adapted to the intestinal tract. Systemic infections up to and including meningitis are observed less frequently (especially in immunocompromised patients, infants and the elderly) (Skirrow & Blaser, 2000). The incubation period is brief (1-7 days) and presumably depends on the infective dose. Besides nearly asymptomatic (clinically inapparent) courses, infected persons suffer from painful gastrointestinal symptoms with sometimes bloody diarrhoea, fever, meningism and myalgias. The acute clinical picture persists for only a few days in most patients. The course of the infection is self-limiting in most cases, although severe losses of fluids and electrolytes must be replaced as required.

Sequelae:

Reactive arthritis (ReA) is among the known sequelae to enteral, and frequently urogenital infections as well caused by certain bacterial and viral pathogens, with onset a few weeks after the primary infection. In addition to urogenital mycoplasmas and chlamydiae, enteral salmonellae, shigellae and yersiniae, Campylobacter spp. are prominent among secondary pathogen diagnoses (Locht & Krogfeldt, 2002; Cox et al., 2003; Hannu et al., 2004). The background pathophysiological mechanism is presumably molecular mimicry of cross-reacting antibodies to antigens of the synovial membrane. Some cases of arthritis of unclear genesis can be correctly diagnosed as post-infection Reactive Arthritis, and not rheumatoid arthritis, on the

basis of the appropriate laboratory parameters.

Similarly to Reactive Arthritis, Guillain-Barré-Syndrom (GBS) may also develop a few weeks after infections, usually enteral or respiratory, caused by certain bacterial and viral pathogens. The presumed level of incidence is between 1:1,000 and 1:10,000. GBS is an acute, immunomediated polyradicular neuropathy caused by an abnormal humoral immune response to the peripheral myelin sheath and/or the neural axon. A number of different viruses (e.g. CMV, EBV, VZV, measles virus, mumps virus), Mycoplasma pneumoniae and bacteria (Borrelia burgdorferi, Haemophilus influenzae) can cause a GBS, but various case-control studies have implicated that mainly Campylobacter spp., and in particular Campylobacter jejuni (Nachamkin et al., 2000; Prendergast et al., 2004; Gilbert et al., 2004; Leonard et al., 2004) as prior agent with a frequency level of 30%, based mainly on serological finding. Direct pathogen detection methods produced negative results in most cases. In pathophysiological terms, pathogen-specific antibodies cross-react with neuronal antigens (e.g. Moran et al., 2000; Yuki et al., 2004), thereby inducing inflammation mediators to invade macrophages, resulting in subsequent local micro-oedemas with transitory or permanent failure of the affected neurones. Neurological failures in GBS therefore include purely motor ("AMAN" = acute motor axonal neuropathy, "AIDP" = acute inflammatory demyelinating polyneuropathy) as well as sensory and mixed types ("ANSAM" = acute motor-sensory axonal neuropathy). Mild and reversible courses are observed as well as severe courses with permanent paralysis. About 5% of GBS cases are terminal. The course of disease appears to be directly proportional to the specific antibody titre. Clinical observations and initial findings from surveillance studies on "flaccid paralysis" (differential diagnosis: poliomyelitis) suggest that, in pathophysiological terms, discrete, transitory paralysis symptoms that take the form of signs of exhaustion in the extremities correspond much more frequently to an inapparent GBS than has been assumed to date.

Principles of Testing

The antigens used in Campylobacter IgG kit are made by genetic engineering techniques. In this way, an optimal presentation without other interfering and cross reacting proteins can be achieved. Only specific antigens with importance for the sensitive serological diagnosis are used in comparison to other ELISA-systems using lysate antigens.

The following recombinant antigens are presented in this kit: PEB4 (cytoplasmic protein, membrane protein), OMP18 (outer membrane protein, peptidoglycan-associated lipoprotein) and P39 (putative ATP/GTP binding protein).

Reagents And Materials Provided

1. Wash buffer (10× the concentration) Contains phosphate buffer, NaCl, detergent and preservatives MIT (0,01%) and Oxypyrion (0,1%), 100 mL

2. Dilution buffer (ready-for-use) Contains protein, detergent and blue dye, preservatives MIT (0,01%) and Oxypyrion (0,1%), 125 mL

3. Chromogenic substrate tetramethylbenzidine (TMB, ready-for-use), 12 mL

4. Stop solution 24,9 % Phosphoric acid (H_3PO_4) , 12 mL

5. Microplate (section marked in blue) coated with recombinant Campylobacter antigens in vacuum-pressure sealed bag, 12×8 wells

6. Positive control (brown screw cap) Preservatives: MIT (0,1%) and Oxypyrion (0,1). 450µL

7. Cutoff control (orange screw cap) Preservatives: MIT (0,1%) and Oxypyrion (0,1). 450µL

8. Negative control (white screw cap) Preservatives: MIT (0,1%) and Oxypyrion (0,1). 450µL

9. Anti-human IgG conjugate (101 times the concentration, blue screw cap) contains NaN_3 (<0,1%), MIT (<0,01%), Chloracetamide (<0,1%). 500 µL

10. Instructions for use 1

11. Evaluation form 1

12. Sealing tape 2 pieces

Materials Required But Not Supplied

Deionised water (high quality), test tubes, micro pipettes, incubator 37°C, microplate photometer.

Storage

2°C - 8°C

Specimen Collection And Preparation

Sample material

The sample material can be serum or plasma that is separated from the coagulum as soon as possible after sampling. Heat-inactivated samples will result in raised background reaction levels and are therefore unsuitable for use. A microbial contamination of the sample has to be avoided at all costs. Insoluble substances must be removed from the sample prior to incubation by centrifugation.

Important:

If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at 2°C - 8°C. Longer storage of the samples is possible at -20°C or below. Repeated freezing and thawing of the samples is not recommended because this may affect the quality of the results. Avoid more than 3 cycles of freezing and thawing.

Reagent Preparation

1. Handling information

A quality guarantee can only be given up to the expiry date on the packages.

Please mind the expire date of the components.

All control sera and conjugates are to be used with the lot noted on the cover of the kit, they must not be used with other parameters or lots.

The sealing tapes are to be used only once.

2. Preparation of the solutions

The test reagents are sufficient for 96 IgA tests. The amounts indicated below refer to processing of a microplate strip with 8 wells. If several microplate strips are used at the same time, the amounts

indicated must be multiplied by the number of microplate strips used. Substrate and stop solution are ready to

use.

a. Preparation of ready-to-use wash buffer

The wash buffer concentrate is diluted 1 + 9 with deionised H_2O. 5 ml concentrate are mixed with 45 ml deionised H_2O per microplate strip with 8 wells.

b. Preparation of conjugate solution

Per each microplate strip with 8 wells, 10 µl anti-human IgG peroxidase conjugate (red cap) or IgA peroxidase conjugate (blue cap) are added to 1 ml dilution buffer in a clean vessel and mixed well (dilution 1 + 100).

4. Storage and stability

Store the reagents at 2°C - 8°C before and after use.

The ready-to-use wash buffer can be prepared in larger amounts. Ready-to-use wash buffer may be stored at 2°C - 8°C for four weeks or at room temperature for one week for use in further tests.

The sample dilutions, controls and conjugate solution must always be prepared freshly.

Assay Procedure

1. Test preparations

Temper all reagents to room temperature (18°C - 25°C) before use for about 30 minutes. To avoid condensation of water in the microplate, it must be brought up to room temperature in the closed bag. After the required section is removed, the bag containing the plate must be reclosed and placed in the refrigerator.

Before use, the control and patient sera and concentrated conjugates must be mixed well and briefly centrifuged if practicable to collect the liquid at the bottom of the tubes.

2. Preparation of samples and controls

Pipette 10 µl of sample or control into 1 ml dilution buffer each and mix well (dilution 1 + 100). A negative control, cutoff control and positive control must be run parallel to each test run and diluted just like the patient samples.

3. Incubation of the samples

The microplate is removed from the pressure-sealed bag.

Pipette 100 µl per well of the diluted samples and diluted controls. One value is tested for each negative control, positive control and patient sample, whereas the cutoff control must be double-tested. It is preferable to pipette one cutoff control at the beginning and again at the end of the series. In manual processing, the microplate is carefully taped over with unused sealing tape and incubated for 1 hour at 37°C.

4. Washing procedure

The wells are emptied completely, then washed four times, each time with 300 µl ready-to-use wash buffer per well. We recommend performing this step with the appropriate ELISA washing equipment. Make absolutely sure that the wash buffer is removed completely between the washing steps. After the last washing step is completed (even if washing equipment is being used) tap the plate over a paper towel to remove any residual liquid from the wells.

5. Incubation with peroxidase conjugate

Pipette 100 µl per well. In manual processing, the microplate is carefully taped over with unused sealing tape and incubated for 30 minutes at 37°C.

6. Washing procedure

The wells are emptied and washed as described under 4.

7. Substrate reaction

The substrate solution is ready to use. Pipette 100 µl per well. It is not necessary to tape the microplate. The microplate is incubated for 30 minutes at room temperature while protecting it from direct sunlight. The time is counted from pipetting of the first well.

8. Stopping the reaction

To stop the reaction, pipette 100 µl of stop solution per well, using the same pipetting scheme as for the substrate solution.

9. Measurement of the extinctions

The extinction in the individual wells are measured in a microplate photometer at 450 nm and at the reference wavelength 650 nm (620 to 650). Blank on air. Measurement should be performed within 60 minutes after stopping the reaction.

Evaluation

1. Validation

Cut off control: The average value of the extinction values for the two cutoffs (at the beginning and end of the series) is obtained.

The test can be evaluated under the following conditions:

The individual extinction values for double-testing of the cutoff control deviate from their average value not more than 20%.

Extinction negative control ≤ 0.150

Extinction cutoff control - Extinction negative control ≥ 0.050

($E_{cutoff} - E_{neg.\ contr.} \geq 0.050$)

Extinction positive control - Extinction cutoff control ≥ 0.300

($E_{pos.\ contr.} - E_{cutoff} \geq 0.300$)

The reproducibility of results can be improved by determining the specific antibodies relative to the cut-off check in U/ml, as the fluctuations from the performance of the test are also included. In validating the test, the positive and negative checks do not need to be evaluated. If necessary, however, they can be carried out for internal quality control purposes. In this case, the results should lie within the target value range given in the analysis certificate or on the label.

2. Evaluation

a. Qualitative evaluation

Cutoff (boundary value): Extinction average for cutoff control

Grey range:

Lower limit = Cutoff

Upper limit = Cutoff + 20% (cutoff x 1.2)

Samples with extinction values above the grey range are to be considered positive.

Samples with extinction values below the grey range are to be considered negative.

Samples with extinction values in the grey range are borderline and should be retested. If they are still borderline after the second test, it is recommended that an additional sample should be taken after a period of time and retested (See **Directions for the interpretation of test results**).

b. Quantitative evaluation

The antibody activity levels in units per ml are assigned to the extinction values using a formula. The measurement units U/ml are arbitrary units, which do not allow conclusions concerning (international) reference values.

U/ml sample = (extinction sample / extinction cutoff) × 20

Grey range:

Lower limit = 20 U/ml

Upper limit = 24 U/ml

U/ml sample > 24 positive test result

U/ml sample < 20 negative test result

20 ≤ U/ml sample ≤ 24 borderline test result

The linearity of the test was determined during the evaluation within the following range:

20 U/ml to 150 U/ml (R^2 = 0.99)

In case of an extinction ≥ 3.0 or a measuring value above the linear range, the result should either be given as > 150 U/ml, or the sample may be diluted and measured again. We recommend to start with a final sample dilution of 1:500 and if necessary further subsequent dilution steps.

3. Directions for the interpretation of test results

In all test interpretations it is important to include any other clinical record data available. Close cooperation between laboratory and the physician in charge of treatment is recommended here as well.

Samples with unclear or questionable results should be rechecked after 2 - 3 weeks in keeping with the clinical situation.

A negative Campylobacter result does not exclude the possibility of a campylobacter infection. False negative results may occur with serum samples obtained at a very early stage after infection in which no antibodies to C.jejuni/C.coli have been produced as yet.

When evaluating the test results, IgG and IgA findings should always be considered together. We generally recommend to check positive and borderline ELISA results by a confirmation test.

Performance Characteristics

1. Intra-Assay-Variance:

A serum was examined on 48 cavities of a microtiterplate. The variation coefficient (VC) was calculated:

VC (IgG) = 4,6%, VC (IgA) = 4,1%.

2. Inter-Assay-Variance:

7 sera of different ODs ware examined in 8 different determinations. The variation coefficient was calculated for each single serum (U/ml):

VC (IgG) < 12%, VC (IgA) < 12 %

Precautions

1. Control sera are from blood donors verified for the absence of antibodies to HIV 1/2, HCV and no Hepatitis Bs-antigen. Since an infection cannot be excluded with absolute certainty despite this precaution, the product must be treated with the same care as the patient sample.
2. Suitable single-use gloves must be worn during the entire test procedure.
3. The conjugates contain sodium azide, MIT (methylisothiazolone) and Chloracetamide. The controls, dilution buffer and wash buffer contain MIT and Oxypyrion. Avoid contact with skin or mucosa.
4. Phosphoric acid is an irritant. Avoid all contact with skin or mucosa.
5. All reagents and materials contaminated with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121°C for at least 1 hour.