BioCLIA® Autoimmune Reagent Kit

ICA

Chemiluminescent Microparticle Immunoassay

Magnetic bead chemiluminescence immunoassay (CLIA) for quantitative determination of anti-ICA antibody in human serum

Key to Symbols Used

REF	Catalog Number		Expiration Date
IVD	For *In Vitro* Diagnostic Use	LOT	Lot Number
	Store between +2°C and +8°C		Consult Instruction for Use
	Manufacturer	EC REP	Authorized Representative in European Union
PRE BUF	Pre Buffer	Σ	Contains Sufficient for < *n* > Tests
CONJ AP G	Conjugate		Chemical Risk Warning
M STREP	Microparticle		Biological Risk Warning

BioCLIA® Autoimmune Reagent Kit, ICA

Intended Use

BioCLIA ICA assay is intended for the in vitro quantitative measurement of antibodies directed to ICA in human serum as an aid in the diagnosis of Insulin - dependent diabetes mellitus (IDDM), Type 1 in conjunction with other laboratory and clinical findings. This kit is used on the instrument of BioCLIA 1200 and BioCLIA® 6500.

Catalog Numbers

MY00144 (50 Tests/kit)

MY00195 (100 Tests/kit)

Summary and Explanation

Insulin - dependent diabetes mellitus (IDDM), Type 1, is caused by the autoimmune destruction of the beta cells of the pancreas. [1, 2] This selective autoimmune pathogenesis causes complete elimination of insulin secretion. The immunological evidence was demonstrated by the presence of specific islet cell autoantibodies in IDDM sera. [3] At least three autoantibodies have been identified against antigenic components of the islet cells in Type 1 diabetics. These autoantibodies are directed specificlly to islet cell antigenic components, glutamic acid decarboxylase and insulin. [4]

Islet Cell Autoantibodies are present in 70% of patients with a recent onset of IDDM compared with 0.1 - 0.5% of the control non-diabetic population. [5] ICA are also detected in first degree relatives of IDDM patients. These individuals comprise the segment of human population who are at a high risk of developing IDDM. Several studies reported that the ICA-positive first degree relatives of IDDM patients subsequently developed diabetes. [6] Other studies also suggested that the presence of serum ICA and IAA is an indicator of the enhanced likelihood to develop IDDM. [7] Therefore, serological detection of ICA may be a powerful tool for early diagnosis of IDDM. The significance of these autoantibodies as markers of IDDM is also illustrated by their presence in nondiabetic individuals who ultimately develop IDDM. The screening of high-risk populations, for all of the three autoantibodies (ICA, IAA and ICA) will help to either prevent or to slow down the onset of the disease. A high-risk (asymptomatic) population, positive for two or more autoantibodies, is vulnerable for developing IDDM, usually in the next 5-7 years. [8]

Principles of the Procedure

BioCLIA ICA assay is a two-step immunoassay using microparticle, enzyme-labeled chemiluminescent technology.

In the first step, the ICA antigen coated magnetic microparticle, human serum and a pre buffer are mixed in a cuvette, which allows anti-ICA antibody to bind to the surface of microparticle. Secondly, after incubation, unbound reagent and sample matrix are removed by washing, and the beads-anti-ICA antibodies immune complexes are kept with the help of a magnetic separator. Third, anti-human IgG conjugated alkaline phosphatase is added. Fourth, after incubation, excess enzyme conjugates are removed by washing and finally the bound enzyme is detected by addition of chemiluminescent substrate. The relative light unit (RLU) intensity is proportional to the amount of anti-ICA IgG. According to a certain specific IgG RLU-concentration standard curve, the RLU tested can be interpreted to anti-ICA IgG concentration in the sample expressed as IU/mL.

For quantitation of anti-ICA antibodies, the BioCLIA ICA assay utilizes a predefined lot specific main calibration curve that is uploaded into the instrument through the reagent cartridge barcode. The main calibration curve is created during manufacturing by using in-house standards that are traceable to the WHO standard material (NIBSC code: 97/550). Based on the main calibration curve, and results obtained by running two calibrators, an instrument specific working curve is created, which is used to calculate International Units (IU)/mL from the RLU obtained for each sample.

Specimen Collection

The appropriate specimen types for BioCLIA ICA reagents are human serum. Cloudy samples should be purified by low-speed centrifugation. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increasing clotting time.

Freshly collected specimens stored in refrigerator (2-8 °C) are valid for testing within 8 days. The unopened specimen should reach to room temperature (18-25°C) before testing, and should not be stored in this temperature condition more than 2 days. All opened/on board specimens should be tested within 10 hours. Three freeze-thaw cycles for specimens do not affect the testing results.

Warnings and Precautions

1. This assay is only for use in the BioCLIA® 1200 and BioCLIA® 6500.

2. This product requires the handling of calibrators, controls and human specimens which contain human sourced materials. It is recommended that all human sourced materials are considered to be potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. 9 Biosafety Level 2 10 or other appropriate biosafety practices 11, 12 should be used for materials that contain or possibly contain infectious agents.

3. Liquid waste and solid waste are temporarily

stored at separate containers. Waste management should also be handled in accordance with standards mentioned in chapter Warnings and Precautions point No. 2

4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing wastes.

5. Once opened, this reagent cartridge must be stored in the instrument's reagent carousel. Avoid spilling the reagents when the reagent cartridge is placed into the instrument.

6. Chemical contamination of the reagents can resulting from improper cleaning or rinsing of the instrument. Residues from common laboratory chemicals such as formalin, bleach, ethanol, or detergent can cause interference in the assay. Be sure to follow the recommended cleaning procedure of the instrument as outlined in the BioCLIA® 1200 and BioCLIA® 6500 operator.

7. Precautions: 

Proclin 300 added in the kit reagents (ICA Microparticle, Conjugate, Pre Buffer) at concentration between 0.0015% - 0.6%.

Storage Instructions

The kit is stable until the expiration date if it is stored and handled as directed. Routine store the kit in refrigerator (2-8 °C). Vial opened reagents or onboard reagents can be used up to 28 running days(2-8 °C). The lyophilized ICA antigen coated microparticles is stable during 28 days after reconstitution when stored between 2- 8 °C. The BioCLIA® 1200 and BioCLIA® 6500 software monitors the expiration of the reagent cartridge. The system will not accept expired reagents. Three freeze-thaw cycles before testing has no effect on the kit reagents.

Materials Supplied

Components are matched in sets. Labels supplied within the kit will be needed for the assay testing.

- **ICA Microparticle** 1 or 2 bottle(s) (1 X sq 4 mL) lyophilized ICA antigen coated microparticles. Dilute with 4ml distilled water per bottle before using.

M	ICA	RCNS	H_2O	DIST

Preservatives: 0.0015% < Proclin 300 < 0.6%.

- **Conjugate** One bottle (6.75/13.5 mL) AP labeled Anti-human IgG in 0.05 M MES (pH6.0) Buffer.

Preservatives: 0.0015% < Proclin 300 < 0.6%.

CONJ	AP	G

- **Pre Buffer** One bottle (2.5/5 mL) 0.05 M Tris (pH7.4) Buffer.

PRE	BUF

Preservatives: 0.0015% < Proclin 300 < 0.6%.

Kit Component Supplied Separately

Additional Materials Required But Not Provided:

- BioCLIA® 1200 (Cat No. MA00139)
- BioCLIA®6500 (Cat No. MA00243)
- BioCLIA Autoimmune Calibrator Set, ICA (Cat No. MY00246, 2 x 1 mL; Cat No. MY00297, 4 x 1 mL)
- BioCLIA Autoimmune Control Set, ICA (Cat No. MY00348, 2 x 1 mL; Cat No. MY00399, 4 x 1 mL)
- BioCLIA Sample Diluent I (Cat No. MY00965)
- BioCLIA System Wash Buffer (Cat No. MY00404)
- BioCLIA System Substrate (Cat No. MY00405)
- BioCLIA Cuvettes (Cat No. MA00138, MA00244)
- BioCLIA Silicon Gasket (Small) (Cat No. MV00195)
- BioCLIA Silicon Gasket (Large) (Cat No. MV00196)
- BioCLIA Substrate Tube Maintenance cleanser (Cat No. MA00140)
- BioCLIA Sample Probe Maintenance cleanser (Cat No. MA00141)
- BioCLIA Micro Cup (Cat No. MA00142)
- Distilled Water

Assay Procedure

Note that, it is important to perform all routine maintenance procedures for optimal performance, such as routine cleaning, calibration and control procedures that are defined in the BioCLIA® 1200 and BioCLIA® 6500 User Manual.

See the BioCLIA® 1200 and BioCLIA® 6500 User Manual for preparation, setup, dilutions, adjustment, assay and quality control procedures.

Reconstitute the lyophilized ICA antigen coated microparticles with distilled water (4ml distilled water per bottle). Blend for more than 30 minutes in low speed, transfer the solution to the supplied empty Microparticle bottle. For the kit with 100 tests, user should transfer both bottles of reconstitute antigen-microparticle solution to supplied empty microparticle bottle before using.

Users should have the periodic calibration procedure for every 28 running days from last calibration. Besides, a calibration procedure should be carried out when a new batch of BioCLIA ICA kit is used.

The control procedure should be done before running the specimens each day. Users also can adjust the control procedure period according to their own lab frequency.

Expected Values

Each Laboratory should establish its own reference ranges.

When the customer see a problem (High CV or unusual values, rerun controls and analyze specimens again.

Result Analysis

With the help of build in master calibration curve and specified two-point calibrator set for instrument, the BioCLIA will automatically calculate the anti-ICA

antibodies concentration of each specimen and interpret the results into IU/mL. The concentration of anti-ICA antibody sample is reported as < 2 IU/mL when it lower than the minimum detection limit, while reported as > 400 IU/mL when it is higher than the range of measurement.

Sample Dilution

The specimens are diluted with BioCLIA Sample Diluent I before testing (dilution ratio 1:20) by the BioCLIA® 1200 and BioCLIA® 6500 automatically.

Cut-Off Value Determination

120 clinical samples, including 60 positive sera and 60 negative sera, were collected and valued. These samples were venous blood from human the aged between 0 - 80, sealed and stored at 2 - 8 oC. Results of 120 clinical samples tested by the BioCLIA ICA kit were analyzed using the receiver-operating characteristic curve (ROC) and the cut-off value was determined at 20 IU/mL.

Test Result Interpretation

Specimen with concentration < 20 IU/mL, interpreted as negative;

Specimen with concentration ≥ 20 IU/mL, interpreted as positive.

Test results only reflect the sample collecting status and should be interpreted/analyzed for diagnosis in conjunction with other laboratory and clinical findings.

Performance Characteristics

APPEARENCE

Kit components are complete with no leakage. No precipitation or floc in liquid reagents. Packing labels are clear and easy to identify.

ACCURACY/SPIKED RECOVERY

The accuracy/spiked recovery was determined by analyzing samples spiked with known amounts of anti-ICA antibodies into certain matrix. Anti-ICA antibody positive samples (low 5 IU/mL, mid 50 IU/mL, high 200 IU/mL) were spiked into two matrixes (20&100 IU/mL) separately at the volume ratio of 1:9, making totally 8 spiked samples and each sample was tested in triplicate. The spiked recovery for the concentration of anti-ICA antibodies was calculatd.*

	Matrix 20 IU/mL			Matrix 100 IU/mL		
Spiked Conc.	Obs	Exp.	Obs/Exp	Obs	Exp.	Obs/Exp
Neat	19.62			98.85		
5IU/mL	18.40	18.5	99.4%	89.75	90.5	99.2%
50 IU/mL	21.87	23.0	95.1%	94.06	95.0	99.0%
200 IU/mL	38.13	38.0	100.4%	108.71	110.0	98.8%

*Representative data; results in individual laboratories may vary from these data.

TRACEABILITY

Anti-ICA antibody concentration can be traced to WHO Standard Anti-ICA, CNS Code: 97/550.

PRECISION

A study based on guidance from CLSI document EP5-A2 was performed for determining the precision of BioCLIA ICA kit. Human serum in the in-house reference panel (low, mid, high) was tested with 10 replicates for intra-assay precision evaluation, while in 4 replicates per sample for intra-assay precision evaluation, while with 4 replicates per sample for inter-assay precision. Each sample tested in individual runs, and 2 runs per day for 10 days, a total of 80 points. Data from this study are summarized in the following table.*

Intra-assay precision: CV ≤ 10%

Intra-Assay	RP1	RP2	RP3	RP4
Mean(IU/ml)	5.02	21.55	199.03	377.32
CV	5.0%	1.6%	6.4%	11.1%

Inter-assay precision: CV ≤ 15%

Inter-Assay	RP1	RP2	RP3	RP4
Mean(IU/ml)	5.05	21.48	199.20	374.02
CV	5.7%	1.4%	6.3%	6.9%

*Representative data; results in individual laboratories may vary from these data.

LIMIT OF BLANK / DETECTION (LOB/LOD)

LOB/LOD was determined consistent with CLSI EP17-A guideline. LOB/LOD of the BioCLIA ICA assay was lower than 0.7 IU/mL, which is below the analytical measuring range of the assay.

ASSAY REPORTABLE RANGE

The BioCLIA ICA kit has a reportable linear range of 2-400 IU/mL. The linear range was determined by diluting a high positive anti-ICA antibody serum sample with a negative sample to several concentrations which covers the entire assay linear range according to the scheme in CLSI EP6-A. The expected value was plotted against the observed value, and linear regression analysis was performed to get slope, intercept and coefficient of correlation (r) values. The results are summarized in the tables below: *

Slope	Intercept	r
1.07	-0.50	0.99

Assay linear range is 5-400 IU/mL. Results below the lower limit will be reported as < 5 IU/mL, while those are above the upper limit will be reported as > 400 IU/mL.

*Representative data; results in individual laboratories may vary from these data.

INTERFERENCE

Bilirubin, hemoglobin, triglycerides, rheumatoid factor (RF), and human anti-mouse antibody (HAMA) will not affect the BioCLIA ICA assay performance when at the level indicated below.

Bilirubin ≤ 40 mg/dL;

Hemoglobin ≤ 150 mg/dL;

Triglycerides ≤ 1,000 mg/dL;

Rheumatoid factor (RF) ≤ 1,000 IU/mL;

Human anti-mouse antibody (HAMA) ≤ 2,000 ng/mL.

METHOD COMPARISON

Method comparison was implemented by comparing BioCLIA ICA assay to the predicated assay.

Clinical Sample		BioCLIA ICA		
		-	+	Total
Predicated Method	-	58	2	60
	+	2	38	40
	Total	60	40	100

Sensitivity	95.0%
Specificity	96.7%
Total agreement	96.0%

Limitations

- The effectiveness of this kit is only confirmed for human serum, the applicability of the other kinds of samples is not verified.
- Reliable and reproducible results will be obtained when the assay procedure is carried out in accordance with the instructions and with adherence to good laboratory practice.
- Clinical diagnosis should not be made on the findings of a single test result, but should be integrated with all clinical and laboratory findings.

References

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Technical Assistance

For technical assistance, contact your National Distributor.

17th April 2019

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