*recom*Line Bordetella pertussis IgG
*recom*Line Bordetella pertussis IgA

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose
The *recom*Line Bordetella pertussis is a qualitative *in vitro* test for detection of IgG and/or IgA antibodies against antigens of Bordetella pertussis in human serum and plasma.

2 Intended use
The *recom*Line Bordetella pertussis facilitates clarification of doubtful ELISA findings and confirmation of positive ELISA findings. The proteins used in this test are immunodominant antigens, whose antibody reactions allow conclusions about the infection status.

3 Test principle
Highly purified recombinant Bordetella pertussis antigens (filamentous haemagglutinin (FHA) and the total toxin (PT) at two concentrations) are fixed on nitrocellulose membrane test 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 and /or IgA), which are coupled to horseradish peroxidase.
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) The reaction control with the strip number that must show a reaction for every serum / plasma sample.
b) The conjugate controls (IgG, IgA) 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 a clear band.
c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each of the antigen bands (see 9.2. Evaluation).

4 Reagents
4.1 Package contents
The reagents in one package are sufficient for 20 (100) tests.
Each test kit contains:

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

4.1.1 *recom*Line Bordetella pertussis IgG
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (10)** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl (5x500 µl)** anti-human IgG conjugate **(hundred times concentrated, green screw cap**) From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.2 *recom*Line Bordetella pertussis IgA
In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2 (10)** tubes, each with 10 numbered test strips
CONJ IgA	**500 µl (5x500 µl)** anti-human IgA conjugate **(hundred times concentrated, colourless screw 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 purchased as needed 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**.
- Subject all ingredients for at least 30 minutes to room temperature (+18 to +25 °C) before beginning the test. The test procedure is carried out at room temperature.
- Identical reagents (see symbol label) of different recomLine and recomBlot tests can be used across all parameters and batches. At the same time, the shelf life of these components is to be noted.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 continue to store at +2 to +8 °C (reseal tube tightly, test strips may not become moist 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 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 substantial changes to the product or the regulations for use by the user, the application may lie outside the purpose given 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 submerged throughout the entire procedure.
- Automation is possible; you will receive further information 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 prepared with inactivated whole bacterial or viral 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 collecting containers must contain suitable disinfectants for 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 of 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.
- Read through the entire instructions for use before carrying out the test and carefully follow them. 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 must be separated as soon as possible from the blood clot after blood sampling to avoid a haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample before incubation.
The use of heat-inactivated, icteric, haemolytic, lipaemic or turbid samples is not recommended.
Caution!
If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at +2 to +8 C. Prolonged storage of the samples is possible at - 20°C or below. Repeated freezing and thawing of samples is not recommended due to the risk of inaccurate results.

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*
This buffer is required for serum and conjugate dilution as well as washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First, 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 are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

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 lightly marred.

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 the 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 strips in **2 ml** ready-to-use **wash buffer A**.	Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of an undiluted sample (human serum or plasma) are pipetted onto the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 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. Cover the incubation tray with plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. Pipette **2 ml** ready for use **wash buffer A** in every well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	Carry out washing stages 8.4a-8.4c three times altogether. Avoid cross-contamination The manufacturer's instructions must be observed during automatic processing.
5	Incubation with conjugate Add **2 ml** ready-to-use **conjugate solution** and incubate for **45 minutes** with gentle shaking.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see 8.4	Carry out washing stages three times altogether (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** ready-to-use **substrate solution** und incubate for **8 minutes** with gentle shaking.	
8	Stopping the reaction Remove substrate solution Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips Dry strip between 2 layers of absorbent paper for **2 hours** before 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 do not use automated interpretation without consideration of the information on interpretation given below.

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 (uppermost line) is clearly stained, dark band
2. Antibody class (second and third bands): the IgG and / or IgA conjugate control band must show a clear staining. In each case, the other conjugate control band may develop a weak, unspecific colouring.
3. Cut-off control (fourth band): weak, but visible staining

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software *recom* scan. The *recom* scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*

1. Note the date and batch number, as well as the detected antibody class, on 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. Now identify the bands of the developed test strip on the basis of the printed control strip of the evaluation sheet and enter this in the evaluation sheet. For this purpose, carry out the assessment of the intensity of the occurring bands on the basis of Table 1 separately for the corresponding immunoglobulin classes.

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 weakly cut-off band)	+/-
Low intensity (equivalent to cut-off band)	+
Strong intensity (stronger than cut-off band)	++
Very strong intensity	+++

Caution!
The band patterns in the *recom*Line Bordetella pertussis IgG and IgA detection can show different intensities. It is possible that the *recom*Line Bordetella pertussis IgG shows stronger and darker bands than the *recom*Line Bordetella pertussis IgA. The intensity of the protein bands is dependent on the concentration of the Bordetella-specific antibodies

9.3 Interpretation of test results

The results of the IgG and IgA detection should be considered as a whole for the assessment of the Bordetella-pertussis immune status (see Table 3). The same interpretation criteria apply to both IgG and IgA antibody classes for FHA and PT (see Table 2).

Table 2: Assessment of the Bordetella pertussis antigens

Antigen	Intensity	Assessment IgG, IgA
FHA/PT/PT-100	lower than "1+" cut-off bands	negative
FHA/PT/PT-100	as strong as or stronger than "1+" cut-off bands	positive

Test interpretation on the basis of filamentous haemagglutinin (FHA):
In the presence of antibodies against Pertussis toxin (IgG and / or IgA), additionally available antibodies against FHA (IgG and / or IgA) do not play any role (see Table 3) in the findings. Antibodies against FHA are assessed only if they occur in isolated form (without antibody against PT).
Different scenarios can be inferred when only FHA (IgG and / or IgA) turns out to be positive:
A: FHA indicates an infection with one of the Bordetella species (for example, Bordetella parapertussis, among others) when considering cross-reactivity's with other bacteria.
B: A very early stage of a Bordetella pertussis infection cannot be excluded. If clinical suspicion persists, a follow-up should be done after about 2 weeks (additional occurrence of antibodies against PT). FHA occurs in different Bordetella species (not only Bordetella pertussis) and is cross-reactive.
In the interpretation of the serological results, it is indispensable to include the case history, clinical symptoms and additionally available laboratory data in the total diagnosis.

Table 3: Interpretation of the Bordetella pertussis antigens

Test interpretation on the basis of Pertussis Toxin (PT) :
Pertussis-Toxin is a specific marker for a **Bordetella pertussis infection**.
PT-100: standardised according to WHO norms. Serums with a positive IgG reactivity against the PT-100 bands have an IgG titre above 100 IU/ml WHO norm → Indication of an acute infection (unless a vaccination was done within the last three years).

IgG **PT-100**	IgG **PT**	IgA **PT**	**Interpretation**
+	+	+	IgG and IgA* antibodies against Bordetella pertussis detectable. Indication of an acute Bordetella pertussis infection.
+	+	-	IgG antibodies against Bordetella pertussis present. Indication of an acute Bordetella pertussis infection. It can also be a titre immediately following a vaccination (within the last three years).
-	+	+	IgG and IgA* antibodies against Bordetella pertussis detectable. Acute infection with Bordetella pertussis possible. Follow-up after about 2 weeks recommended (titre increase).
-	-	+	IgA* antibodies against Bordetella pertussis detectable. Early stage of an acute infection with Bordetella pertussis possible. Follow-up after about 2 weeks recommended (titre increasen IgA, sero-conversion in IgG).
-	+	-	IgG antibodies against Bordetella pertussis detectable. Indication of a previous Bordetella pertussis infection / vaccination. A very early infection stage cannot be excluded. If clinical suspicion persists, a follow-up should be done after about 2 weeks.
-	-	-	No indication of a Bordetella pertussis infection / vaccination. If clinical suspicion persists, a follow-up should be done after about 2 weeks.

*IgA antibodies occur only rarely after a vaccination

10 Limitations of the method - restrictions

- Serological test results must always be seen in the context of the clinical picture of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- The results of the IgG and IgA detection should be considered as a whole for the assessment of the Bordetella-pertussis immune status.
- Samples with unclear results should be followed-up after about 2 weeks depending upon the clinical condition.
- Following a primary infection with Bordetella pertussis, IgG antibodies occur at the earliest 2 - 3 weeks after onset of illness and reach their maximum after about 8 weeks. IgA antibodies can be measured already after 1 - 2 weeks. A pertussis infection in the early stage can show isolated positive IgA findings as a result. While IgA antibodies are often not detectable later than 6 months after an infection, IgG antibodies can persist for years. The exclusive detection of specific IgG antibodies is consistent not only with an earlier pertussis infection, but also with a status after pertussis vaccination.
- The detection of specific IgA antibodies can be regarded as an indication of a fresh infection, as IgA is normally not formed after a vaccination. Nevertheless, it is to be noted that IgA is detectable in babies in the first 6 months of life only rarely and / or in low concentration.
- Antibodies against Bordetella pertussis are also found in unvaccinated youth and adults. It is assumed that repeated sub-clinical infections are responsible for this.
- A negative result does not exclude the possibility of a Bordetella infection in general. Incorrect negative results can occur if the sampling is done before the initial reaction of the immune system.
- Dark test strips: Some patient samples can produce a 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 Sensitivity

43 patient serums were tested. An acute infection with Bordetella pertussis had been diagnosed for all patients based on the clinical symptoms as well as IgG and IgA-positive ELISA findings against pertussis toxin.

	FHA IgG	FHA IgA	PT-100 IgG	PT IgA
positive	42 (97,6 %)	39 (90,6 %)	42 (97,6 %)	18 (41,9 %)
negative	1 (2,3 %)	4 (9,4 %)	1 (2,3 %)	25 (58,1 %)
Total	43 (100 %)	43 (100 %)	43 (100 %)	43 (100 %)

11.2 Specificity

Specificity relates to the data from healthy blood donors (see 11.3), wherein a reference line assay was used for defining the seronegative blood donors. It is 100%.

11.3 Detection rate

100 plasmas of healthy unselected blood donors in the *recom*Line Bordetella pertussis were tested.

	FHA-positive	PT-100-positive	PT-positive
IgG	48 %	3 %	18 %
IgA	23 %	---	0 %

11.4 Analytical specificity

The analytical specificity is defined as the capacity of the test to determine the analytes exactly in the presence of potential interference factors in the sample matrix or cross reactions with potentially interfering antibodies.
a) Interferences: Control studies on potentially interfering factors showed that the efficiency of the test is not influenced by haemolysis, bilirubinaemia or lipaemia of the sample.
b) Cross reactions: Potentially interfering samples (autoimmune diseases, fresh EBV infections, rheumatism-factor-positive and samples from pregnant women) were tested.
Positive PT-100-reactivity was observed in five of 70 serums summarised under a) and b). Two of these five samples were examined in two commercially available Bordetella pertussis tests. The positive PT-100-reactivity was confirmed for both serums, so as to confirm the presence of a real pertussis infection. Cross reactions could be excluded here.

12 Literature

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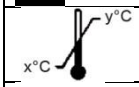
We will be glad to send you further reading on Bordetella diagnostics on request.

13 Explanation of symbols

	Content is sufficient for <n> applications Number of applications
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line Bordetella pertussis IgG** ***recom*Line Bordetella pertussis IgA**	Item. No. **5772 (5770)** Item. No. **5773 (5779)**
Instructions for use valid from	GARLBP006EN 2023-03

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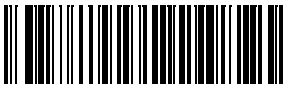

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