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Electronic Instruction For Use: version



**911MX\_4**

## ORG 911MX Anti-Borrelia IgM Abs.

### INTENDED PURPOSE

Anti-Borrelia IgM Abs. for Alegria® is an ELISA-based test system for the quantitative measurement of IgM class antibodies against *Borrelia burgdorferi sensu lato* in human serum or plasma samples. This product is intended for professional in vitro diagnostic use only. The Alegria® Anti-Borrelia IgM Abs. offers test strips with integrated rheumatoid factor (RF) absorbent.

The test detects antibodies against the primary causes of Lyme disease in Asia, Europe, and the USA and is used to confirm the diagnosis in cases of suspected disease. In later stages of the infection, detection of *Borrelia*-specific antibodies is essential to confirm clinical suspicion of Lyme disease.

### SYMBOLS USED

	In vitro diagnostic medical device
	Manufacturer
	Catalogue number
	Sufficient for ... determinations
	Batch code
	Use by
	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
	CE marked according to 98/79/EC
	Electronic Instruction For Use: version

	Alegria® Test Strips
	Wash Buffer
	System Fluid
	Ready to use

### SUMMARY AND EXPLANATION OF THE TEST

*Borrelia burgdorferi sensu lato* (s. l.) are the main causative organisms of Lyme disease in Asia, Europe, and the USA. Strains present in Europe are *Borrelia burgdorferi sensu lato* gathering B. afzelii, B. garinii, B. burgdorferi sensu stricto, B. spielmanii, B. bavariensis being pathogenic species. Potentially pathogenic species are B. valaisiana and B. lusitaniae and only in the USA B. burgdorferi sensu stricto.

Transmission to humans is caused by ticks of the genus Ixodes. Erythema migrans is the first clinical sign in approximately 60% of cases, occurring several days to weeks after infection; possibly attended with mild flu-like symptoms. Spreading of the pathogen via the blood stream or the lymphatic system may be followed by several complications in later stages of the disease, especially neuroborreliosis (ca. 20%) and Lyme arthritis (ca. 10%). In untreated patients the disease may take a severe course leading to persistent chronic infection. On the other hand, infection with B. burgdorferi can proceed without any clinical symptom. Diagnosis of Lyme disease should be based on careful evaluation of medical history.

Determination of antibodies also plays a crucial role. In many cases antibodies are not yet detectable in the early phase of the disease (erythema migrans). In later stages detection of *Borrelia*-specific antibodies is essential to confirm clinical suspicion of Lyme disease. Detection of intrathecal antibody production is conclusive for the diagnosis of neuroborreliosis. In Lyme arthritis extremely high titres of IgG antibodies are found, with IgM antibodies being completely absent. Progression of Lyme disease can be prevented by administration of antibiotics.

### PRINCIPLE OF THE TEST

Highly purified recombinant antigen (VlsE, OspC, Flagellin intern) of *B. burgdorferi* s.s., *B. afzelii* and *B. garinii* is bound to reaction wells.

The Alegria® assay features barcoded 8-well-microstrips, called Alegria® Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria® Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and a test specific control. Furthermore each strip has two antigen-coated wells which serve as reaction wells for one control and one patient sample.

The determination is based on an indirect enzyme linked immune reaction with the following steps: Antibodies present in positive samples bind to the antigen coated on the surface of the two reaction wells forming an antibody antigen complex. After incubation, a first washing step removes unbound and unspecific bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolysis and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm.

The Alegria® Test Strip is based on the proprietary SMC®-Technology (Sensotronic Memorized Calibration); information about the assay, analysis and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria® Test Strip.

The Alegria® Test Strip can be used with the diagnostic instrument Alegria® - a fully automated Random Access Analyser. By means of SMC®-Technology data encoded on the barcode are transferred from the Alegria® Test Strip to the instrument and the assay is automatically processed and evaluated. The instrument reads the date of expiry and rejects further processing if the Alegria® Test Strip is out of date.

Rheumatoid factors (RF) may influence the result of an IgM determination in serology of infectious diseases. This is avoided by using RF absorbent. The Alegria® assay offers test strips with integrated rheumatoid factor (RF) absorbent. Absorption occurs during sample processing in the Alegria® analyser. No sample preparation or predilution is required.

### WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- System fluid contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous
- Enzyme conjugate, control and sample buffer contain ProClin 300 0.05% as preservative.

This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex.

Wear protective glasses. Used according to intended use no dangerous reactions known.

- Conditions to avoid: Since substrate solution is light-sensitive. Store Alegria® strips in the dark.

- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

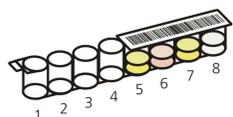
### CONTENTS OF THE KIT

▽ 24 ORG 911MX

Sufficient for 24 determinations

ALEGRIA TEST STRIPS

Alegria® Test Strips are modules of 8 wells each composed of:



Wells 1 + 2: empty and not coated (wells for the sample dilution)

Wells 3 + 4: coated with antigen (reaction wells)

Well 5: Control; yellow; containing test specific antibodies, PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.

Well 6: Enzyme Conjugate; light red; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%.

Well 7: Sample Buffer: yellow; containing PBS, BSA, detergent, RF absorbent and preservative sodium azide 0.09% and ProClin 300 0.05%.

Well 8: TMB Substrate: clear; containing 3,3', 5,5'-Tetramethylbenzidin.

Code on barcode: **Borrel IgM Ab** on printout: **Borr-MX**

WASH

1x 20 ml

Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.

SYSTEM FLUID

1x 2.5 ml

System Fluid, contains acid; 1000 x concentrate

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Alegria® Instruction for Use: Alegria® Mini-DVD

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Certificate of Analysis

### STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store Alegria® Test Strips sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 15 months from day of production.  
Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and System Fluid are stable for at least 30 days when stored at 2-8°C.  
Once transferred to the reagent container we recommend consumption on the same day.

### MATERIALS REQUIRED

- Vortex mixer
- Pipettes for 10 µl
- Measuring cylinder for 1000 ml and 2500 ml
- Distilled or deionized water

### SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.

- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

### PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- All materials must be at room temperature (20-28°C) prior to use.
- To avoid carryover or contamination, change the pipette tip between samples.

### PREPARATION OF REAGENTS

WASH

Dilute the content of the Wash Buffer concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Transfer the diluted Wash Buffer into the instrument reagent container. If only one Alegria run is to be performed on one day we recommend transferring only 500 ml diluted Wash Buffer.

SYSTEM FLUID

Dilute the content of the System Fluid concentrate (1000x) with distilled or deionized water to a final volume of 2500 ml prior to use. Transfer the diluted System Fluid into the instrument reagent container.

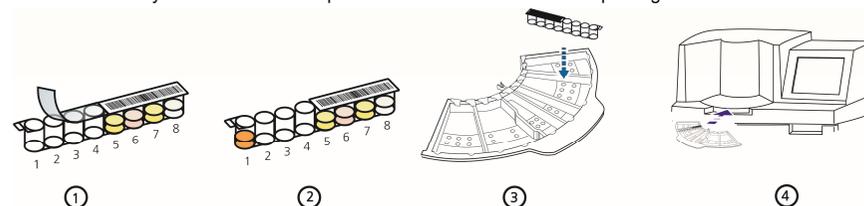
ALEGRIA TEST STRIPS

Take the required number of Alegria® Test Strips out of the clip bag and let them reach room temperature (20-28°C). Do not remove foil covering the empty wells until you are ready to start the assay.

### TEST PROCEDURE

Alegria® Test Strips with SMC® technology are used with the diagnostic instrument Alegria®. Detailed information about operating the instrument can be taken from the Instrument User Manual.

- (1) Remove the foil from the empty wells 1 to 4 of the Alegria® Test Strip.  
**Do not remove foil with printed barcode, covering wells 5 to 8.**
- (2) Pipette 10 µl undiluted sample at the bottom of well 1.
- (3) Insert the strip into the SysTray.
- (4) Place loaded SysTrays into the correct position in the Alegria® instrument and start run. All further steps will be done automatically. The test run is completed when the instrument starts printing the results.



### CALIBRATION

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

### CALCULATION OF RESULTS

By means of SMC® Technology (Sensotronic Memorized Calibration), all test data are transferred to the system through individual barcodes on the Alegria® Test Strip. Calculation and interpretation of results will be performed automatically.

### PERFORMANCE CHARACTERISTICS

#### Measuring range

The calculation range of this Alegria® assay is: 0 - 200 U/ml

## Expected values

The cut-off of this Alegria<sup>®</sup> assay is: 25 U/ml

## Interpretation of results

Negative: < 20 U/ml  
Borderline: 20 - 25 U/ml  
Positive: > 25 U/ml

## Limitations of the procedure

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above reference ranges should be regarded as guidelines only. It is recommended that each laboratory establishes its own normal and pathological ranges for antibodies in patient samples.

A negative result does not rule out an infection, since the serum can be sampled too early for the antibodies to be detectable. A positive result does not rule out the presence of another infectious pathogen as the cause of disease.

## Linearity

Not applicable, this is a nonlinear assay, because sample buffer contains RF-absorb.

## Detection limit

The lowest amount of detectable antibody is: 5.5 U/ml

## Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
	Mean [U/ml]	% CV
	7.7	4.1
1	23.6	2.3
2	103.3	3.8

Inter-Assay		
	Mean [U/ml]	% CV
	8.1	4.0
1	23.3	4.0
2	105.7	4.9

## Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

No interference has been observed in bacterial or viral infections with *T. pallidum*, *Chlamydia sp.*, *Yersinia sp.*, Parovirus B19, or acute EBV infection. Nor have any interfering effects been observed in rheumatoid diseases associated with elevated titers of rheumatoid factors or antinuclear antibodies.

In samples from patients with acute EBV infection a higher rate of seroprevalence was found possibly due to polyclonal stimulation of B-lymphocytes in these patients. Interference by rheumatoid factors is avoided by the integration of RF absorb in the dilution buffer of the Alegria<sup>®</sup> test strips.

## Seroprevalence

Analysis of 100 healthy blood donors from Germany showed 3 positive results equivalent to 3 % seroprevalence.

## Study results

### Comparative Study

224 samples were tested against a comparative method. The study population contained samples from two clinical laboratories. Borderline results were not validated.

In the reference method 40 samples were found positive. Based on reference method 32 of 40 samples were also positive in the Alegria<sup>®</sup> Anti-Borrelia IgM Abs. assay, thus yielding a sensitivity of 80.0%.

In the reference method 184 samples were found negative. Based on reference method 181 of 184 samples were negative in the Alegria<sup>®</sup> Anti-Borrelia IgM Abs. assay, thus yielding a specificity of 98.4%.

213 of 224 samples showed matching results which concludes in 95.1 % agreement.

Furthermore 10 samples from clinically defined patients as having Borrelia infection were analysed. Nine of ten were analysed correctly in the Anti-Borrelia IgM Abs. assay.

		Comparative Method		
		Pos	Neg	
ORG 911MX	Pos	32	3 *	
Anti-Borrelia IgM Abs.	Neg	8 °	181	
		40	184	224
Sensitivity:	80.0	%		
Specificity:	98.4	%		
Overall agreement:	95.1	%		

\* two samples positive in Western Blot

° six samples negative, two samples borderline in Western Blot

## Clinical Study

84 clinical samples were analysed with the Alegria assay.

Stage 1: 21 serum samples from patients with clinical suspicion of stage 1 Lyme borreliosis after tick-bite (11 cases with erythema migrans)

Stage 2: 20 serum samples from patients with neuroborreliosis (acute and past)

Stage 3: 43 serum samples from patients with arthritis

Samples		Anti-Borrelia IgM				Anti-Borrelia IgG				IgM + IgG		
Disease	Clinics	number	positive	% positive	borderline	% borderline	positive	% positive	borderline	% borderline	reactive*	% reactive
Stage 1	Borreliosis after tick-bite	21	15	71.4%	2	9.5%	10	47.6%	3	14.3%	18	85.7%
Stage 2	Neuroborreliosis	20	6	30.0%	3	15.0%	14	70.0%	1	5.0%	15**	75.0%
Stage 3	Arthritis	43	9	20.9%	1	2.3%	39	90.7%	4	9.3%	43	100.0%

\* reactive: borderline or positive

\*\* 5 samples being negative with the serological assays showed a positive CSF reaction

## REFERENCES

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- Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 2007; 49(1):13-21.
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treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2006; 43(9):1089-134.

Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 911MX\_IFU\_EN\_QM122190\_2017-08-30\_3* Reason for revision: *Introduction electronic IFU on homepage*

