ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49-51
55129 Mainz - Germany
Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com

Electronic Instruction For Use: version

911G_4

ORG 911G Anti-Borrelia IgG

INTENDED PURPOSE

Anti-Borrelia IgG for Alegria® is an ELISA based test system intended for the quantitative measurement of IgG 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 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

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 24	Sufficient for ... determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
911G_4	Electronic Instruction For Use: version

ALEGRIA TEST STRIPS	Alegria® Test Strips
WASH	Wash Buffer
SYSTEM FLUID	System Fluid
RTU	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 senso 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, DbpA, OspC, p83/p100) 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 hydrolisation 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.

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, classifiaction 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:

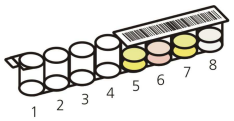
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste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

Σ 24	ORG 911G	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 IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%. Well 7: Sample Buffer: yellow; containing PBS, BSA, detergent, 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 IgG*** on printout: ***Borr-G***
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
	1	Alegria® Instruction for Use: Alegria® Mini-DVD
	1	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 dessicated in the clip bag provided.
- Shelf life of the unopended 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.

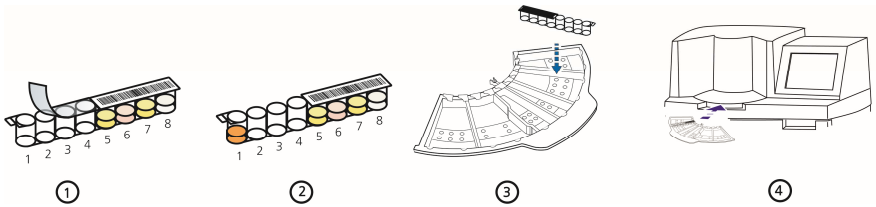
ALEGRIA TEST STRIPS

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® 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

Three patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay. Activity for each dilution was calculated by means of SMC® Technology.

Sample	Dilution	Observed	Expected	O/E
		U/ml	U/ml	[%]
1	1:100	159.8	159.8	100
	1:200	80.9	79.9	101
	1:400	38.6	40.0	97
	1:800	19.7	20.0	99
2	1:100	115.9	115.9	100
	1:200	56.9	58.0	98
	1:400	26.5	29.0	92
	1:800	13.8	14.5	95
3	1:100	98.7	98.7	100
	1:200	54.0	49.4	109
	1:400	25.8	24.7	105
	1:800	13.5	12.3	110

Detection limit

The lowest amount of detectable antibody is: 4.5 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		
Sample	Mean [U/ml]	% CV
1	6.1	5.4
2	24.2	6.3
3	155.0	5.4

Inter-Assay		
Sample	Mean [U/ml]	% CV
1	8.8	5.7
2	25.8	6.4
3	150.7	5.4

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 rheumatic diseases associated with elevated titers of rheumatoid factors or antinuclear antibodies.

Seroprevalence

Analysis of 100 healthy blood donors from Germany showed 9 positive results equivalent to 9% seroprevalence.

Study results

Comparative Study

194 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 56 samples were found positive. Based on reference method 51 of 56 samples were also positive in the Alegria® Anti-Borrelia IgG assay, thus yielding a sensitivity of 91.1%.
In the reference method 138 samples were found negative. Based on reference method 133 of 138 samples were negative in the Alegria® Anti-Borrelia IgG assay, thus yielding a specificity of 96.4%.
184 of 194 samples showed matching results which concludes in 94.8 % agreement.
Furthermore 10 samples from clinically defined patients as having Borrelia infection were analysed. These were analysed correctly in the Anti-Borrelia IgG assay (9 samples positive and 1 sample borderline).

		Comparative Method Pos	Comparative Method Neg	
ORG 911G	Pos	51	5 *	
Anti-Borrelia IgG	Neg	5 °	133	
		56	138	194
Sensitivity:	91.1 %			
Specificity:	96.4 %			
Overall agreement:	94.8 %			

* two samples positive in Western Blot
° all 5 samples negative 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

1. Ang CW, Notermans DW, Hommes M, Simoons-Smit AM, Herremans T. Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 2011; 30(8):1027-32.
2. Bratton RL, Whiteside JW, Hovan MJ, Engle RL, Edwards FD. Diagnosis and treatment of Lyme disease. Mayo Clin Proc 2008; 83(5):566-71.

3. Nau R, Christen HJ, Eiffert H. Lyme disease--current state of knowledge. Dtsch Arztebl Int 2009; 106(5):72-81.
4. Rizzoli A, Hauffe H, Carpi G, Vourc HG, Neteler M, Rosa R. Lyme borreliosis in Europe. Euro Surveill 2011; 16 (27):pii=19906.
5. Schulte-Spechtel U, Fingerle V, Goettner G, Rogge S, Wilske B. Molecular analysis of decorin-binding protein A (DbpA) reveals five major groups among European Borrelia burgdorferi sensu lato strains with impact for the development of serological assays and indicates lateral gene transfer of the dbpA gene. Int J Med Microbiol 2006; 296 Suppl 40:250-66.
6. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 2007; 49(1):13-21.
7. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS et al. The clinical assessment, 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 911G_IFU_EN_QM122189_2017-08-30_3* Reason for revision: *Introduction electronic IFU on homepage*