VIROTECH Yersinia enterocolitica IgG LINE Immunoblot

(Y. enterocolitica IgG LINE-32)

Order No.: WE242G32

(Y. enterocolitica IgG LINE-96)

Order No.: WE242G96

VIROTECH Yersinia enterocolitica IgA LINE Immunoblot

(Y. enterocolitica IgA LINE-32)

Order No.: WE242A32

(Y. enterocolitica IgA LINE-96)

Order No.: WE242A96

FOR IN VITRO DIAGNOSIS ONLY

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REV 21 / VIROTECH Yersinia enterocolitica IgG & IgA LINE Immunoblot EN

Contents

1. Intended Use

Line Immunoblot test kit for qualitative detection of specific IgG/ IgA antibodies in human serum which are directed against antigens of the 70kb virulence plasmid of pathogenic Yersinia species. Detection of reactive bands contributes towards the diagnosis of Yersinia-associated sequelae (for example reactive arthritis). The test is not suitable for diagnosis of acute enteritic 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 – either with or without preceding enteritis – 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 – 6 months when the course is uncomplicated. In chronic yersiniosis, the IgA reactivity can persist for 2 – 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.

In testing of German blood donors, IgG antibodies could be detected in 41% of cases (16).

3. Principle of Test

Pathogen antigen proteins are transferred onto a nitrocellulose membrane by a special spraying process. The nitrocellulose membrane is then cut up into individual strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immuncomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis conjugated anti-human IgG-, respectively IgA-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violette precipitates at each site („antigen bands“) where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interprete the presence of specific IgG-, respectively IgA-antibodies.

4. Package Contents

4.1 Kit for 32 determinations

1.	**Nitrocellulose test strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**2x**	16 strips
2.	**IgG resp. IgA Cut off Control,** human serum, prediluted	**1x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**2x**	50 ml
4.	**IgG- resp. IgA- Conjugate** (100x conc.) **Anti-human**-(goat)-Alcalic Phosphatasis, with preservative	**1x**	0,7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
	Evaluation Record sheet for the notation and deposit of the results	**1x**	1 pcs.

4.2 Kit for 96 determinations

1.	**Nitrocellulose test strips** with applied antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**3x**	32 strips
2.	**IgG resp. IgA Cut off Control,** human serum, prediluted	**2x**	1.0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**4x**	50 ml
4.	**IgG- resp. IgA- Conjugate** (100x conc.) anti-human-(goat)-Alcalic Phosphatasis, with preservative	**3x**	0,7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**3x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**3x**	1 pcs.

Also available on request:

IgG resp. IgA - Positive control, human serum, prediluted, 1.0 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE242P60 resp. IgA: WE242P40)

IgG/IgA- Negative control, human serum, prediluted, 1.0 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG/IgA: WE242N20)

5. Storage and Stability of the Testkits and the Components

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. **Nitrocellulose test strips** : Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelflife
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months

Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	*or* room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic foreceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additional required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic foreceps
9. Aqua dest. or deionised water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

9. Test Procedure

Precise adherence to the user manual is the prerequisite for obtaining correct results.

9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipaemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000 x g), pipette clear supernatant and use for testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality Aqua dest./deionised and bring up to room temperature (20-25°C) before usage.

3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
   The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well. Both the concentrated and the dilute dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.
5. **IgG-, resp. IgA Conjugate**
   Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: The nitrocellulose test strips may only be tested in the approved Ig class (see label on blot booklet and indication on each individual test strip).

For the correct performance and evaluation of the Yersinia enterocolitica LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moistured completely within one minute and can be incubated in supine, lateral position or face-down position.
5. **15 µl patient serum or plasma** or **100 µl of the cut-off or positive / negative control** added by pipetting, if at all possible at the upper marked end of the strip. Incubate the patient serum and control for **30 minutes** on the rocking platform. Ensure that no cross-contamination occurs between individual patient samples during pipetting and subsequent pouring away.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulosis paper.
7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for 30 minutes on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with Aqua dest./deionised**.**
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1.5 ml portions of ready-to-use **substrate solution** into the channels and develop for **10 ± 3 minutes** on the rocking platform.
14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **Aqua dest./deionised** each**.**
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the enclosed evaluation record sheet for evaluation. The high specificity bands annotated on the record sheet facilitate evaluation of the patient samples.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot Processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE strip is fitted out with two controls:

1. **Serum control:**
   Only after the incubation with patient serum the serum incubation band appears below the markline.
2. **Conjugate control:**
   The LINE strip is fitted out with a conjugate control band which appears after incubation with the respective conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed nitrocellulose test strip.

The position of the serum band and conjugate control band may be found on the record sheet.

10.1 Usage of the cut-off control

Any bands which are of a lower intensity than the YopD cut-off band of the cut-off control are not included in the evaluation. The prevalence band YopDD, which is only applied onto the IgG test strips, is likewise not included in the evaluation. A YopDD band ≥ than cut-off with negative serology is indicative of prior contact with Yersinia.

10.2 Meaning of the Antigens

Antigen/ notation	Molecular weight	Meaning of antigens	Specificity of antibody in LINE
YopM (2a)	48 kD	"Yersinia outer proteins" (Yop) are plasmid-coded proteins which are released by the bacteria and are formed by all species of Yersinia which are pathogenic to humans. V antigen or also LcrV is governed by the same regulatory mechanisms as Yops and was only named differently for historical reasons.	Both IgG and IgA antibodies against "Yersinia outer proteins" and V-Ag are highly specific for infection with pathogenic Yersinia.
YopH (2b)	45 kD		
YopB (3)	42 kD		
V-Ag (17)	38 kD		
YopD (4a)	36 kD		
YopE (5)	27 kD		

No cross-reactions occur with Salmonella thyphimurium, Campylobacter jejuni or Brucella (13, 14).

The YopDD band (the final band on the test strip), which is only present on the IgG strip, is not included in the evaluation. A YopDD band associated with a negative finding of an intensity ≥ than the cut-off band is indicative of prior contact with Yersinia.

10.3 Interpretation Criteria

The interpretation of serological results should always include the clinical picture, epidemiological data and all further available laboratory findings.

Band Pattern	Meaning	Assessment
No bands ≥ cut-off band	No serological notice of a Yersinia infection or past Yersiniosis.	negative
One band ≥ cut-off band	Antibodies against Yersinia are detectable.	suspicious

(except YopD)	Weakened reaction at convalescence or persisting antibodies or just beginning infection. The request of a follow-up control is recommended.	
Isolated YopD (36 kD) ≥ cut-off band	Antibodies against Yersinia are detectable. More than 90% of patients having Yersinia-arthritis develop IgA-antibodies against YopD. It may also be a recent infection or persisting antibodies.	positive
Two bands ≥ cut-off band	Antibodies against Yersinia are detectable. An infection is likely. IgG- and IgA-antibodies may persist for years.	positive
YopDD (prevalence) ≥ cut-off band	Positive prevalence band. Indicative of prior contact with Yersinia	

If possible the IgG-results should also be considered for judging the IgA-results as strong band intensities and a large number of bands in IgG and IgA are the typical serological picture of a Yersinia-induced reactive arthritis (8).
Yersinia enterocolitica LINE is specifically adjusted in the IgG range such that only clear titers are detected. The YopDD prevalence band, which is indicative of prior contact with Yersinia, provides an indication of general prevalence in IgG.

10.4 Limits of the Test

1. Both the IgA and IgG LINE results should be included in the diagnosis of patients where Yersinia is suspected.
2. The persistance of IgA-antibodies after successful treatment may be between 6 months and 3 years. The IgG-antibodies normally persist many years.
3. Although they do not play any role in differential diagnosis cross reactions with sera against directed Pseudomonas aeruginosa cannot theoretically be completely excluded. Functionally similar “type III secretion machines“ [YopB and YopD homologous to PopB and PopD] can be found with P. aeruginosa, which is an opportunistic pathogen, that normally only infects immunologically compromised individuals (18, 19).
4. The Yersinia enterocolitica LINE is not intended for diagnosing acute enteric diseases.
5. In rare cases patient sera may show “inverse” bands (dark background, white bands), these are not to be interpreted, means the immunoblot is in those cases not interpretable. The serum should be checked using other serological methods.

11. Performance Data

11.1 Sensitivity

Sensitivity was determined by testing clinically characterized sera in Yersinia enterocolitica LINE and in a recombinant Yop D ELISA as reference.

Sensitivity
Combined sera (n=84)

Yersinia enterocolitica IgG Line Immunoblot	**Yersinia enterocolitica Yop D ELISA**		
	negative	**suspicious**	**positive**
negative	12	1	0
suspicious	2	0	0
positive	5	1	63

Yersinia enterocolitica IgA Line Immunoblot	Yersinia enterocolitica Yop D ELISA		
	negative	**suspicious**	**positive**
negative	17	4	3
suspicious	2	0	0
positive	11	2	45

Sensitivity is >99.9% for the Yersinia enterocolitica IgG Line Immunoblot and 93.8% for the IgA Line Immunoblot. Suspicious sera were not taken into account in the calculation.

11.2 Specificity and Prevalence (Expected Values)

Specificity was determined by testing blood donor sera in Yersinia enterocolitica LINE.

Yersinia enterocolitica IgG Line Immunoblot (blood donor sera, n=69)	
positive	8
suspicious	1
negative	60

Yersinia enterocolitica IgA Line Immunoblot (blood donor sera, n=87)	
positive	9
suspicious	2
negative	76

Specificity is 88.2% for the Yersinia enterocolitica IgG Line Immunoblot and 89.4% for the IgA Line Immunoblot. Yop DD reveals a prevalence rate of >40% in IgG.
Suspicious sera were not taken into account in the calculation.

11.3 Intra-Assay-Precision (Repeatability)

Repeatability was determined by incubating 32 nitrocellulose test strips in a test batch with a serum in IgG and IgA. The bands in each case exhibit uniform intensities over the entire nitrocellulose sheet.

11.4 Inter-Assay-Precision (Reproducibility)

Reproducibility was determined by testing 3 sera. The determination was performed in 10 different test batches of 3 test subjects. The sera used were a negative serum, a serum with high band intensity and a cut-off control.
The specified serological values were exactly matched in all the independent tests.

12. Literature

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13. Symbols

 => refer to user manual!

14. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl Patient serum/ plasma / 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	**3 x 5 minutes**	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	**30 minutes**	with 1,5 ml working dilution (1 + 100)
Washing	**3 x 5 minutes** **1 x 1 minutes**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml substrate solution each
Stopping	**3 x without incubation in between**	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515ml	3,03ml	4,545ml	6,06ml	7,575ml	9,09ml	11,11ml	12,12ml	14,14ml	15,15ml

Number of strips	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17ml	18,18ml	20,2ml	21,21ml	23,23ml	24,24ml	26,26ml	27,27ml	29,29ml	30,3ml

Number of strips	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	32,32ml	33,33ml	35,35ml	36,36ml	38,38ml	39,39ml	41,41ml	42,42ml	44,44ml	45,45ml

Number of strips	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47ml	48,48ml	50,5ml	51,51ml	53,53ml	54,54ml	56,56ml	57,57ml	59,59ml	60,6ml