Insudex® IA-2 Test

Islet Antigen-2 (IA-2) Autoantibodies

Product Information Sheet
Instructions for Use

INSUDEX®

Diabetes Detection Redefined

REF 2048

CE IVD

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INTENDED USE

The Insudex® IA-2 Test is for the semi-quantitative determination of Islet Antigen-2 (IA-2) 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 (LADA). The IA-2 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]. IA-2 autoantibody detection is part of current autoantibody testing in children due to the high frequency of IA-2 Ab positivity in the at-risk childhood population[4].

IA-2 Ab 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]. IA-2 Ab and GAD Ab 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 and IA-2 autoantibodies are detected in high percentage of autoantibody-positive individuals with putative type-2 diabetes[7].

IA-2 Ab 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. IA-2 Ab positivity indicates an obese type-1 diabetes, while IA-2 and 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® IA-2 Test Key Card and these instructions. The test strip utilizes a combination of IA-2 antigen to detect anti-IA-2 antibodies in the sample and rabbit anti-IA-2 antibodies to serve as a procedural control. Each device contains IA-2-colloidal gold conjugate pre-dried on a pad. IA-2 antigen (on the Test Line) and rabbit anti-IA-2 polyclonal antibody (on the Control Line) are coated and immobilized on a nitrocellulose membrane.

PRINCIPLES OF THE TEST

The Insudex® IA-2 device utilizes an antibody bridging method. The device depends on the ability of IA-2 autoantibodies to act bivalently and form a bridge between IA-2 coated on the nitrocellulose membrane and liquid phase IA-2-colloidal gold. During the test procedure, IA-2 Ab in the specimen react with the conjugate (IA-2-colloidal gold conjugate) and form a complex. This complex migrates by capillary action along the test strip to the IA-2 test line; if IA-2 Ab are present in the sample, the complex is captured onto the test line, where, if IA-2 Ab are present at concentrations above the cutoff, a red line becomes visible indicating a positive result. If IA-2 Ab 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 IA-2-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® IA-2 Test is available in 20 test kits.
Each product contains:

- Insudex® IA-2 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® IA-2 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® IA-2 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® IA-2 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 (Serum)

1. Obtain the serum sample (30 µL required).
2. Remove one foil pouch, one Sample Buffer vial and one empty mixing tube from product box. 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 mixture up 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 product box. 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 and Sample 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® IA-2 Test Calibration Card (Serum or Fingerstick whole blood) which is supplied along with the cassette box and place it in front of the reader at approximately 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 'ACTIVATE BARCODE 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 it will 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 the touch 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 results of 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 38 IU/mL be considered positive and values less than 38 IU/mL be considered negative. As with all *in vitro* diagnostic assays, each laboratory should establish its own reference range(s) for the diagnostic evaluation of patient results.

COMPARISION TO PREDICATE

Sera collected from 227 subjects were tested using candidate device Insudex® IA-2 Test and predicate device Kronus IA-2b ELISA. The sample cohort included:

- 88 Type I diabetics (newly diagnosed and/or confirmed Type I diabetes)
- 46 Type II diabetics

- 93 patients (non-diabetic) with other autoimmune diseases, including Hashimoto's thyroiditis, SLE, kidney disease, myasthenia gravis, and rheumatoid arthritis.

Predicate device Kronus IA-2b ELISA values >7.5 U/mL and candidate device Insudex® IA-2 Test values >38.0 IU/mL (based on WHO NIBSC 97/550 reference standard) are considered as positive.

Method comparison of Insudex® IA-2 Test against predicate Kronus IA-2 Ab ELISA

Candidate device Insudex® IA-2 Test	**Predicate device Kronus IA-2 Ab ELISA**		
	+	-	Total
+	25	5	30
-	2	195	197
Total	27	200	227

Method comparison agreement for this cohort showed a positive percent agreement (PPA) of 92.5% (25/27 samples), a negative percent agreement (NPA) of 97.5% (195/200 samples) and an overall percent agreement (OPA) of 96.9% (220/227 samples).

Clinical sensitivity and specificity.
Sera collected from 332 were tested Insudex® IA-2 Test. The sample cohort included:

- 163 Type I diabetics (newly diagnosed and/or confirmed Type I diabetes)
- 46 Type II diabetics
- 30 control non-diabetic subjects
- 93 patients (non-diabetic) with other autoimmune diseases, including Hashimoto's thyroiditis, SLE, kidney disease, myasthenia gravis, and rheumatoid arthritis.

Of the 163 TIDM patient sera 102 (62.5%) (95% C.I.= 54.6%-69.9%) were positive in the Insudex® IA-2 Test. Using the clinical cut-of 38 IU/mL used in TIDM, Type 2 diabetes and other autoimmune diseases Insudex® IA-2 Test has a clinical sensitivity of 62.5% (95% C.I.= 54.6%-69.9%) and specificity of 97.0% (95% C.I.=92.8%-98.9%) and 80.1% of overall agreement.

The positive predictive and negative predictive values for the test are 95.3% (95% C.I. = 88.9%-98.2%%) and 72.8% (95% C.I. = 66.4%-78.4%), respectively.
A representative population of healthy controls (169 subjects) to determine the reference range was used to derive the Insudex® IA-2 Test cut-off value of 38 IU/mL.

Samples tested for clinical specificity

Condition	Number of Samples Evaluated	Number of Insudex® IA-2 Test Negative Results	Number of Insudex® IA-2 Test Positive Results
Type 2 diabetes	46	44	2
Hashimoto's Thyroiditis	20	19	1
SLE	15	15	0
Chronic Kidney disease	9	9	0
Grave's Disease	19	19	0
Myasthenia Gravis	10	10	0
Rheumatoid Arthritis	20	19	1
Healthy controls	30	30	0

PERFORMANCE CHARACTERISTICS

Precision

Lot-to-Lot Reproducibility

Insudex® IA-2 Test Lots		Concentration (IU/mL)				
		0	37	59	106	183
Lot#E100401	Mean	4.9	43.5	71.1	113.5	194.0
	SD	5.4	9.6	12.8	16.5	29.2
	%CV	110.6	22.1	18.0	14.6	15.1
Lot#E100501	Mean	4.6	39.4	65.1	106.3	185.5
	SD	5.7	7.3	8.5	14.0	24.0
	%CV	122.3	18.6	13.0	13.2	12.9
Lot#E100601	Mean	6.7	32.0	58.5	102.8	210.0
	SD	3.4	6.4	11.5	17.5	35.9
	%CV	50.5	20.0	19.7	17.0	17.1

Within-Lab Reproducibility

Lot E100401							
Sample	Concentration (IU/mL)↓	Within-Run			Day-to-Day		
		Mean	SD	%CV	Mean	SD	%CV
1	0	2.8	2.8	98	4.9	5.4	110.6
2	37	53.2	6.2	11.6	43.5	9.6	22.1
3	59	64.1	8.1	12.7	71.1	12.8	18
4	106	110.1	8.7	7.9	113.5	16.5	14.6
5	183	212.5	21.9	10.3	194	29.2	15.1

Lot E100501							
Sample	**Concentration (IU/mL)↓**	**Within-Run**			**Day-to-Day**		
		Mean	SD	%CV	Mean	SD	%CV
1	0	2.7	3.9	145.9	5	6	122.3
2	37	45.7	6.8	14.8	39	7	18.6
3	59	68.9	3.5	5.1	65	8	13
4	106	118.5	13.3	11.2	106	14	13.2
5	183	223.3	39.2	17.6	186	24	12.9
Lot E100601							
Sample	**Concentration (IU/mL)↓**	**Within-Run**			**Day-to-Day**		
		Mean	SD	%CV	Mean	SD	%CV
1	0	5.9	2.3	39.6	7	3	50.5
2	37	37.6	3.1	8.4	32	6	20
3	59	59.1	5.9	9.9	59	12	19.7
4	106	99.6	16.3	16.3	103	17	17
5	183	236.8	28.2	11.9	210	36	17.1

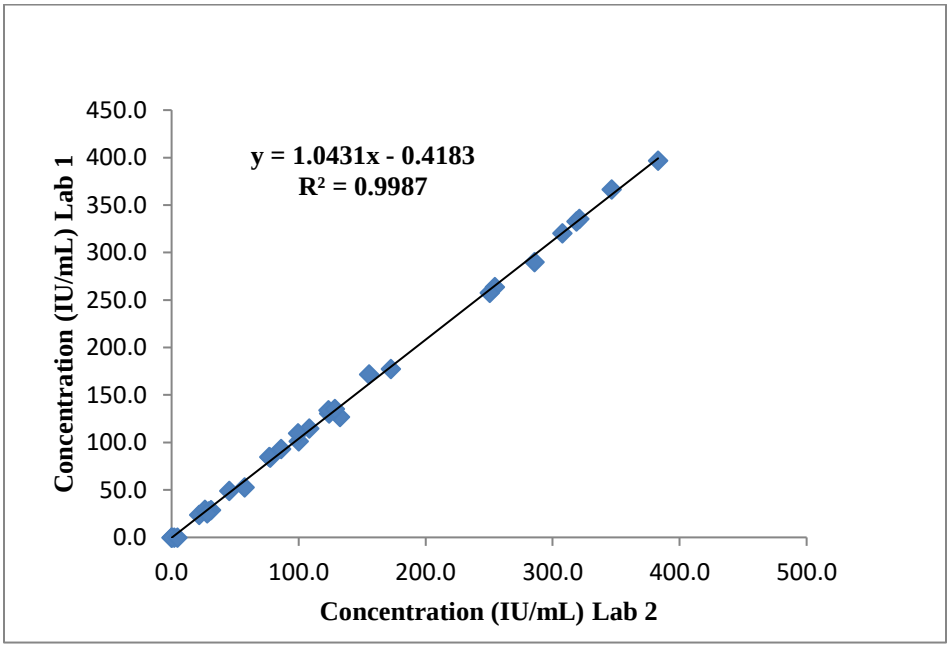
Operator-to-Operator Reproducibility

Lot#E100401		**Target Sample Concentration (IU/ml)**				
		0	**37**	**59**	**106**	**183**
Operator A	**Mean**	4.8	37.6	58.5	111.2	158.6
	SD	4.8	8.8	4.7	14.4	6.8
	CV%	100%	23%	8%	13%	4%
Operator B	**Mean**	1.9	44.4	77.1	117.0	199.7
	SD	2.8	7.6	12.5	11.0	16.1
	CV%	147%	17%	16%	9%	8%

Reader-to-Reader Reproducibility

Lot#E100401	**Replicates**	**Target Sample Concentration (IU/ml)**				
		0	**37**	**59**	**106**	**183**
Reader 996E	**Mean**	4.8	37.6	58.5	111.2	158.6
	SD	4.8	8.8	4.7	14.4	6.8
	CV%	100.4	23.4	8.1	12.9	4.3
Reader 6F26	**Mean**	4.3	38.7	64.2	114.0	157.4
	SD	4.1	11.0	2.8	7.5	12.1
	CV%	93.3	28.4	4.4	6.6	7.7
Reader 45CC	**Mean**	0.3	36.8	62.8	114.1	159.4
	SD	0.7	7.8	3.1	8.0	16.0
	CV%	223.6	21.1	4.9	7.0	10.0

Site-to-Site Correlation



Analytical Sensitivity

- Limit of Blank (LoB) = Avg. Blank + (SD of blank)*1.645 = 22.6 IU/mL
- Limit of Detection (LoD) calculated at 33.7 IU/mL as LoB + (SD of low concentration sample)*1.645 – the higher of the two calculations
- Limit of Quantitation (LoQ) = Lowest concentration of the measurand with percent CV less than or equal to 20% - the higher of the two calculations; summary table is below.

Limit of Quantitation Summary Data

Lot E100501	Mean (IU/mL)	SD	%CV
Low Positive 1	37.9	6.29	17
Low Positive 2	47.3	6.08	13
Low Positive 3	60.7	9.011	15
Low Positive 4	66.4	5.58	8
Lot E100601	**Mean (IU/mL)**	**SD**	**%CV**
Low Positive 1	32.8	3.55	11
Low Positive 2	41.3	4.98	12
Low Positive 3	51.8	6.24	12
Low Positive 4	61.2	7.19	12

LoQ Lot # E100501 = 37.9 IU/mL
LoQ Lot # E100601= 32.8 IU/mL

Test result display on Insudex® IA-2 reader

Result	Report
<38.0 IU/mL	Not detect, result <38 IU/mL
>38 IU/mL	IA-2 Ab detected, Positive, report result

Analytical Specificity

The high, clinically feasible levels listed for bilirubin (20 mg/dL), hemoglobin (500 mg/dL), insulin (1000 mg/mL) and intralipid (3000 mg/dL) represent their levels in serum or plasma, prior to the 1:6 dilution in sample buffer that is part of the test protocol. Compounds were determined not to interfere in the assay if the recovery rates did not deviate 20% from the control value.

Effect of potential interferents

Potential interferents	Replicate	Analyte Concentration Tested (IU/mL)→ Analyte Concentration Observed (IU/mL)↓			
		0	**59**	**106**	**183**
None (Control)	R1	15	63	100	182
	R2	7	66	103	241
	R3	7	73	108	194
	R4	5	72	105	184
	R5	0	77	104	222
	Average	6.5	70.1	104.1	204.4
	SD	5.2	5.4	3	25.9
	%CV	0.8	7.8	2.9	12.7
Bilirubin (20mg/dL)	R1	10	56	91	145
	R2	13	65	108	184
	R3	10	71	96	204
	R4	11	61	102	196
	R5	2	65	100	189
	Average	9.3	63.4	99.3	183.4
	Stdev	4.2	5.6	6.2	22.7
	%CV	0.5	8.8	6.2	12.4
Hemoglobin (500 mg/dL)	R1	0	61	116	194
	R2	0	66	110	165
	R3	0	69	121	173
	R4	7	79	98	218
	R5	0	74	124	208
	Average	1.4	69.6	114.1	191.6
	Stdev	3	7.1	10.3	22.2
	%CV	2.2	10.1	9	11.6
Insulin (10mg/mL)	R1	2	71	109	167
	R2	5	70	125	215
	R3	17	93	114	218
	R4	16	81	129	217
	R5	7	77	127	180

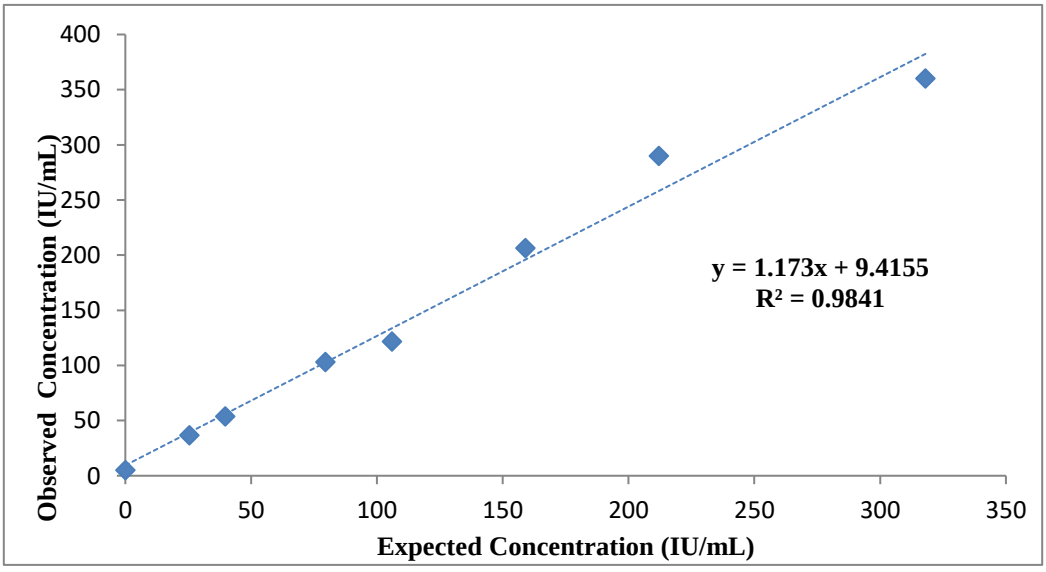
	Average	9.2	78.4	120.6	199.4
	Stdev	6.8	9.2	8.9	24.1
	%CV	0.7	11.8	7.4	12.1
Intralipid (1000 mg/dL)	R1	17	63	117	166
	R2	0	67	114	236
	R3	8	66	108	193
	R4	2	66	125	201
	R5	5	67	111	209
	Average	6.4	65.9	115.1	200.7
	Stdev	6.9	1.8	6.4	25.2
	%CV	1.1	2.8	5.6	12.6

Dilution and Recovery

Insudex® IA-2 calibrators were diluted with IA-2 antibody negative serum and assayed in the Insudex® IA-2 test. The results are shown in the table below. The recoveries ranged from 113.2% to 148.0 % with a mean recovery of 129.2%.

		Expected Concentration (IU/mL)							
Observed Concentration (IU/mL) ↓	**Replicate #**	**318**	**212**	**159**	**106**	**80**	**40**	**25**	**0**
	R1	277	271	197	112	83	45	25	0
	R2	337	301	253	107	94	52	45	4
	R3	466	297	169	146	133	65	40	11
	Average	360	290	207	122	103	54	37	5
	Stdev	97	16	43	21	26	10	11	6
	%CV	27	6	21	17	25	19	29	NA
	% Recovery	113.2	136.8	130.2	115.1	128.7	135	148	NA

The correlation between expected and observed concentrations for samples in the linearity test.



Hook Effect

Three high IA-2 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 380 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
	Contains sufficient for single test
	Contains sufficient for 20 tests

Manufactured for Diabetomics, Inc. by:

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