Insudex® GAD Test

Glutamic acid decarboxylase
(GAD) Autoantibodies

Product Information Sheet

Instructions for Use

INSUDEX®

Diabetes Detection Redefined

REF 2022, 2023, 2024

CE IVD

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INTENDED USE

The Insudex® GAD Test is for the semi-quantitative determination of glutamic acid decarboxylase (GADAb) autoantibodies in human serum and blood. The assay is useful as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes) and Latent Autoimmune Diabetes in Adults (LADA). The GAD Test is not to be used alone and is to be used in conjunction with other clinical and laboratory findings.

INTRODUCTION, BACKGROUND, AND CLINICAL SIGNIFICANCE

Antibodies directed against pancreatic beta-cell antigens are important analytes that play a crucial role in many aspects of diabetes diagnosis and the enabling of precision medicine in diabetes therapy. The major diabetes-associated autoantibodies employed in current screening tests are directed against GAD, IA-2, and insulin (1).

Prospective studies have shown that the number of autoantibodies present is associated with risk for the development of frank type-1 diabetes in children with a high-risk genetic background (2,3). GAD autoantibody detection is a mainstay of current autoantibody testing in children due to the high frequency of GADAb positivity in the at-risk childhood population, comprising 76% in 2 to 17-year olds and 61% of 13 to 34-year olds at diagnosis (4).

GADAb status is also important in the differential diagnosis of adult-onset diabetes, which can be either adult-onset type-1 diabetes, classical type-2 diabetes, or Latent Autoimmune Diabetes in

Adults (LADA), which typically presents as type-2 diabetes but has an autoimmune component that is associated with faster progression to insulin dependency (5,6). GADAb positivity in conjunction with insulin deficiency is indicative of adult-onset type-1 diabetes. Autoantibody positivity in patients with apparent type-2 diabetes based on age or BMI is suggestive of LADA rather than classical type-2 diabetes. GAD autoantibodies are detected in 96% of autoantibody-positive individuals with putative type-2 diabetes (7).

GADAb status is also important in correctly characterizing children or adolescents with obesity, who may be incorrectly diagnosed as type 1 based on age or type 2 based on BMI. GADAb positivity indicates an obese type-1 diabetes, while GADAb-negative status can indicate early-onset type 2 diabetes (8,9).

SUMMARY OF THE TEST

The device consists of: A foil pouch containing a plastic cartridge housing a test strip with a desiccant, a vial containing sample buffer, a mixing vial, the Insudex® GAD Test Key Card and these instructions. The test strip utilizes a combination of GAD antigen to detect anti-GAD antibodies in the sample and rabbit anti-GAD antibodies to serve as a procedural control. Each device contains GAD-colloidal gold conjugate pre-dried on a pad. GAD antigen (on the Test Line) and rabbit anti-GAD polyclonal antibody (on the Control Line) are coated and immobilized on a nitrocellulose membrane.

PRINCIPLES OF THE TEST

The Insudex® GAD device utilizes an antibody bridging method. The device depends on the ability of GAD autoantibodies to act divalently and form a bridge between GAD coated on the nitrocellulose membrane and liquid phase GAD-colloidal gold. During the test procedure, GADAb in the serum specimen react with the conjugate (GAD-colloidal gold conjugate) and form a complex. This complex migrates by capillary action along the test strip to the GAD test line; if GADAb are present in the sample, the complex is captured onto the test line, where, if GADAb are present at concentrations above the cutoff, a red line becomes visible indicating a positive result. If GADAb are not present in the sample above the cutoff, no line is visible on the test line, indicating a negative result. The control line should develop regardless of the test line result, if the test was correctly used and/or performed correctly, since GAD-colloidal gold conjugate is present in excess to bind to the antibodies at the control line, resulting in a visible red line. At the completion of the test, the intensity of the test line is determined with the Insudex® Reader.

MATERIALS PROVIDED

The Insudex® GAD Test is available in three configurations of 5, 20, and 50 tests.
Each product contains:

- Insudex® GAD Test Cartridge in foil pouch with desiccant (1 per test)
- Vial containing Sample Buffer (1 vial with 1 mL Sample Buffer per test)

- Empty tubes for mixing sample with buffer (1 per test)
- Instructions for use (this document) (1 per box)
- Insudex® GAD Test Key Card (2 per box, 1 for serum and 1 for fingerstick whole blood).

MATERIALS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Insudex® Reader, REF 2005
- Pipette(s) and tips capable of delivering 50-135 µL
- Quality Control Materials – Please refer to the Product Insert for the Insudex® GAD Control Kit for directions for use.

STORAGE AND HANDLING

- Store in a cool, dry place between 2°– 8 °C, (35.6°– 46.4 °F). Test cartridges and buffer vials must be fully brought to room temperature before opening and using. Do not freeze.
- The test has only been verified to perform accordingly through a temperature range of 20 to 30 °C.
- Keep away from heat and direct sunlight.
- Do not open pouch until sample is ready to be tested.
- Use test cartridge as soon as you have removed it from the foil pouch.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Intended for professional use only.
- The assay is useful as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes).
- Test results are meant to be interpreted only by the Insudex® Reader.
- Do not use kit or kit components beyond the expiration dates specified on the product and component labeling.
- Storage or use of kit materials at temperatures except as specified may result in diminished test performance and may give inaccurate results.
- Use only calibrated pipettes.
- Always use separate pipette tips, Sample Buffer vials, and mixing vials for each patient
- Do not reuse test cartridges. Discard test cartridge after using.
- **Do not ingest any of the supplied materials.**
- **Caution: Handle and dispose of all materials coming in contact with blood or serum according to universal precautions and guidelines.**

SPECIMEN COLLECTION AND PREPARATION

- The Insudex® GAD Test is designed for use with serum or fingerstick whole blood.

- If testing frozen samples allow specimen to thaw completely and thoroughly mix prior to sampling.
- Serum samples may be stored at room temperature for up to 24 hours and stored at 2-8 °C for up to 1 week.

INSTRUCTIONS FOR TESTING

IMPORTANT: Read all instructions carefully before testing. Read the Reader User Guide thoroughly before starting testing.

Sample Preparation

Sample Preparation (Serum)

1. Obtain the serum sample (30 µL required).
2. Remove one foil pouch, one Sample Buffer vial and one empty mixing tube from productbox. Allow each to come to room temperature.
3. Open the Sample Buffer vial by unscrewing cap.
4. Pipette 135 µL of Sample Buffer into the empty mixing tube.
5. Add 30 µL serum to buffer vial and mix by aspirating the sample and Sample Buffer mixtureup and down 6-8 times.
6. Open the foil pouch, remove the cartridge and place it on a flat surface.
7. Immediately proceed to **TESTING** below

Sample Preparation – Fingerstick whole blood

1. Remove one foil pouch, one Sample Buffer vial and one empty mixing tube from productbox. Allow each to come to room temperature.
2. Open the Sample Buffer vial by unscrewing cap.
3. Pipette 135 µL of Sample Buffer into the empty mixing tube.
4. Add 30 µL fingerstick whole blood to the mixing tube and mix by aspirating the sample andSample Buffer mixture up and down 6-8 times.
5. Immediately proceed to **TESTING** below

Testing

Preparation of the Reader

1. Place the Reader on a level and flat surface. Turn on the Reader and allow it to perform its self-check.
2. Press the 'NEW SCAN' button on the Main screen.
3. Push on the 'CASSETTE CONFIG' button to load the Cassette Configuration to be used in the next scan.
4. Take the appropriate Insudex® GAD Test Key Card (Serum or Fingerstick whole blood) which is supplied along with the cassette box and place it in front of the reader atapproximately 5 cm distance.

5. Push on ‘ACTIVATE BARCODE READ’. The barcode reader will start the scanning using a light that will disappear when the barcode has been read. You may need to move the barcode around a little to capture the information.
6. In the new screen, the new Cassette Configuration data are shown. Drag up and down on the touchscreen to see all data. Push on the ‘NEW SCAN’ button to return to the scanning screen. If the Cassette Configuration data is not correct, push on the ‘CASSETTE CONFIG’ button to scan another configuration.
7. Push on the ‘CONFIRM CASSETTE’ button to confirm that current Cassette Configuration matches the cassette you are going to process.
8. Place the barcode on the bottom of the cassette in front of the barcode reader.
9. Push on the text ‘Introduce your sample/patient id’ and a virtual keyboard will appear. Key-in an identification text and push the ‘Enter’ button. Alternatively, press ‘ACTIVATEBARCODE READ’ to scan in the sample ID.
10. Open the foil pouch, remove the cartridge and place it on a flat surface.
11. Pipette 150 µL of the diluted sample into the sample well of the cartridge.
12. Place the cartridge into the Reader drawer with the arrow on the cartridge pointing forward and the combed handle at the rear. Insert the drawer into the Reader as far as itwill go.
13. Press the ‘Timed Scan’ option.
14. The results of the scan will be displayed when the process ends. Drag up and down on thetouch screen and press on the different tabs to see all data.
15. If you have a ticket/label printer connected and configured, press the ‘PRINT’ button in order to obtain a ticket/label containing the identification data and the summary resultsof the most recent scan.
16. After completing the test, discard the cartridge into a biohazard container.

EXPECTED RESULTS

Results are reported in International Units per milliliter (Units are NIBSC 97/550). It is recommended by Diabetomics that values greater than or equal to 37 IU/mL be considered positive and values less than 37 IU/mL be considered negative. It is the responsibility of each laboratory to establish its own reference ranges for the population of patients it serves, as expected values are affected by many different factors.

COMPARISION TO PREDICATE

A set of 550 sera were tested, including 286 Type I diabetics (newly diagnosed and/or confirmed Type I diabetes); 59 Type II diabetes mellitus, 112 control non-diabetic subjects and serum from 93 patients with other autoimmune diseases including Hashimoto's thyroiditis, SLE, kidney disease, Grave's disease, myasthenia gravis, and rheumatoid arthritis. These samples were run in

both Insudex® GAD and the Kronus GADAb ELISA. 128 samples were randomly selected from 384 samples that gave a negative result in the Insudex® GAD test. 55 samples were randomly selected from 166 samples that gave a positive result in the Insudex® GAD test. Using the cut-off value of 29.09 IU/mL, method comparison was done with the remaining 367 samples. Method comparison agreement for this study showed a positive percent agreement (PPA) of 88 % (106/121 samples), a negative percent agreement (NPA) of 98 % (240/246 samples) and an overall percent agreement (OPA) of 94% (346/367).

Method comparison of Insudex® GAD POC against predicate Kronus GADAb ELISA

Kronus GADAb ELISA	POC Insudex® GAD		
	-	+	Total
-	240	6	246
+	15	106	121
Total	255	112	367

Based on a negative cut-off value of 29.09 IU/mL, the Insudex® GAD POC compared to the predicate device has a clinical sensitivity of 88 % (95 % C.I.= 82-93 %), and a clinical specificity of 98 % (95 % C.I.= 96-99 %). The calculated positive predictive value and negative predictive value are 95% (106/112 samples) (95% C.I.= 90-99%) and 94% (240/255 samples) (95% C.I.= 91-97%), respectively.

Individuals with other autoimmune diseases were assayed in the Insudex® GAD test and the predicate Kronus GAD Autoantibody ELISA Kit. Results of that study are provided below.

Condition	Number of Samples Evaluated	Number of Insudex GAD Positive Results	Number of Kronus ELISA Positive Results
Hashimoto's Thyroiditis	19	1	1
SLE	15	1	1
Kidney disease	10	0	0
Grave's Disease	20	1	1
Myasthenia Gravis	10	0	0
Rheumatoid Arthritis	19	2*	0

* samples identified as weak positive

PERFORMANCE CHARACTERISTICS

Precision

Four samples, ranging from 0-553 IU/mL were assayed on three individual lots over twenty days

with four replicates per standard per day. A total of 80 strips were tested per standard per lot. These data were used to evaluate lot-to-lot variability, day-to-day variability, and within run variability. Two separate operators assayed the same set of prepared standards on the same day on the same lot of strips on one reader to evaluate operator to operator variability. To evaluate reader to reader variability, the same set of prepared standards were assayed on the same day with the same lot of strips, and these strips were read on three different Readers. Reader to Reader variability was tested on three lots of strips.

Lot-to-Lot Reproducibility

		Sample Concentration (IU/ml)			
		0	**102**	**360**	**553**
Lot#IAG090301	**Mean**	0.0	122.4	372.4	563.0
	SD	0.0	6.0	2.9	12.0
	% CV	0.0	4.9	0.8	2.1
Lot#IAG090401	**Mean**	0.0	120.2	373.7	579.0
	SD	0.0	9.3	10.3	2.3
	% CV	0.0	7.7	2.8	0.4
Lot#IAG091801	**Mean**	0.0	113	354.8	561.2
	SD	0.0	15.7	13.7	29.6
	% CV	0.0	13.9	3.9	5.3

Within-Lab Reproducibility

Lot IAG090301		**Within-Run**			**Between-Day**		
Sample	**Concentration (IU/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**
1	**0**	0.0	0.0	0.0	0.0	0.0	0.0
2	**102**	122.4	6.0	4.9	107.8	23.6	21.9
3	**360**	372.4	2.9	0.8	368.4	18.4	5.0
4	**553**	563.0	12.0	2.1	561.7	23.2	4.1
Lot IAG090401		**Within-Run**			**Between-Day**		
Sample	**Concentration (IU/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**
1	**0**	0.0	0.0	0.0	0.0	0.0	0.0
2	**102**	120.2	9.3	7.7	108.0	15.2	14.1
3	**360**	373.8	10.3	2.8	357.3	21.0	5.9
4	**553**	579.0	2.3	0.4	554.4	23.0	4.2
Lot IAG091801		**Within-Run**			**Between-Day**		
Sample	**Concentration (IU/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**

1	**0**	0.0	0.0	0.0	0.0	0.0	0.0
2	**102**	113	15.7	13.9	125.5	26.4	21.0
3	**360**	354.8	13.7	3.9	368.5	25.1	6.8
4	**553**	561.2	29.6	5.3	562.8	30.1	5.4

Operator-to-Operator Reproducibility

	Lot# IAG090301	**Sample Concentration (IU/mL)**			
		0	**102**	**360**	**553**
Operator A	**Mean**	0.0	122.5	372.4	562.7
	SD	0.0	5.9	3.0	12.2
	% CV	0.0	4.9	0.8	2.2
Operator B	**Mean**	0.0	114.9	359.8	563.0
	SD	0.0	6.3	18.6	14.9
	% CV	0.0	5.5	5.2	2.7

Reader-to-Reader Reproducibility

	Lot# IAG090301	**Sample Concentration (IU/mL)**			
		0	**102**	**360**	**553**
Reader 393	**Mean**	0.0	122.5	372.4	562.7
	SD	0.0	5.9	3.0	12.2
	% CV	0.0	4.9	0.8	2.2
Reader DC20	**Mean**	0.0	117.7	366.3	552.5
	SD	0.0	10.0	3.0	10.4
	% CV	0.0	8.5	0.8	1.9
Reader 47C6	**Mean**	0.0	113.3	367.1	561.5
	SD	0.0	6.2	4.0	10.7
	% CV	0.0	5.5	1.1	1.9

Site-to-Site reproducibility

Comparison	**Correlation**	**R^2**
Lab1 vs Lab 2	y = 0.9895x + 15.681	R^2 = 0.9836
Lab1 vs Lab 3	y = 0.9708x + 4.8472	R^2 = 0.9957
Lab2 vs Lab 3	y = 0.9657x - 3.9943	R^2 = 0.9807

Analytical Sensitivity

Limit of Blank (LoB) is 0 IU/mL, calculated as the 95th percentile from 60 determinations of an anti-GAD antibody negative sample.

Limit of Detection (LoD) is 36.4 IU/mL, calculated from the pooled standard deviation of 15 determinations of four low positive samples.

Limit of Quantitation (LoQ) is 76 IU/mL (estimated dose measurable with imprecision <25%), calculated from the pooled standard deviation of 15 determinations of four low positive samples.

Test result display on Insudex® GAD reader

Result	Report
<37.0 IU/mL	Not detect, result <37 IU/mL
>37 IU/mL	GAD Ab detected, Positive, report result

Analytical Specificity

In accordance with "EP07-A2 Interference testing in clinical chemistry approved guideline" four potential clinically relevant interferents (bilirubin, hemoglobin, triglycerides, and insulin) were tested at recommended concentrations. Neither the overall performance nor the results of the Insudex® GAD test were altered by the presence of high levels of bilirubin, hemoglobin, triglycerides, and insulin, as compared to the control set.

Dilution and Recovery

Insudex® GAD calibrators were diluted with GAD antibody negative serum and assayed in the Insudex® GAD test. The results are shown in the table below. The recoveries ranged from 95.2% to 136.4% with a mean recovery of 116.1%.

		Expected Concentration IU/mL							
	Replicate #	**0**	**62**	**102**	**129**	**247**	**360**	**472**	**553**
Observed Concentration IU/mL	**R1**	0	66	127	175	295	413	518	638
	R2	0	68	136	169	300	383	497	612
	R3	0	42	125	184	303	387	524	662
	Average	0	59	129	176	299	395	513	637
	Stdev	0	14	6	8	4	16	14	25
	% CV	0	24	5	4	1	4	3	4
	% Recovery	N/A	95.2	126.5	136.4	121.1	109.7	108.7	115.2

Hook Effect

Three high GAD antibody positive samples were diluted in normal human serum to determine if there was any hook effect. No hook effect was observed for back calculated concentrations of up to 116,480 IU/mL.

LIMITATIONS OF THE PROCEDURE

- Interpretation of Results: Interpretation of test results requires a properly qualified and experienced medical practitioner. Interpreting the clinical significance of these test results requires consideration of additional factors, such as the patient's overall health, the results of other tests, patient age, and the presence of other autoimmune diseases, etc.

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ORDERING INFORMATION AND TECHNICAL SERVICE

For technical assistance or to place an order, contact:

GLOBAL

Diabetomics, Inc.

Phone: +1 503-924-5110

Email: enquiry@diabetomics.com

Website: www.InsudexPOC.com

EC REP

EMERGO EUROPE

Prinsessegracht 20

2514 AP The Hague

The Netherlands

Diabetomics, Inc.

2345 NE Overlook Drive

Suite #140

Hillsboro, OR 97006, USA

EXPLANATION OF SYMBOLS

	Consult Instructions for Use
	Storage Temperature
IVD	For in vitro Diagnostic Use
SN	Serial Number
	Manufacturer
	Separate collection for waste of electrical and electronic equipment
	Caution
REF	Component Number
EC REP	Authorized Representative in the European Community
	Date of Manufacture
CE	European Conformity
	Do Not Reuse
LOT	Batch Code, Lot Number
	Use By Date

Manufactured for Diabetomics, Inc. by:

Diabetomics Medical (P) Ltd.
Plot # 26 A / 26B
Industrial Estate
Muppireddipally (V), Toopran (M)
Medak,Telangana – INDIA

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