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Electronic Instruction For Use: version

914GL_4

ORG 914GL Anti-VZV IgG Liquor

INTENDED PURPOSE

Anti-VZV IgG Liquor for Alegria® is an ELISA based test system intended for the comparative quantitative measurement of IgG class antibodies against varicella zoster virus in human serum or plasma samples and in cerebrospinal fluid (CSF). This assay is for the detection of IgG antibody synthesis in the central nervous system. The assay is not suitable for detection of anti-VZV IgG in serum or plasma samples only. This product is intended for professional in vitro diagnostic use only.

An elevated VZV antibody index characterises varicella zoster ganglionitis, often accompanied by facial nerve paresis. VZV-antibody detection helps to distinguish neuroborreliosis. In chronic diseases with CNS involvement, e. g. multiple sclerosis (MS) or Lupus with neurological manifestations, an elevated antibody index of anti-measles virus, anti-rubella virus and / or anti-varicella zoster virus can be detected in the CSF. This MRZ reaction is detected in about 90% of MS patients.

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
914GL_4	Electronic Instruction For Use: version

ALEGRIA TEST STRIPS	Alegria® Test Strips
WASH	Wash Buffer
SYSTEM FLUID	System Fluid
RTU	Ready to use

PRINCIPLE OF THE TEST

Purified VZV glycoprotein isolated from virus cell cultures 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.

A CSF and a corresponding serum sample from the same patient should be tested in the same run. To run both in parallel, two strips are needed – one for the CSF and one for the serum.

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 unspecifically 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.

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 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:

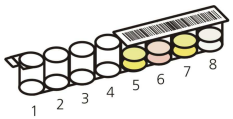
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste 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 914GL	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: ***VZV SerCSF IgG*** on printout: ***VZV-L-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 9 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, pipettes for variable volumes 10-100 µl and 100-1000µl
- Measuring cylinder for 1000 ml and 2500 ml
- Distilled or deionized water

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.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Blood and CSF sample should be taken from a patient at the same day.
- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Specimens should be clear and non-hemolyzed.
- 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 specimens. This may result in variable loss of antibody activity.
- Testing of heat-inactivated specimens is not recommended.

SPECIMEN PREPARATION

- CSF and serum or plasma samples should have a similar protein concentration for this assay.
- Since CSF is much lower concentrated than serum or plasma, pre-dilution of serum samples is needed.
- In order to obtain values within the measuring range pre-dilution of serum/plasma samples may be different for each parameter depending on seroprevalence.
  Even CSF samples may be pre-diluted or used in lower volume, if a high antibody concentration is expected.

- It is recommended to pre-dilute a serum or plasma sample with ready to use Wash Buffer.
  Specimen preparation is dependent on the sample panel and may differ from laboratory to laboratory.
  Empirical values for pre-dilutions (serum/plasma) and volumes (CSF):

		pre-dilution	serum/plasma + Wash Buffer	CSF
ORG 901GL	EBV (VCA) IgG	1:8 1:16	10 µl + 70 µl 10 µl + 150 µl	30 µl
ORG 905GL	HSV-1/2 IgG	1:8 1:16	10 µl + 70 µl 10 µl + 150 µl	30 µl
ORG 909GL	Measles IgG	1:8 1:16	10 µl + 70 µl 10 µl + 150 µl	30 µl
ORG 911GL	Borrelia IgG	1:4	20 µl + 60 µl	60 µl
ORG 911ML	Borrelia IgM	1:4	20 µl + 60 µl	60 µl
ORG 914GL	VZV IgG	1:8 1:16	10 µl + 70 µl 10 µl + 150 µl	30 µl
ORG 919GL	Rubella IgG	1:8 1:16	10 µl + 70 µl 10 µl + 150 µl	30 µl

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. Set apart 50 ml for sample dilution.
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.

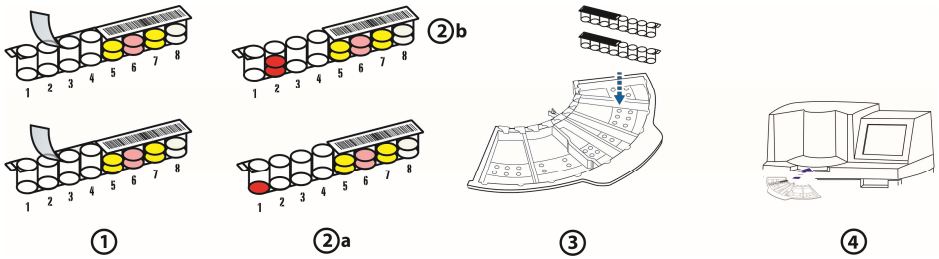
TEST PROCEDURE

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.
Use one Alegria® Test Strip for CSF and one for serum. Different processing:

(2a) Serum Strip: Pipette 10 µl pre-diluted serum or plasma at the bottom of well 1.
(2b) CSF Strip: Pipette 30 µl undiluted CSF at the bottom of well 2.

(3) Insert the strips 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.

PERFORMANCE CHARACTERISTICS

Measuring range

The calculation range of this Alegria® assay is: 10 - 200 U/ml

Detection limit

The lowest amount of detectable antibody is: 10 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in wash buffer to demonstrate the dynamic range of the assay.

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three serum samples from the results of 20 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 serum samples from the results of 2 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean	CV
	[U/ml]	[%]
1	11.3	10.6
2	36.7	13.0
3	126.1	5.0

Inter-Assay		
Sample	Mean	CV
	[U/ml]	[%]
1	10.1	12.9
2	36.8	13.9
3	130.8	10.7

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. However for practical reasons it is recommended that grossly hemolyzed or lipemic serum/plasma samples should be avoided. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine).
No interference has been observed in viral infections with HSV, EBV, Parovirus B19, measles or mumps.
Nor have any interfering effects been observed in rheumatic diseases associated with elevated titers of rheumatoid factors or antinuclear antibodies.

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 of results will be performed automatically.

CALCULATION OF ANTIBODY INDEX (AI)

The calculation of the antibody index requires the analysis of albumin and total IgG in serum and in CSF by alternative test methods that are not included in this test kit. Total IgG and albumin concentrations should be measured with methods that are commonly used in neurological laboratories.

Usually, the application of undiluted CSF and a 1:4 pre-dilution of the serum sample as recommended before will be adequate to determine the antibody concentration in the samples. Occasionally, the results may be higher than the upper limit of the measuring range (> 200 U/ml) for individual samples. These samples have to be retested at higher dilutions for a correct calculation of the antibody concentration. In case of CSF samples lower volumes of undiluted CSF can be pipetted into well 2 or a pre-dilution can be done with ready to use Wash Buffer.
Possible pre-dilutions and pipetted volumes as well as the resulting dilution factors are shown in the dilution table below:

sample	pre-dilution	sample + diluent	well 1: µl	well 2: µl	dilution factor
serum	1:4	20 µl + 60 µl	10 µl	---	381
serum	1:8	10 µl + 70 µl	10 µl	---	762
serum	1:16	10 µl + 150 µl	10 µl	---	1524
serum	1:32	10 µl + 310 µl	10 µl	---	3048
serum	1:64	10 µl + 630 µl	10 µl	---	6096
serum	1:128	10 µl + 1270 µl	10 µl	---	12192
sample	pre-dilution	sample + diluent	well 1: µl	well 2: µl	dilution factor
CSF	---	---	---	60 µl	4
CSF	---	---	---	30 µl	7
CSF	---	---	---	15 µl	13
CSF	1:2	50 µl + 50 µl	---	60 µl	8
CSF	1:4	20 µl + 60 µl	---	60 µl	16
CSF	1:8	10 µl + 70 µl	---	60 µl	32
CSF	1:16	10 µl + 150 µl	---	60 µl	64
CSF	1:32	10 µl + 310 µl	---	60 µl	128

Antibody index calculation:
Antibody index (AI) calculation will provide information about VZV specific intrathecal antibody production (i.e. antibodies produced in CSF). Antibody index ranges from 0.6 to 1.3 normally (see interpretation of results).
For AI calculation, the concentrations of anti-VZV specific IgG in serum and in cerebrospinal fluid (CSF) are needed first, corrected by dilution factors. With these, the IgG specific ratio ($Q_{IgG\ spec}$) is determined:

$Q_{IgG\ spec}$ = [$IgG_{CSF\ spec}$] / [$IgG_{serum\ spec}$]
= (U/ml_{CSF} * dilution factor) / (U/ml_{serum} * dilution factor) for dilution factors see table

Definition of AI: $\mathbf{AI = Q_{IgG\ spec} / Q_{IgG}}$ (1)

(Q_{IgG} = [IgG_{CSF}] / [IgG_{serum}] = the ratio of total IgG. Needs to be measured separately by other means!)

Clinical amendment:
The AI ratio (1) takes into consideration changes in the blood-liquor barrier function but does not correct for a large local synthesis of polyspecific IgG in the CNS, which increases Q_{IgG} and leads to falsely low AI values.

For a safer clinical diagnosis of various neurological diseases, it is therefore important to differentiate between
a) infections with a monospecific intrathecal immune reaction that indicates the causative antigen and
b) diseases, where antibodies have been synthesized as a secondary polyspecific immune reaction by an unspecific stimulation of B-cell lines.

To avoid such false-negative results with the standard AI calculation (1), H.Reiber suggested a threshold function (derived from empirical clinical data) to be used for a corrected AI calculation (with the additional demand of measuring Albumin: Q_{Alb} = [Alb_{CSF}] / [Alb_{serum}]):

$$Q_{lim}\ (IgG) = 0.93 \times \sqrt{(Q_{Alb})^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$$

Depending on that threshold, AI calculation differentiates between two cases:

$AI = Q_{IgG\ spec} / Q_{IgG}$ (if $Q_{IgG} < Q_{Lim\ (IgG)}$) (monospecific immune reaction) (2)
$AI = Q_{IgG\ spec} / Q_{Lim\ (IgG)}$ (if $Q_{IgG} > Q_{Lim\ (IgG)}$) (polyspecific immune reaction) (3)

Equation 2 is used if Q_{IgG} represents the barrier conditions referring to a predominantly blood-derived CSF protein fraction without a significant local polyspecific IgG synthesis in the CNS, i.e., if $Q_{IgG} < Q_{Lim\ (IgG)}$.
Equation 3 is used if $Q_{IgG} > Q_{Lim\ (IgG)}$.
To facilitate these calculations a computerized table can be used. ORGENTEC Diagnostika has prepared such a table under Excel and provides it to interested customers using the Alegria® Liquor assays on request.

Interpretation of results

0.6 ≤ AI < 1.3	normal range
1.3 ≤ AI < 1.5	indeterminate
AI ≥ 1.5	intrathecal synthesis of specific VZV antibody

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. In addition, every decision for therapy should be taken individually.

For serum or CSF samples showing values below the lower limit of the measuring range (documented test result < 10 U/ml), an antibody index cannot be calculated. Depending on results' constellation (e.g. elevated antibody values in CSF) a rerun with a lower dilution of the serum sample may be considered. AI values < 0.6 may indicate methodic errors. All partial results should be checked for validity.

The above reference ranges are according to the work of H. Reiber and should be regarded as guidelines only. It is recommended that each laboratory establish its own normal and pathological ranges for antibodies in patient samples.

A normal AI result does not rule out an infection. When the sample is taken early in the disease course antibody index may still be in the normal range. An elevated AI does not rule out the presence of another infectious pathogen as the cause of disease. Elevated AI values may persist for years after an infection.

Study results

		Comparative Method		
		AI > 1.5	AI < 1.5	
ORG 914GL	AI > 1.5	22	1	
	AI < 1.5	0	30	
		22	31	53

Sensitivity:	100.0	%
Specificity:	96.8	%
Overall agreement:	98.1	%

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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 914GL_IFU_EN_QM141897_2016-03-10_3* Reason for revision: *Introduction electronic IFU on homepage*