ICA Screen ELISA

Catalog number:
ICA31-K01

Enzyme immunoassay for screening
of **Islet cell autoantibodies** in human serum

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v. 1.0

INTENDED USE

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune process which destructs the insulin-secreting pancreatic beta cells. This is caused by simultaneous action of specific auto-reactive CD4+ and CD8+ T lymphocytes. Even before the onset of type 1 diabetes autoantibodies against different antigens of the islet cells can be detected in the serum of those patients. This process may take years to complete and may occur at any time at all ages.
These autoantibodies represent the most important markers to identify persons with increased risk to develop diabetes at a time when all metabolic tests available still show normal results.
Islet cell autoantibodies (ICA) have been the first serological markers described for type 1 diabetes mellitus. These antibodies belong to the IgG subtype and are directed against a variety of islet cell antigens. They are detected by means of immunohistochemical methods using slides from the pancreas.
Although there have been several international workshops since the first description in 1974 these methods still reveal some methodological problems. Especially the standardization of this technique is very difficult. In spite of this it is considered as the "gold standard" and used in many routine laboratories.
After discovery of the two main protein antigens of ICA - the enzyme glutamic acid decarboxylase (GAD65) and the islet cell protein IA2 which belongs to the family of tyrosine phosphatases – it becomes possible to measure these specific autoantibodies by means of sensitive radio ligand assays. Recently also ELISA (anti-GAD ELISA and Anti-IA2 ELISA) tests became available.
The early diagnostics of type 1 diabetes can be achieved by the combination of tests of different autoantibodies. Patients who were found to be positive for two or more autoantibodies have a considerably higher risk to develop diabetes.
In addition to the above mentioned ELISAs it is possible for the first time to determine ICA also by the semi-quantitative ICA Screen ELISA Assay Kit screen which allows measuring ICA by a reproducible and reliable method that can replace the determination of ICA by indirect immunfluorescence.

LITERATURE

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- Batstra MR, HJ Aanstoot and P Herbring: Prediction and diagnosis of Type 1 Diabetes using ß-cell autoantibodies; Clin Lab 2001; 9+10, 497-507

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PRINCIPLE of the Assay

ICA Screen ELISA Assay Kit is an enzyme immunoassay for the semi-quantitative determination of islet cell autoantibodies (ICA) in human serum.
The ICA Screen ELISA Assay Kit system uses the ability of IC autoantibodies to act di-valently, that is, to form a bridge between autoantigens (highly purified material from primate source) coated onto the plate and liquid phase autoantigen-Biotin. In the first step IC autoantibodies from the sample bind to autoantigens coated on the microtiter plate. In a second step IC-autoantigen-Biotin binds to this complex which correlates with the amount of IC autoantibodies in patient's serum. Unbound IC-autoantigen-Biotin is removed by washing.
The bound IC-autoantigen-Biotin then is quantified by addition of Streptavidin- peroxidase and a colorogenic substrate (TMB) followed by reading the optical density (OD) at 450 nm.

SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Do not use lipaemic or grossly hemolytic serum samples. Plasma should not be used.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, initially aliquot samples and keep at -20 °C.

TEST COMPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate** 12 breakable strips per 8 wells coated with IC-autoantigens	vacuum sealed with desiccant
B WASHB	**Concentrated wash buffer** sufficient for 1250 ml	**125 ml** concentrate
D CONJ	**Streptavidin-peroxidase** (SA-POD) sufficient for 14.0 ml	**0.7 ml** concentrate
E SUB	**Substrate (TMB)** (3,3´,5,5´-Tetramethylbenzidine)	**15 ml** ready for use
F STOP	**Stop solution** (0.25 M sulfuric acid)	**12 ml** ready for use
G BUF D	**Diluent for SA-POD (D)**	**15 ml** ready for use
H START	**IC-autoantigen-Biotin**	**3 vials** lyophilized
J BUF H	**Diluent for IC-autoantigen-Biotin (H)**	**2 x 15 ml** ready for use colored blue
K ENH	**Enhancer**	**4 ml** ready for use colored red
CI CONTROL	**Negative control** Binding index: see leaflet	**0.7 ml** ready for use
CC CONTROL	**Cut-off control**	**0.7 ml** ready for use
CII CONTROL	**Positive control** Binding index: see leaflet	**0.7 ml** ready for use

Materials required

- Precision pipettes 10 - 100 µl
- Multi-channel pipette
- Disposable pipette tips
- 8 channel wash comb with a vacuum pump or microplate washer
- Micro plate reader with optical filters for 450 nm and 620 or 690 nm
- Graduated cylinders
- Distilled or de-ionized water
- Absorbent paper or paper towel
- foil

Size and storage

ICA Screen ELISA Assay Kit has been designed for 96 determinations. This is sufficient for the analysis of 45 unknown samples as well as for control sera assayed in duplicates.
The expiry date of each component is reported on its respective label that of the complete kit on the box label. Please note that the washing procedure is crucial. Insufficient washing will result in poor precision and falsely elevated OD readings
Upon receipt, all components of the ICA Screen ELISA Assay Kit have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow samples and all test components to reach room temperature prior to assay (at least 30 minutes). Take care to agitate serum samples gently in order to ensure homogeneity.

Please, handle carefully with the following components:

A Allow the sealed microplate to reach room temperature **before** opening. Unused wells should be stored refrigerated (2° - 8° C) and protected from moisture in the original bag carefully resealed. Use within 4 weeks.

B Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer (B) 1 + 9 with distilled or de-ionized water. For example, dilute 50 ml of the concentrate with 450 ml of distilled water. B should be free of crystals before dilution, otherwise dissolve by warming up to max. 37 °C. The diluted washing solution can be stored at 2 - 8 °C up to 4 weeks.

D Prepare a sufficient amount of Streptavidin-peroxidase solution by diluting SA-POD concentrate (D) 1 + 19 (0.25 ml SA-POD concentrate with 4.75 ml diluent for SA-POD (G)**.** The SA-POD solution prepared is stable up to 16 weeks at 2 - 8 °C.

E Avoid exposure of substrate solution (E) to light.

H Prepare a sufficient amount of IC-autoantigen-Biotin solution by reconstitution of one vial lyophilized IC-autoantigen-Biotin (H) with 5.5 ml diluent for IC-autoantigen-Biotin (J) directly prior to use. Use within the day of reconstitution.

ASSAY PROCEDURE

- Duplicates are recommended.

1. Pipette into the corresponding wells according to assay scheme
   - **50 µl** controls (CI, CC, CII)
   - **50 µl** patient's samples

2. Pipette **25 µl** Enhancer (K) into each well.
   Cover the plate, shake for 5 seconds > 400 rpm and incubate **over night** (at least 16 h) at 2 - 8 °C.

3. Prepare sufficient amount of reagents (B, D / G, E, H/J) and allow the covered plate to reach **room temperature** (at least 30 minutes preferable while shaking – possible precipitates will disappear).

4. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash **3** times with **300 µl** washing solution (diluted from B) with 5 sec. soaking time each.

5. Add **100 µl** of reconstituted IC-autoantigen-Biotin solution (prepared from H and J) to each well.
   Cover the plate and incubate for **60 min** at room temperature
   (18 - 25 °C) while shaking > 400 rpm.

6. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash **3** times with **300 µl** washing solution (diluted from B) with 5 sec. soaking time each.

7. Add **100 µl** diluted SA-POD (prepared from D and G) to each well. Cover the plate and incubate for **20 min** at room temperature (18 - 25 °C) while shaking > 400 rpm.

8. Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash **3** times with **300 µl** washing solution (diluted from B) with 5 sec. soaking time each.

9. Add **100 µl** substrate solution (E) to each well and shake for 5 seconds. Incubate for **20 min** in the **dark** at room temperature.

10. Add **100 µl** stop solution (F) after exact 20 min to each well. Shake the plates for 5 seconds > 400 rpm.

11. Read the optical density **at 450 nm** versus 620 nm (690 nm) within **5 minutes** after adding the stop solution.

DATA PROCESSING

Results are interpreted by calculating the binding index (BI):

BI = OD_{sample} / $OD_{cut\text{-}off\ control}$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

Comparison to JDF and WHO units are available.

TYPICAL EXAMPLE

Do not use for evaluation!

Wells	OD (a)	OD (b)	OD (mean)	BI
Negative control CI	0.176	0.188	0.182	0.5
Cut-off control CC	0.398	0.346	0.372	1
Positive control CII	1.315	1.291	1.303	3.5
Patient 1	1.718	1.776	1.747	4.7
Patient 2	0.108	0.116	0.112	0.3
Patient 3	0.291	0.281	0.286	0.8

REFERENCE VALUES

ICA Screen ELISA Assay Kit	Binding Index
negative	≤ 0.7
grey zone	0.7 - 1.0
positive	≥ 1.0

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum ICA levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide only a guide.

CHARACTERISTIC ASSAY DATA

Specificity and sensitivity

Using a cut-off of BI = 1.0 the ICA Screen ELISA Assay Kit shows a sensitivity of 98.3 % for type 1 diabetic patients and a specificity of 100 %.

Detection limits
The functional sensitivity was determined at a BI < 0.5.

Intra - and inter-assay variation

Intra-assay n= 10			Inter-assay n= 5		
Sample no.	BI	CV (%)	Sample no.	BI	CV (%)
1	0.4	3.3	4	0.5	4.8
2	0.9	3.1	5	1.0	4.1
3	2.3	2.5	6	3.8	3.3

ICA Screen ELISA Assay Kit

ASSAY SCHEME

Bring all reagents to room temperature. Gently mix all reagents to ensure homogeneity.

Step	Activity	Material	Control sera (C I, CC, C II)	Patients 1, 2 etc.
1	Pipette	Samples	50 µl	50 µl
2	Pipette	Enhancer (K)	25 µl	25 µl
	Incubate	Microtiter plate (A)	Incubate at 2 - 8 °C **for at least 16 h**	
3	Prepare	Reagents B, D/G, E, H/J shaking for at and sealed plate	bring to **room temperature** (plate preferable while least 30 minutes)	
4	Aspirate or decant	Microtiter plate (A)	put sharply onto absorbent tissue	
	Pipette	Washing solution (diluted from B)	3 x 300 µl	3 x 300 µl
5	Pipette	IC-autoantigen-Biotin solution (made from H and J)	100 µl	100 µl
	Incubate	Microtiter plate (A)	**1 hour** at room temperature **with shaking (> 400 rpm)**	
6	Aspirate or decant	Microtiter plate (A)	put sharply onto absorbent tissue	
	Pipette	Washing solution (diluted from B)	3 x 300 µl	3 x 300 µl
7	Pipette	SA-POD solution (made from D and G)	100 µl	100 µl
	Cover and incubate	Microtiter plate (A)	**20 min** at room temperature **with shaking (> 400 rpm)**	
8	Aspirate or decant	Microtiter plate (A)	put sharply onto absorbent tissue	
	Pipette	Washing solution (diluted from B)	3 x 300 µl	3 x 300 µl
9	Pipette	Substrate (E)	100 µl	100 µl
	Incubate	Microtiter plate (A)	**20 min** at room temperature **in the dark**	
10	Pipette and mix	Stop solution (F)	100 µl	100 µl
11	Measure OD		at 450 nm against 620 nm (690 nm) within 5 min	

SAFETY PRECAUTIONS

- **This ICA Screen ELISA Assay Kit is for research use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/v) of sodium azide as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this ICA Screen ELISA Assay Kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the ICA Screen ELISA Assay Kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
  - In any case GLP should be applied with all general and individual regulations to the use of this kit.

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.