*recom*Line Tropical Fever IgG
*recom*Line Tropical Fever IgM

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

*recom*Line Tropical Fever IgG, IgM is an immunoassay for qualitative detection of IgG and IgM antibodies against Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) viruses in human serum or plasma.

2 Field of Application

Dengue, Chikungunya and Zika viruses are primarily transmitted by mosquitoes from the *Aedes* genus. Dengue is widespread in tropical and subtropical regions around the world and is one of the most common viral diseases transmitted by mosquitoes with an estimated 390 million infections each year.
Since the circulation areas of DENV, ZIKV and CHIKV partly overlap and the pathogens can induce similar clinical symptoms in the early stages, misdiagnoses are possible.
While CHIKV belongs to the family of togaviruses / genus alphavirus, DENV and ZIKV are flaviviruses / genus flavivirus. The close genetic relationship between the flaviviruses makes serological differentiation difficult.
The *recom*Line Tropical Fever IgG, IgM is a line immunoassay. In contrast to ELISA test systems, the separate line-up of the antigens, allows the identification of specific antibodies against single antigens from Dengue, Chikungunya and Zika viruses. Virus-like particles (VLP) are used to detect CHIKV. To detect and differentiate DENV and ZIKV, NS1 (non-structural protein 1) and a variant of the envelope (E) protein (Equad), which has a higher specificity due to targeted mutations, are used.

3 Test Principle

Highly purified recombinant antigens are fixed to nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample, whereby specific antibodies are binding to the pathogen antigens on the test strips.
2. Unbound antibodies are then washed away.
3. The strips are incubated in a second step with anti-human immunoglobulin antibodies (IgG and/or IgM) that are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then washed away.
5. Specifically bound antibodies are detected in a colour reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band appears on the strip at the corresponding position.

Control bands are located at the top end of the test strip:

a) The reaction control below the strip number for which every serum/plasma sample must show a reaction.
b) The conjugate controls (IgG, IgM) are used to check the conjugate and strip type used (Ig class specific). If the IgG-specific test strips are used to identify IgG antibodies, the IgG conjugate control band shows a clear reaction; for the IgM-specific test the IgM control band must show a positive reaction.
c) 'Cut-off control': The intensity of this band enables an evaluation of the reactivity of the individual antigen bands (see 9.2 Evaluation).

4 Reagents

4.1 Package Contents

The reagents in one pack are sufficient for 20 (100) assays.

Each set of reagents contains:

WASHBUF A 10 X	**100 ml (5× 100 ml)** wash buffer (**10× concentrate**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml (5× 40 ml)** chromogenic substrate tetramethylbenzidine **(TMB, ready-to-use)**
MILKPOW	**5 g (5× 5 g)** skimmed milk powder
INSTRU	**1** instructions for use
EVALFORM	**1 (5)** evaluation form(s)

4.1.1 *recomLine Tropical Fever IgG*

In addition to the components listed under point 4.1, each set of reagents contains:

TESTSTR	**2 (10)** tubes each with 10 consecutively numbered test strips
CONJ IgG	**500 µl (5× 500 µl)** anti-human IgG conjugate **(100× concentrate, green cap)** From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)

4.1.2 *recomLine Tropical Fever IgM*

In addition to the components listed under point 4.1, each set of reagents contains:

TESTSTR	**2 (10)** tubes each with 10 consecutively numbered test strips
CONJ IgM	**500 µl (5× 500 µl)** anti-human IgM conjugate **(100× concentrate, purple cap)** From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)

4.2 Additionally Required Reagents, Materials and Equipment

- Incubation trays (can be purchased from MIKROGEN as required)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotatory device
- Vacuum pump or similar device
- Measuring cylinder 50 ml and 1000 ml
- Micropipettes with single-use tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for biohazardous substances

5 Shelf Life and Handling

- Store reagents between +2°C and +8°C before and after use, **do not freeze**.
- Before starting the test, allow all reagents to sit at room temperature (+18°C to +25°C) for at least 30 minutes. The test procedure is carried out at room temperature.
- Wash buffer, milk powder, dilution buffer, conjugates and TMB can be exchanged between the different *recom*Line and/or *recom*Blot test systems if these components carry the same symbol. In doing so, attention must be paid to the shelf life of these components.
- Before use, mix the concentrated reagents and patient sera thoroughly. Avoid foam formation.
- Only open the tubes with the test strips just before use to prevent water condensation. Strips that are not needed must be left in the tube and continue to be stored at +2°C to +8°C (reseal tube firmly, test strips must not be moist before the start of the test!).
- The strips are identified with a consecutive number and a test abbreviation.
- The packages have an expiry date, after which no further guarantee of quality can be given.
- Keep the kit components away from direct sunlight throughout the test procedure. Note: The substrate solution (TMB) is light sensitive.
- The test must only be performed by trained, authorised and qualified personnel.
- Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by MIKROGEN.
- Cross-contamination of the patient samples or conjugates in the kit can lead to false test results. Add patient samples, test strips and conjugate solution carefully. Make sure that any incubated liquids are not carried over to other wells. Carefully remove liquids.
- The strips must be completely wetted and submersed throughout the entire procedure.
- Automation is possible. Further details are available from MIKROGEN.

6 Warnings and Safety Precautions

- Only use for in vitro diagnostics.
- All blood products must be treated as potentially infectious.
- The test strips were manufactured with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasitic antigens.
- After adding patient or control material, the strips must be considered to be potentially infectious and handled appropriately as such.
- Suitable single-use gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide (NaN_3), MIT (methylisothiazolinone), Oxypyrion, chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membranes. Sodium azide (NaN_3) can form explosive azides if it comes into contact with heavy metals such as copper and lead.
- All aspirated liquids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens or be autoclaved. All reagents and materials that come into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentration specifications and incubation times of the manufacturer must be followed.
- Only use incubation trays once.
- Handle strips carefully with a pair of plastic forceps.
- Do not replace or mix the reagents with reagents of other manufacturers.
- Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and Preparation of Reagents

7.1 Sample Material

The sample material can be either serum or plasma (citrate, EDTA, heparin, CPD) and must be separated as soon as possible after collection to avoid haemolysis. Microbial contamination of the sample must absolutely be avoided. Non-soluble substances must be removed from the sample prior to incubation.
Use of heat-inactivated, icteric, haemolytic, lipaemic or cloudy samples is not recommended.
Caution!
If the tests are not carried out immediately, the sample material can be stored for up to 2 weeks at 2°C to 8°C. It is possible to store the samples for longer periods at -20°C or lower. Repeated freezing and thawing of the samples is not recommended due to the risk of false results. More than 3 freezing and thawing cycles should be avoided.

7.2 Preparation of Solutions

7.2.1 *Preparation of the ready-to-use wash buffer A*
This buffer is required for the serum and conjugate dilution and the wash steps.
Before the dilution, the volume of wash buffer A must be determined for the corresponding number of tests to be carried out.
The skimmed milk powder is first pre-dissolved in wash buffer A concentrate and then this mixture is made up to the final volume with deionised water (dilution 1 + 9). The required quantity for a defined number of test strips is calculated using the following formulae (device-specific dead volumes are not taken into account):

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

Ready-to-use wash buffer A can be stored at +2°C – +8°C for four weeks. The ready-to-use wash buffer A has no odour and is slightly turbid.

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

The required quantity for a defined number of test strips is calculated using the following formulae:

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

The conjugate quantities are calculated without dead volumes. Depending on the processing (manually or with a device), please prepare additional conjugate solution for 1 to 3 strips.

8 Test Procedure

No.	Implementation	Note
1	Before starting the test, allow all reagents to sit at 18°C – 25°C (room temperature) for at least 30 minutes.	The test is carried out at room temperature.
2	Prepare the test strips Place strips in **2 ml** ready-to-use **wash buffer A**. **Important:** IgG and IgM strips are not interchangeable!	Do not handle the strips with bare hands – use forceps. The strip number faces upwards. For each strip one well in an incubation tray (see 4.2) is required. The strips must be completed submersed.
3	Sample incubation	
a)	**20 µl** of an undiluted sample (human serum or plasma) are added by pipette to the test strip for each incubation mixture. (dilution 1 + 100)	Pipette the sample onto one end of the submersed strip in wash buffer A and mix as quickly as possible by gently shaking the incubation tray.
b)	Incubate for **1 hour** with gentle shaking.	Cover the incubation tray with the plastic lid and place on the shaker.
4	Wash	Carry out wash steps 8.4a–8.4c a total of three times.
a)	Carefully remove the plastic lid from the incubation tray.	Avoid cross-contamination.
b)	Carefully aspirate the serum dilution from the individual wells.	With automated processing, follow the directions of the device manufacturer for this step.
c)	Pipette **2 ml** ready-to-use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then aspirate off the wash buffer A.	
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 the plastic lid and place on the shaker.
6	Wash See point 8.4	Carry out wash steps a total of three times (see 8.4a–8.4c).
7	Substrate reaction Add **1.5 ml substrate solution** and incubate for **8 minutes** with gentle shaking.	
8	Stopping the reaction Remove the substrate solution. Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips Dry the strips before the evaluation for **2 hours** between 2 layers of absorbent paper.	Carefully remove the strips from the water with a pair of plastic forceps. Store the strips protected from light.
Caution! **Incubation solutions must not be carried over to other wells. Avoid splashing, particularly when opening and closing the cover.**		

9 Results

Caution:
Do not use the automated interpretation without noting the information described below about the interpretation.

9.1 Validation – Quality Control

The test can only be evaluated when the following criteria are satisfied:
1. Reaction control band (upper line) is clearly stained, dark band detectable.
2. Antibody class (second band): the IgG or IgM conjugate control band must be clearly stained.
3. Cut-off control (third band): weak but visible staining.

9.2 Evaluation

The test strips can be evaluated visually or with a computer using the *recom*Scan test strip analysis software. The *recom*Scan software is intended to help with test strip interpretation. Additional information and corresponding instructions for computer-aided analysis are available from MIKROGEN upon request. The following instruction refers to the visual analysis.

9.2.1 *Evaluation of band intensity*

1. Note the date and batch number along with the antibody class that was detected in the attached evaluation form.
2. Enter the sample identification numbers in the evaluation form.
3. Now adhere the associated test strip with a glue stick into the corresponding field in the evaluation form. Align the test strip with the reaction control band at the indicated marker line. Then adhere the test strip to the left of the marking line using transparent adhesive tape (do not stick over the reaction control band!). Adhering the entire test strip with glue stick or adhesive tape can lead to changes in the staining.
4. Now identify the bands for the developed test strips using the printed control strip from the evaluation form and enter these into the evaluation form. Carry out the evaluation of the intensity of the emergent bands separately for the relevant immunoglobulin classes using Table 1.

Table 1: Evaluation of the band intensity relative to the cut-off band

Colour intensity of the bands	Evaluation
No reaction	-
Very low intensity (less than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
Strong intensity (stronger than the cut-off band)	++
Very strong intensity	+++

9.3 Interpretation of the Test Results

1. The serological evaluation of a Dengue, Chikungunya and/or Zika virus infection should always be carried out in the context of the results of the IgG and IgM tests, the time of the infection (or of the sample collection after the onset of clinical symptoms) and the whereabouts (endemic / non-endemic region) in the previous 6 weeks.
2. For primary flavivirus infections minimal cross-reactions between the genetically related flaviviruses are reported.
   For secondary flavivirus infections sera from patients who have previously been infected with other flaviviruses (Yellow Fever virus (YFV), West Nile virus (WNV), tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV), Usutu virus)) can cross-react in serological assays. In this context, the serological results should be interpreted on the basis of an expert assessment. A single determination from the acute phase should be considered a preliminary result. It is recommended to test a second sample 1 to 2 weeks after the initial sampling. For secondary flavivirus infections, it must be considered that the IgM response can be low or completely absent. At the same time, significant IgG increases for the previous flavivirus infection are possible with parallel or delayed (IgM)/IgG seroconversion for the secondary flavivirus infection.
3. Differentiating between Dengue and Zika with the *recom*Line Tropical Fever is possible using the specific NS1 or Equad antigen reactivities and is done in two steps.
   If DENV and/or ZIKV NS1 is positive, differentiation is exclusively made via the NS1 reactivities by directly comparing the NS1 band intensities (see Table 3). If the positive DENV NS1 band is stained considerably stronger than the ZIKV NS1 band, the interpretation is presumptive Dengue and vice versa. Equally strong DENV and ZIKV NS1 reactivities are typical for secondary flavivirus infections with DENV and/or ZIKV. In this constellation, clear differentiation is not possible. In this case we recommend testing a follow-up sample.
   If DENV and ZIKV NS1 reactivities are both absent or below cut-off, positive bands for DENV and/or ZIKV Equad are used for differentiation. If the positive DENV Equad band is stained considerably stronger than the ZIKV Equad band, the interpretation is presumptive Dengue and vice versa. Equally strong band intensities do not allow specification. Again, we recommend in this case examining a follow-up sample.
4. For both IgG and IgM antibody classes, the same interpretation criteria apply. The criteria for the test interpretation can be found in Tables 2–4.

Table 2: Test interpretation for Flavivirus

At least one virus-specific antigen band (DENV NS1 and/or DENV Equad and/or ZIKV NS1 and/or ZIKV Equad) reacts with the same (+) or stronger intensity than the cut-off band.	Flavivirus positive
No virus-specific antigen bands for DENV and ZIKV (-) or bands with weaker (+/-) intensity than the cut-off band.	Dengue / Zika negative

Table 3: Test interpretation for Dengue and Zika virus differentiation

IgG and/or IgM NS1 reactivity	**IgG and/or IgM NS1 antigen pattern**	**Interpretation**
NS1 positive: DENV NS1 AND/OR ZIKV NS1 bands react with the same (+) or stronger intensity as the cut-off band.	DENV NS1 band is stained with considerably greater intensity than the ZIKV NS1 band.	Flavivirus positive, presumptive Dengue
	DENV NS1 AND ZIKV NS1 bands are stained with equal intensity without a clearly visible difference.	Flavivirus positive
	ZIKV NS1 band is stained with considerably stronger intensity than the DENV NS1 band.	Flavivirus positive, presumptive Zika
IgG and/or IgM NS1 reactivity	**IgG and/or IgM Equad antigen pattern**	**Interpretation**
NS1 negative: DENV NS1 AND ZIKV NS1: No bands (-) or bands with weaker (+/-) intensity than the cut-off band.	DENV Equad band is stained with the same (+) or stronger intensity than the cut-off band and is stained with considerably stronger intensity than the ZIKV Equad band.	Flavivirus positive, presumptive Dengue
	DENV Equad AND/OR ZIKV Equad bands react with the same (+) or stronger intensity than the cut-off band and are stained with equal intensity without a clearly visible difference.	Flavivirus positive
	ZIKV Equad band is stained with the same (+) or stronger intensity than the cut-off band and is stained with considerably stronger intensity than the DENV Equad band.	Flavivirus positive, presumptive Zika

Table 4: Test interpretation for Chikungunya virus

The CHIKV VLP antigen band reacts with the same (+) or stronger intensity as the cut-off band.	Chikungunya positive
No CHIKV VLP antigen band (-) or band with weaker intensity (+/-) than the cut-off band.	Chikungunya negative

10 Limitations and Restrictions of the Method

- Serological test results must always be considered in the context of other medical assessments of the patient. The therapeutic consequences of the serological finding must be related to the clinical data.
- When interpreting the test results, it is important to consider possible cross-reactions. Dengue and Zika viruses are members of the flavivirus family. The literature describes cross-reacting antibodies against partial antigens that are common to the flavivirus family.
- A negative *recom*Line Tropical Fever test result cannot rule out an infection with Dengue, Chikungunya and/or Zika viruses. Particularly in an early stage of infection (< 7 days after the onset of symptoms), antibodies might not present or are not present in detectable quantities. With existing clinical suspicion of an infection with Dengue, Chikungunya and/or Zika viruses and negative serological results, further sampling and testing should be carried out again after one to two weeks.
- A positive result in the IgG and/or IgM does not mean that an active disease is present in every case.
- Malaria may lead to polyclonal activation of B lymphocytes. This can lead to a non-specific IgM reactivity in the *recom*Line Tropical Fever IgM. It is recommended, where applicable, to carry out further tests for differential diagnosis to rule out malaria.
- Dark test strips: Some patient samples can produce a dark generalised or patterned staining over the entire nitrocellulose strip. There are a number of different factors associated with the patient serum that are responsible for this. Evaluating these strips is generally only possible with limitations. Thus, ‘inverse’ bands (white

bands on a dark background) must be rated negatively. The corresponding serum should be checked using another serological method.

11 Performance Characteristics

11.1 Diagnostic Sensitivity

The diagnostic sensitivity for DENV and ZIKV (see Table 5) was determined using predefined positive samples from regions in which Dengue is endemic and not endemic.

Table 5: Diagnostic sensitivity for DENV and ZIKV IgG and IgM

***recom*Line Tropical Fever**	**Predefined positive samples**			
	DENV		**ZIKV**	
	IgG (n=68)	**IgM (n=13)**	**IgG (n=26)**	**IgM (n=18)**
Flavivirus positive	68	13	26	18
DENV/ZIKV negative	0	0	0	0
Diagnostic sensitivity	**100%**	**100%**	**100%**	**100%**

Additionally, predefined positive samples from regions in which Dengue is endemic and not endemic were investigated in terms of the differentiation of DENV and ZIKV (see Table 6).

Table 6: Differentiation of DENV and ZIKV IgG and IgM in different sample collectives

Collectives tested flavivirus-positive with *recom*Line Tropical Fever	**Flavivirus differentiation**					
	Presumptive Dengue		**Presumptive Zika**		**No differentiation**	
	IgG	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**
Dengue, endemic regions (positive, IgG=38, IgM=31)	71%	90.3%	0%	0%	29%	9.7%
Dengue, non-endemic regions (positive, IgG=10, IgM=6)	100%	100%	0%	0%	0%	0%
Zika, endemic regions (positive, IgG=4, IgM=9)	0%	0%	25%	100%	75%	0%
Zika, non-endemic regions (positive, IgG=53, IgM=15)	0%	0%	100%	100%	0%	0%

The diagnostic sensitivity for CHIKV (see Table 7) was determined using predefined positive samples from regions in which Dengue is endemic and not endemic.

Table 7: Diagnostic sensitivity for CHIKV IgG and IgM

***recom*Line Tropical Fever**	**Defined positive CHIKV samples**	
	IgG (n=70)	**IgM (n=20)**
CHIKV positive	70	20
CHIKV negative	0	0
Diagnostic sensitivity	**100%**	**100%**

11.2 Diagnostic Specificity

The diagnostic sensitivity was determined using 100 blood donors from Germany (see Table 8).

Table 8: Diagnostic specificity of the *recom*Line Tropical Fever IgG, IgM

***recom*Line Tropical Fever**	**Blood donors from Germany (n=100)**					
	DENV		**CHIKV**		**ZIKV**	
	IgG	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**
Negative	100	100	100	100	100	100
Positive	0	0	0	0	0	0
Diagnostic specificity	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Potential cross-reactions to other related flaviviruses (apart from DENV and ZIKV) as well as malaria-positive samples and pregnant women were investigated (see Tables 9 and 10).

Table 9: Testing of potentially cross-reactive samples, positive for flaviviruses or malaria, and samples from pregnant women with the *recom*Line Tropical Fever IgG, IgM and presentation of the results for DENV and ZIKV

***recom*Line Tropical Fever**	**Flavivirus positive**					
	Presumptive Dengue		**Presumptive Zika**		**No differentiation**	
	IgG	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**
Flaviviruses*						
YFV (n=9)	1**	0	0	0	0	0
TBEV (n=12)	1**	0	0	0	0	0
JEV (n=7)	0	0	0	0	0	0
WNV (n=1)	0	0	0	0	0	0
Usutu virus (n=1)	0	0	0	0	0	0
Specificity	**93.3%**	**100%**	**100%**	**100%**	**100%**	**100%**
Pregnant women (n=50)	1**	1	0	0	0	0
Specificity	**98%**	**98%**	**100%**	**100%**	**100%**	**100%**
Malaria (n=20)	-	1	-	0	-	1
Specificity	**-**	**95%**	**-**	**100%**	**-**	**95%**

*The flavivirus panel includes samples from persons vaccinated against YFV, TBEV, and JEV and samples that have tested positive for *anti*-JEV (IgG, IgM), *anti*-WNV (IgG, IgM) and *anti*-Usutu (IgG).
**Sample also tested positive with two other commercial DENV antibody detection tests.

Table 10: Testing of potentially cross-reactive samples, positive for flaviviruses or malaria, and samples from pregnant women with the *recom*Line Tropical Fever IgG, IgM and presentation of the results for CHIKV

***recom*Line Tropical Fever**	**CHIKV positive**	
	IgG	**IgM**
Flaviviruses*		
YFV (n=9)	0	0
TBEV (n=12)	0	0
JEV (n=7)	0	0
WNV (n=1)	0	0
Usutu virus (n=1)	0	0
Specificity	**100%**	**100%**
Pregnant women (n=50)	0	0
Specificity	**100%**	**100%**
Malaria positive (n=20)	-	3
Specificity	**-**	**85%**

*The flavivirus panel includes samples from persons vaccinated against YFV, TBEV, and JEV and samples that have tested positive for *anti*-JEV (IgG, IgM), *anti*-WNV (IgG, IgM) and *anti*-Usutu (IgG).

11.3 Analytical Specificity

The analytical specificity is defined as the ability 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) Interference: Control studies of potentially interfering factors have shown that the test performance is not influenced by anticoagulants (CPD, sodium citrate, EDTA, heparin), haemolysis (≤ 500 mg/dL haemoglobin), bilirubinaemia (≤ 20 mg/dL conj./unconj. bilirubin) or lipaemia (≤ 1000 mg/dL).
b) Cross-reactions: Potential interference of antibodies against other flaviviruses (YFV, WNV, TBEV, JEV, Usutu virus) were investigated in control studies (see Tables 9 and 10). Although very few cross-reactions were detected, they cannot be completely ruled out. Conditions that can be attributed to atypical immune system activity (rheumatoid factor, pregnancy, EBV, CMV, malaria, anti-nuclear autoantibodies) were also tested. Malaria, EBV, CMV and anti-nuclear autoantibodies can lead to a non-specific IgM reactivity in the *recom*Line Tropical Fever IgM. A specificity of 98% (Flavivirus detection) and 100% (Chikungunya virus detection) was demonstrated for pregnant women in control studies. No cross-reactions were detected for samples positive for rheumatoid factor.

12 Literature

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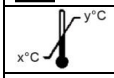
13 Explanation of Symbols

	Content is sufficient for <n> formulations Number of formulations
WASHBUF A 10 X	Wash buffer A (10× concentrate)
SUBS TMB	Chromogenic substrate tetramethylbenzidine
MILKPOW	Skimmed milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
CONJ IgM	Anti-human IgM conjugate
EVALFORM	Evaluation form
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
IVD	In vitro diagnostic agent
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 Data

***recom*Line Tropical Fever IgG** ***recom*Line Tropical Fever IgM**	Article no. **7872 (7870)** Article no. **7873 (7879)**
Instructions for use Valid from	GARLTF003EN 2023-03

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GARLTF003