VIROTECH Yersinia enterocolitica IgG/IgA ELISA
(Y. enterocolitica IgG/IgA ELISA)

Order No.: EC142.00

Color Coding: green metallic/transparent

FOR IN VITRO DIAGNOSIS ONLY

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Contents

1. Intended Use

The VIROTECH Yersinia enterocolitica ELISA is intended for the qualitative and semiquantitative detection of specific IgG/IgA antibodies which are directed against antigens of the 70 Kb virulence plasmid of pathogenic Yersinia in human serum. The antigen used is a mixture of purified native Yops (Yersinia outer membrane proteins) and recombinant YopB, YopD and YopE. Antigens YopD and YopE have been found to be the immunodominant antigens in infections in IgG, IgM and IgA; in reactive arthritis, they are the dominant antigens in IgA. As a consequence, they allow reliable diagnosis (11, 13, 14, 15). The determination of increased antibody levels makes a contribution to diagnosis of Yersinia induced arthritis. The test is not intended for diagnosing acute enteric diseases.

2. Diagnostic Relevance

The genus Yersinia belongs to the family of the *Enterobacteriaceae*. Along with the human pathogens Y. enterocolitica, Y. pestis and Y. pseudotuberculosis, it includes other non-pathogenic species (1).

Yersinias occur worldwide in temperate and subtropical climates. The most important bacterial reservoirs for Y. enterocolitica and Y. pseudotuberculosis, the causes of enteral yersiniosis, are latent infections in warm-blooded wild, working and domestic animals (especially domestic pigs), whose excreta lead to contamination of the environment (2, 3). Transmission to humans is mainly alimentary.

Y. enterocolitica infections are manifested after an incubation period of approximately 14 days mainly as mesenteric lymphadenitis, which presents clinically as enteritis, pseudoappendicitis, ileitis or colitis (4). Extramesenteric forms (20-30% of cases), local infections after dissemination, septic forms and the lymphadenopathy syndrome ï either with or without preceding enteritis ï can also occur.

Reactive arthritis (often in HLA B27 positive patients) (5, 6) and erythema nodosum (7) are among the commonest immunopathological complications of Y. enterocolitica infection. Reactive arthritis typically occurs after a symptom-free interval of 1 to 3 weeks, especially in the joints of the lower half of the body (8).

Important factors in the pathogenicity of the yersinias are associated with the presence of a virulence plasmid, which among other things codes for virulence-associated release proteins, also called YOPs (Yersinia outer membrane proteins).

These YOPs are produced only by human pathogenic yersinia strains (DNA sequence homology 70 - 100%). The detection of antibodies to these release proteins is therefore a highly specific and very sensitive method for the serological diagnosis of all forms of yersiniosis (9).

In the acute phase (10-14 days after infection), class-specific antibodies (IgM, IgA and IgG) to the different release proteins can usually be detected (10). However, antibodies are not always produced against all yersinia antigens. All antibody classes are also not always produced (11).

IgM antibodies occur in close temporal relationship with the clinical manifestation of possible immunopathological complications (reactive arthritis; erythema nodosum) and persist in most cases for only 1-3 months or disappear regularly within 6 months (12).

The IgA response is also important, as this antibody class is nearly always detectable in active yersiniosis. The IgA reactivity can last approximatly 2 ï 6 months when the course is uncomplicated. In chronic yersiniosis, the IgA reactivity can persist for 2 ï 3 years, and even longer in individual cases (12). Corresponding serological tests therefore make sense only at longer intervals of 4 - 6 months. IgG antibodies persist for years.

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

Serological examinations should be proceeded as two-test-approach. In a first step the Ig-class-specific, rather sensitive focussed ELISA is recommended. In a second step positive ELISA-results should be confirmed by the more specific Yersinia enterocolitica LINE.

4. Package Contents (IgG and IgA Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgA negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgA cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgA positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgA-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3ô5,5ôTMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.

2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgA-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 ï 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgA-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$\text{VE (positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut - off control)}} \times 10$$

$$\text{VE (patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut - off control)}} \times 10$$

9.3 Interpretation Scheme IgG and IgA

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 ï 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. The VIROTECH Yersinia enterocolitica ELISA is not intended for diagnosing acute enteric diseases.
3. Both IgA and IgG Western Blot results should be taken into consideration for the diagnosis of patients sera suspected to have a Yersinia infection.

10. Performance Data

10.1 Sensitivity and Specificity

To determine the sensitivity and specificity 143 sera were tested in IgG. The VIROTECH Yersinia enterocolitica LINE is used as reference.

Finding	VIROTECH Yersinia enterocolitica ELISA IgG		
	Negative	Borderline	Positive
Negative	65	6	3
Borderline	10	7	3
Positive	4	7	38

Referring to findings a sensitivity of 90,5 % resp. a specificity of 95,6 % for IgG have been obtained.
Borderline results have not been considered for the calculation of the sensitivity and specificity.

To determine the sensitivity and specificity 144 sera were tested in IgA. The VIROTECH Yersinia enterocolitica LINE is used as reference.

Finding	VIROTECH Yersinia enterocolitica ELISA IgA		
	Negative	Borderline	Positive
Negative	117	1	2
Borderline	7	2	0
Positive	9	1	5

The findings for IgA give a specificity of 98.3 %.
Because of the low number of positive sera, no statement can be made about the percentage specificity.
Borderline results have not been considered for the calculation of the sensitivity and specificity.

10.2 Prevalence (Expected Values)

The following table shows the results of the examination of 80 blood bank sera in IgG and IgA:

n=80	IgG		IgA	
	No.	%	No.	%
Negative	52	65	77	96,2
Borderline	11	13,7	2	2,5
Positive	17	21,3	1	1,3

10.3 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation for IgG is < 9% .

10.4 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs by different persons in different laboratories.

The obtained variation coefficient values are lower than 15%.

11. Literature

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Preparation of Patient Samples and Washing Solution

∑ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

IgG/IgA-Samples ï Dilution 1:101

e.g.:

10 µl serum/plasma + 1000 µl Dilution Buffer

(Serum Dilution Buffer is ready to use)

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇓		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇓		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgA
⇓		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇓		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇓		
Stopping		**50 µl Stopping Solution** shake carefully
⇓		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**