*recom*Line Yersinia IgG 2.0
*recom*Line Yersinia IgA [IgM] 2.0

MIKROGEN
DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

*recom*Line Yersinia IgG, IgA [IgM] is an immunoassay for the qualitative determination of IgG, IgA and IgM antibodies to Yersinia enterocolitica and Yersinia pseudotuberculosis in human serum or plasma.

2 Intended use

*recom*Line Yersinia IgG, IgA [IgM] can be used as a screening or confirmatory test.
*recom*Line Yersinia IgG, IgA [IgM] is a line immunoassay. In contrast to ELISAs, this testing principle allows the separate lining up of individual antigens and thus the determination of specific antibodies to individual Yersinia enterocolitica and Yersinia pseudotuberculosis antigens. The test uses recombinantly produced antigens: Yersinia Outer Proteins (YOP-M, YOP-D, YOP-E), V-AG, PsaA and MyfA. Through the use of species-specific antigens, such as PsaA (specific to Y. pseudotuberculosis) and MyfA (specific to Y. enterocolitica), it is also possible to distinguish between Y. enterocolitica and Y. pseudotuberculosis infections.

3 Test principle

Highly purified recombinant Yersinia antigens are fixed on nitrocellulose membrane strips.
1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG, IgA and/or IgM), which are coupled to horseradish peroxidise (HRP).
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:
a) Reaction control is located below the strip number, and needs to show a reaction for each serum/plasma sample.
b) The conjugate controls (IgG, IgA, IgM) are used for the inspection of the antibody class detected. If, for example, the test strip is used for the detection of IgG antibodies, the IgG conjugate will show this clearly on the band.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4 Reagents
4.1 Package contents
The reagents in one package are sufficient for 20 (200) tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml (10x100 ml)** Wash Buffer A (**10-fold concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml (10x40 ml)** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g (10x5 g)** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1 (10)** Evaluation form

4.1.1 *recomLine Yersinia IgG*
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (20)** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl (10x 500 µl)** anti-human IgG conjugate (**100-fold concentration, green cap**) From rabbit, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.2 *recomLine Yersinia IgA*
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (20)** tubes, each with 10 numbered test strips
CONJ IgA	**500 µl (10x500 µl)** anti-human IgA conjugate (**100-fold concentration, colourless cap**) From rabbit, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

Also available for the determination of IgM antibodies is *recom*Line Yersinia IgA:

CONJ IgM Art. No. 10015	**500 µl** anti-human IgM conjugate (**100-fold concentration, purple cap**) From rabbit, contains NaN$_3$ (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.2 Materials required but not supplied
- Incubation trays (can be ordered from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling
- Store reagents at +2 to +8°C before and after use, **do not freeze**.
- Leave all ingredients at room temperature (+18 to +25°C) for at least 30 minutes before starting the test. The test procedure is carried out at room temperature.
- Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different *recom*Line and *recom*Blot test systems, if these components carry the same symbols. Consider the shelf life of these components.
- Mix the concentrated reagents and samples thoroughly before use. Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and store at +2 to +8°C (reseal tube tightly to prevent test strips from absorbing moisture before the test!).
- The strips are marked with the serial number, as well as the test code.
- The packages bear an expiration date. After this has been reached, no guarantee of quality can be offered.
- Protect the kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In case of significant changes to the product or the regulations for use by the user, the application may lie outside the purpose indicated by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells. Carefully remove liquids.
- The strips must be completely wetted and immersed throughout the entire procedure.
- Automation is possible; further information can be obtained from MIKROGEN.

6 Warnings and precautions
- For *in vitro* diagnostic use only
- All blood products must be treated as potentially infectious.
- The test strips were manufactured with inactivated whole cell lysates and / or recombinant produced bacterial, viral or parasitic antigens.

- After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion and chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
- All siphoned liquids must be collected. All collection containers must contain suitable disinfectants for the inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods stated by the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Carefully read the instructions for use prior to the test and then follow the individual steps indicated. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and preparation of reagents

7.1 Samples
The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation.
The use of heat-inactivated, icteric, haemolytic, lipaemic or turbid samples is not recommended.
Caution!
If tests are not carried out immediately, samples can be stored for up to 2 weeks at +2 to +8°C. More extended storage of the samples is possible at -20°C or lower. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results. Avoid more than 3 cycles of freezing and thawing

7.2 Preparation of solutions
7.2.1 *Preparation of ready-to-use wash buffer A*
This buffer is required for sample and conjugate dilutions, as well as for the washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First of all, the skimmed milk powder is dissolved in wash buffer A concentrate and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips need to be calculated using the following formulae (device-specific dead volume has not been taken into account):

Reagent	Formula	Example: 5 strips
Skimmed milk powder [g]	= number of strips x 0.1	0.5 g
Wash buffer A concentrate [ml]	= number of strips x 2	10 ml
Deionised water [ml]	= number of strips x 18	90 ml
Ready-to-use wash buffer A [ml]	= number of strips x 20	100 ml

Ready-to-use wash buffer A can be stored for 4 weeks at +2°C to +8°C. The ready-to-use wash buffer A is odourless and slightly turbid.

7.2.2 *Preparation of conjugate solutions*
The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.
One part of conjugate concentrate is diluted with 100 parts of ready-to-use wash buffer A (1 + 100).

The quantities required for a defined number of test strips are to be calculated according to the following formula:

Reagent	Formula	Example: 5 strips
Conjugate concentrate [µl]	= number of strips x 20	100 µl
Ready-to-use wash buffer A [ml]	= number of strips x 2	10 ml

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

No.	Execution	Note
1	Subject all reagents for at least 30 minutes to 18-25°C (room temperature) before beginning the test.	The test procedure is carried out at room temperature.
2	Prepare test strips
Place the strips in **2 ml** of ready-to-use **wash buffer A**.	Do not touch the strips with bare hands - use tweezers instead. The strip number points upward. Place each strip in a separate well in the incubation tray (see 4.2).The strips must be completely immersed.
3
a)	Incubation of samples
20 µl of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100)	Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray.
b)	Incubate for **1 hour** while gently shaking.	Cover the incubation tray with plastic cover and place in the shaker.
4
a)	Washing
Carefully remove the plastic cover from the incubation trays.	Carry out washing stages 8.4a-8.4c three times in all. Avoid cross-contamination.
b)	Gently siphon the serum dilution from the individual wells.	The manufacturer's instructions must be observed during automatic processing.
c)	Pipette **2 ml** of ready-for-use **wash buffer A** into each well, wash for 5 minutes while gently shaking and then siphon off the wash buffer A.	
5	Incubation with conjugate
Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** while shaking gently.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing
see 8.4	Carry out the washing stages three times in all (see 8.4a-8.4c).
7	Substrate reaction
Add **1.5 ml** of ready-to-use **substrate solution** and incubate for **8 minutes** while shaking gently.	
8	Stopping the reaction
Remove the substrate solution. Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips
Dry the strip between 2 layers of absorbent paper for **2 hours** prior to analysis.	Carefully remove strips from water using plastic forceps. Store strip away from light.

Caution!
Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.

9 Results
Caution:
Please read the information on interpretation below, before using automated interpretation.

9.1 Validation – Quality Control
An analysis of the test can be carried out if the following criteria have been fulfilled:
1. Reaction control band (top line) with clearly visible stain, dark band
2. Antibody class (second, third and fourth bands): the IgG, IgA and/or IgM conjugate control band must show clearly visible staining.
3. Cut-off control (fourth band): weak, but visible staining

9.2 Evaluation
The test strips can be analysed visually or by using a computer - by making use of the test strip analysis software *recom*Scan. The *recom*Scan software has been designed to support the evaluation of test strips. Further information and related instructions for computer-assisted analysis are available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*
1. Enter the date, batch number and detected antibody class to the attached evaluation form.
2. Enter the sample identification numbers in the evaluation sheet.
3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Then identify the bands of the developed test strip using the printed control strip of the evaluation sheet for comparison purposes and enter them to the evaluation sheet. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	Assessment
No reaction	-
Very low intensity (lower than cut-off band)	+/-
Low intensity (equivalent to cut-off band)	+
Strong intensity (stronger than cut-off band)	++
Very strong intensity	+++

9.3 Interpretation of test results
The test result is determined by adding the point values in Table 2 of the individual bands reactive ≥ cut-off (i.e. with a minimum grade of +). The resulting total is entered in the column with the sigma (summation) symbol.

The positive, unknown or negative assessment of the sample can then be directly determined using Table 3 and entered in the assessment column of the evaluation sheet.

Table 2: Antigen points' rating

Antigen	Points
Yop M	1
V-AG	1
PsaA	1
Yop D	3
MyfA	1
Yop E	1

Table 3: Test Interpretation

Points' total	Assessment
0-1	negative
2	inconclusive
≥ 3	positive

Species are differentiated by antigens PsaA and MyfA, but only with positive test results (see tables 3 and 4), and only in the IgG.

Table 4: Differentiation

Differentiation	Criteria
Y. pseudotuberculosis	• Test result is positive <u>and</u> • PsaA reacts ≥ cut-off <u>and</u> • MyfA reacts < cut-off
Y. enterocolitica	• Test result is positive <u>and</u> • MyfA reacts ≥ cut-off <u>and</u> • PsaA reacts < cut-off
differentiation not possible	• Test result is positive <u>and</u> • and none of the criteria for y. pseudotuberculosis or y. enterocolitica apply

10 Limitations of the method - restrictions
- Serological test results must always be considered in the context of other medical assessments of the patient. The therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- After fresh infections, IgM and IgA antibodies disappear within three to six months. IgG antibodies persist for years. In chronic yersiniosis and with immunopathological complications, IgG reactivity and IgA titre are present for many years. When evaluating IgA reactivity, it is important to also include the results of the IgG determination and possibly also of the IgM determination.
- Samples with inconclusive results should be followed up after 3-5 weeks, subject to the clinical situation.
- Isolated IgA-positive results require careful interpretation. They may indicate a fresh infection or long-term persistent antibodies.

- The typical serological image of a Yersinia-induced reactive arthritis is the high IgG and IgA titre along with weak or missing IgM antibody reactivity.
- <u>Dark test strips:</u> Some patient samples can produce dark, uniform or patterned staining across the entire nitrocellulose strip. Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance
11.1 Diagnostic sensitivity

*recom*Line Yersinia	Earlier positive findings in two reference tests		
	IgG (n=122)	IgA (n=68)	IgM (n=61)
negative	0	2	0
borderline	0	0	0
positive	122	66	61
sensitivity	100%	97%	100%

11.2 Differentiation between Y. enterocolitica and Y. pseudotuberculosis through determination of species-specific IgG antibodies

*recom*Line Yersinia	Yersinia enterocolitica* (n=59)	Yersinia pseudotuberculosis ** (n=63)
positive for Y. enterocolitica (MyfA antigen)	41	0
positive for Y. pseudotuberculosis (PsaA antigen)	0	51
Differentiation possible in % of the samples	**69%**	**81%**

* Classification as Y. enterocolitica samples with positive Widal test result
* Classification as Y. pseudotuberculosis samples with positive culture and PCR results

11.3 Diagnostic specificity

*recom*Line Yersinia	Earlier negative findings in two reference tests		
	IgG (n=95)	IgA (n=134)	IgM (n=109)
negative	95	134	108
borderline	0	0	0
positive	0	0	1
Specificity	100%	100%	99%

11.4 Detection rate

*recom*Line Yersinia	n	IgG positive	IgA-positive	IgM positive
Blood donor serums	114	47 (41,2%)	13 (11,4%)	2 (1,8%)

Differentiation of IgG positive samples (n = 47):
7/47 were linked to a Y. enterocolitica infection
4/47 were linked to a Y. pseudotuberculosis infection
Differentiation was not possible for the remainder of the samples, since these blood samples – mostly infections from the distant past – only showed YOP D reactivity.

11.5 Analytical specificity
Analytical specificity is defined as the capacity of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.
a) <u>Interferences:</u> Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia or cycles of freezing and thawing do not affect the performance of the test.
b) <u>Cross-reactions:</u> The potential interference of antibodies with other organisms (Treponema sp.) has been investigated in control studies. Also tested were conditions caused by atypical activity of the immune system (antinuclear autoimmune antibodies, rheumatoid factor, pregnancy, fresh herpes simplex infection). There was no evidence of cross-reactions.

12 Literature

1. Hammer, M.et al. Postenteritische reaktive Arthritiden and Spondarthritiden. Deutsches Ärzteblatt 1995, 92(41):2738-2749
2. Cremer J. et al. Immunoblotting of Yersinia plasmid-encoded released proteins: A tool for serodiagnosis. Electrophoresis 1993, 14:952-959
3. Larsen J. H. et al. The determination of specific IgA-Antibodies to Yersinia Enterocolitica and their role in enteric infections and their complications. Acta Pathol Microbiol Immunol Scand B. 1985, 93(5):331-9
4. Sieper J. Disease Mechanisms in reacitive Arthrirtis. Current Rheumatology Reports 2004, 6:110-116
5. Sieper J. et al: Diagnosing reactive Arthritis. Arthritis and Rheumatism 2002, 46(2):19-327
6. Perdikogianni, C. et al. Yersinia enterocolitica infection mimicking surgical conditions. Ped. Surgery. 2006, 22:589-592
7. Zheng H. et al. Yersinia enterocolitica infection in diarrheal patients. Eur J Clin Microbiol Infect Dis 2008, 27(8):741-52
8. Antonopoulos et al. An emergency diagnostic dilemma: a case of Yersinia enterocolitica colitis mimicking acute appendicitis in a ß-thalassemia major patient: the role of CT and literature review. Emerg Radiol 2008, 15(2):123-6
9. Jalava K, et al. An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with Yersinia pseudotuberculosis. J Infect Dis 2006, 94(9):1209-16

Further Information on Yersinia diagnostics is available on request.

13 Explanation of symbols

Symbol	Description
Σ	Content is sufficient for <n> applications Number of applications
WASHBUF A 10 X	Wash Buffer A (**10 times concentration**)
SUBS TMB	Chromogenic substrate Tetramethylbenzidin
MILKPOW	Skimmed milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
ADD	Additional reagent, available on request
CONJ IgA	Anti-human IgA conjugate
CONJ IgM	Anti-human IgM conjugate
EVALFORM	Evaluation form
INSTRU	Instructions for use
[i]	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
(do not freeze symbol)	Do not freeze
REF	Order number
(hourglass symbol)	Use by Expiry date
(thermometer symbol)	Store at x°C to y°C
(factory symbol)	Manufacturer

14 Manufacturer and version information

GARLYE010