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User's Manual

Campylobacter jejuni IgG ELISA Kit

REF **DEIA-XY90**

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 jejuni IgG tests is qualitative immunoassays for the demonstration of human antibody classes IgG directed against Campylobacter jejuni. The assays are recommended for the sensitive detection of such antibodies in various kinds of samples.

General Description

Worldwide there are more than 20 different Campylobacter species. In the industrialised countries Campylobacter jejuni is the second most common cause of intestinal inflammation. The primary reservoirs for the organisms are wild warm blooded animals and farm animals. Campylobacter infections in humans are primarily a result of food contamination, inadequately heated poultry being the primary source of infection. The incubation time is generally between two and seven days. ELISA test-systems are especially suited for the differential analysis of immunoglobulin classes directed against the virus.

Principles of Testing

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies in the field of infectious serology. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colourless substrate pnitrophenylphosphate is then converted into the coloured product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with eight antigen coated single wells, 12 pieces, (altogether 96):
   The coating material is inactivated.
2. Standard serum (ready-to-use), 2 × 2 ml:
   Human serum in protein containing phosphate buffer;
   negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab;
   preservative: < 0.1 % sodium azide;
   colouring: Amaranth O.
3. Negative control serum (ready-to-use), 2 ml:
   Human serum in protein containing phosphate buffer;
   negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab;
   preservative: < 0.1 % sodium azide;

colouring: Lissamin Green V.

4. Anti-human IgG conjugate (ready-to-use), 13 ml:
   Anti-human IgG polyclonal antibody,
   conjugated to alkaline phosphatase, stabilised with protein stabilisation solution;
   preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane.
5. Washing solution concentrate (sufficient for 1000 ml), 33.3ml:
   Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7,4;
   preservative: < 0.1 % sodium azide.
6. Dilution buffer (ready to use), 2 × 50 ml:
   Protein containing phosphate buffer with Tween 20;
   preservative: < 0.1 % sodium azide;
   colouring: 0.01 g/l Bromphenol blue.
7. Stopping solution (ready to use), 15 ml:
   < 0.1 N sodium hydroxide, 40 mM EDTA
8. Substrate (ready-to-use), 13 ml:
   Para-nitrophenylphosphate in solvent free buffer;
   preservative: < 0.1 % sodium azide
9. Quality control certificate, 1 page

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtitre plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm - 690 nm (e.g. 650 nm)
3. Incubator 37 °C
4. Moist chamber
5. Distilled water

Storage

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	unopened after opening at 2 – 8 °C in closed aluminum bag with desiccant	see expiry date; 6 months
Control sera / Standard sera	unopened after opening at 2 – 8 °C	see expiry date 6 months
Conjugate	unopened after opening at 2 – 8 °C	see expiry date 6 months
Dilution buffer	unopened after opening at 2 – 8 °C	see expiry date 6 months
Washing solution	unopened / after opening at 2 – 8 °C working dilution at 2 – 8 °C working dilution at room temperature	see expiry date 2 weeks 1 week
Substrate	unopened after opening at 2 – 8 °C	see expiry date 6 months
Stopping solution	unopened after opening at 2 – 8 °C	see expiry date 6 months

Specimen Collection And Preparation

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

Dilution of Samples

Before running the test, all samples (V1) must be diluted in dilution buffer (V2) as follows:

$V_1 + V_2 = 1+1000$	add	10 µl	sample
	each to	1000 µl	dilution buffer (1 + 100)
	each to	20 µl 180 µl	of first dilution dilution buffer (1 + 9)

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

Sample Storage

The samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

Reagent Preparation

Bring all reagents to room temperature before testing.

1. Microtiter Test Strips

The microtiter test strips in frames are packed with a desiccant in an aluminum bag. Take unrequired cavities out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions.

2. Control Sera / Standard Sera

Control and standard sera are ready-to-use and must not be diluted any further. For each test run - independent of the number of microtiter test strips to be used - control and standard sera must be included. The standard sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

3. Anti-human IgG AP-Conjugate (ready-to-use)

Conjugates with the same concentration and of the same immunoglobulin class are interchangeable. Avoid contamination of ready-to-use conjugates e. g. by using sterile tips.

4. Washing Solution

Dilute washing buffer concentrate (V1) 1:30 with aqua dest. to a final volume of V2.

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33.3 ml	1000 ml
1.0 ml	30 ml

5. Dilution Buffer for Samples (ready-to-use)

6. Substrate (ready-to-use)

Avoid contamination of the ready-to-use substrate solution e. g. by using sterile tips.

7. Stopping Solution (ready-to-use)

Assay Procedure

1. Place the required number of **cavities in the frame** and prepare a protocol sheet.
2. Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

IgG well no.	
well A1	Substrate blank
well B1	Negative Control
well C1	Standard serum
well D1	Standard serum
well E1	Sample 1....

3. **Sample incubation** for 60 minutes (+/- 5 min) at 37 °C (+/- 1°C) in moist chamber
4. After incubation **wash** all wells with washing solution (by automated washer or manually):

(1) aspirate or shake out the incubation solution

(2) fill each well with 300 µl washing solution

(3) aspirate or shake out the washing buffer

(4) repeat the washing procedure 3 times (altogether 4 times!)

(5) dry by tapping the microtiter plate on a paper towel

5. **Addition of conjugate**

   Add 100 µl of the ready-to-use IgA/IgG/IgM conjugate to the appropriate wells (except substrate blank)

6. **Conjugate incubation** for 30 minutes (+/- 1 min)* at 37 °C (+/- 1 °C) in moist chamber.
7. After incubation **wash** all wells with washing solution (see above)
8. **Addition of substrate**

   Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)

9. **Substrate incubation** for 30 minutes (+/- 1 min)* at 37 °C (+/- 1 °C) in moist chamber.
10. **Stopping of the reaction**

    Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.

11. **Read extinction**

    Read optical desity (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

Quality Control

The substrate blank must be < 0.25 OD

The negative control must produce a negative test result.

The mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific qualitycontrol certificate.

The variation of OD-values of the standard serum may not be higher than 20%.

If these criteria are not met, the test is not valid and must be repeate

Calculation

A lot specific quality control certificate is included in the test kit so that the obtained OD values can be interpreted qualitatively. The substrate blank must be substracted from all OD values prior to evaluation.

To fix the cut-off ranges multiply the mean value of the measured standard OD with the lot specific correction factor from the quality certificate. Then add and substract the lot specific grey zone percentage mentioned on the quality certificate to obtain the upper and lower cut-off. The following numbers are an example only, the valid data you will find in the lotspecific QC certificate which comes with each kit.

Lot specific correction factor: 0.805

Lot specific grey zone: 15%

If the measured mean absorbance value of the standard serum is 0.84 OD, the range of the cut-off is:

Lower cut-off: (0,84 * 0,805) -15% = OD 0,575

Upper cut-off: (0,84 * 0,805) +15% = OD 0,778

Interpretation Of Results

A positive test result confirms the presence of specific antibodies. A negative result indicates that no relevant antibodies against the pathogen are present in the sample, but does not exclude the possibility of an acute reaction. In case of a borderline result a reliable evaluation is not possible. A definitive determination can only be achieved by testing paired serum samples, taken at one to two weeks intervals, in parallel.

Evaluation

For qualitative interpretation of serum samples a lot specific correction factor as well as a lot specific grey zone is calculated by manufacturer for each kit lot. These values can be found on the lot specific quality certificate included in each test kit.

For test run control a standard serum Is used in each Individual test run. For this control serum a reference value with a validity range is determined by the quality control of the manufacturer. Within this range a correct cut-off interpretation is ensured.

Sensitivity

The sensitivity exceeded 99%.

Specificity

To determine detection of cross-reactive antibodies directed against different parameters sera were analyzed with Campylobacter jejuni IgG and a commercially available anli-Campylobacter IgG ELISA. Positive sera (10 sera each) for Helicobacter pylori lgG, Yersinia lgG, Parvovirus IgG and Borrelia IgG have been tested as well as sera positive for rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Within this internal evaluation no potential cross-reactivities have been observed. Other cross-reactivities cannot be ruled out in general.

Reproducibility

Sample	Mean Value (OD)	Intraassay (CV %)	Mean Value (OD)	Interassay (CV %)
Serum 1	0.541	3.7	0.493	6.9
Serum 2	0.628	3.0	0.640	6.0
Serum 3	1.219	2.4	1.225	5.2

Interferences

To determine the influence of interfering substances, sera with different reactivities were analyzed with the

Campylobacter jejuni IgG. No interferences have been detected for sera with concentrations up to 2.00 g/L hemoglobin, 11.50 g/L lipemia/triglyceride or 0.201 g/L bilirubin (conjugated and unconjugated).

Precautions

Procedural Notes

1. Optimum results can only be achieved if the instructions are strictly followed. The components of the kit must not be exchanged for reagents of other manufacturers. Standard and control sera are defined exclusively for the test kit to be used and must not be used in other lots.
2. Each test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.
3. There are three different conjugate concentrations for immunoglobulin class (IgG), indicated on the C of A as + (low), ++ (medium) and +++ (high). Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.
4. Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).
5. Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results. Avoid exposure of reagents to strong light during storage and incubation.
6. Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!
7. Reagents must be tightly closed after use to avoid evaporation and contamination. Take care not to mix-up the caps of the bottles and/or vials.
8. The immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

Safety Measures

The Campylobacter jejuni IgG ELISA Kits are designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and human specimens should be handled carefully, using established good laboratory practice.

1. This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV-ab, HBs-Ag (Hepatitis B-Virussurface Antigen) and anti-HCV-ab, they should be considered potentially infectious.
2. Do not pipette by mouth.
3. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
4. Wear disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.
5. Sample material and other potentially infectious material should be decontaminated after the test run.

6. Reagents should be stored safely and be inaccessible to unauthorized access e.g.children.
7. Stopping solution: corrosive (C); causes acid burn (R34)
   Use safety glasses, gloves and laboratory coat while handling!