Insudex® IAA Test

Insulin Autoantibodies

Product Information Sheet
Instructions for Use

INSUDEX®

Diabetes Detection Redefined

REF 2049

CE IVD

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INTENDED USE

The Insudex® IAA Test is for the semi-quantitative determination of Insulin autoantibodies in human serum and blood. The assay is useful as an aid in the diagnosis of Type I diabetes mellitus (autoimmune mediated diabetes). The IAA 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]. IAA are most closely associated with risk of early-onset T1D, and are particularly useful in predicting risk of T1D in children [3]. Recent studies have shown that electro-chemiluminescent assays that employ proinsulin as the target antigen empirically recognize IAA that are more predictive of disease risk [4,5]. It is thought that this approach is more selective for high-affinity IAA resulting from autoimmunity to insulin or proinsulin rather than low-affinity IAA that may result from autoimmunity to cross-reacting antigens that are not associated with T1D risk and which are

routinely detected in standard radioimmunoassay's. The reason for this is postulated to be because use of proinsulin rather than mature insulin as the capture antigen allow more target epitopes to be accessible due to the target size of proinsulin, rather than the detection of proinsulin vs insulin-directed autoantibodies. Proinsulin-based assays may also identify a set of high-titer IAA patients who respond better to prophylactic insulin therapy [6]. The Insudex® IAA test exploits this aspect of T1D risk-related IAA by employing synthetic proinsulin as the target antigen rather than mature insulin.

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® IAA Test Key Card and these instructions. The test strip utilizes a combination of proinsulin antigen to detect anti-IAA antibodies in the sample and rabbit anti-IAA antibodies to serve as a procedural control. Each device contains proinsulin-colloidal gold conjugate pre-dried on a pad. Proinsulin antigen (on the Test Line) and rabbit anti-IAA polyclonal antibody (on the Control Line) are coated and immobilized on a nitrocellulose membrane.

PRINCIPLES OF THE TEST

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

- Insudex® IAA 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® IAA 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® IAA 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® IAA 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® IAA 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 Units per milliliter. It is recommended by Diabetomics that values greater than or equal to 30 U/mL be considered positive and values less than 30 U/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.

CLINICAL SENSITIVITY AND SPECIFICITY

Sera collected from 319 subjects were tested with Insudex® IAA Test. The sample cohort included:

- 135 Type I diabetics (newly diagnosed and/or confirmed Type I diabetes)
- 52 Type II diabetics
- 67 control non-diabetic subjects
- 65 patients (non-diabetic) with other autoimmune diseases, including Hashimoto's thyroiditis, Grave's disease, SLE and rheumatoid arthritis.

Of the 135 TIDM patient sera 76 (56.2%) (95% C.I.= 47.5%-64.7%) were positive in the Insudex® IAA Test. Using the clinical cut-of 30 U/mL used in TIDM, Type 2 diabetes and other autoimmune

diseases Insudex® IAA Test has a clinical sensitivity of 56.2% (95% C.I.= 47.5%-64.7%) and specificity of 96.7% (95% C.I.=92.6%-98.6%).

The positive predictive and negative predictive values for the test are 92.6% (95% C.I. = 84.1%-96.9%%) and 74.8% (95% C.I. = 68.7%-80.2%), respectively.
A representative population of healthy controls (172 subjects) to determine the reference range was used to derive the Insudex® IAA Test cut-off value of 30 U/mL.

Samples tested for clinical specificity

Condition	Number of Samples Evaluated	Number of Insudex® IAA Test Negative Results	Number of Insudex® IAA Test Positive Results
Type 2 diabetes	52	50	2
Hashimoto's Thyroiditis	17	16	1
SLE	16	15	1
Grave's Disease	20	20	0
Rheumatoid Arthritis	13	13	0
Healthy controls	67	65	2

PERFORMANCE CHARACTERISTICS

Precision

Lot-to-Lot Reproducibility

Insudex® IAA Test Lots		Concentration (U/mL)				
		0	23.4	46.8	95.6	374.4
Lot F030701	Mean	3.7	26.9	47.7	91.9	378.2
	SD	0.79	3.24	8.51	18.42	63.43
	%CV	21%	12%	18%	20%	17%
Lot F040801	Mean	1.7	23.9	47.2	95.3	376.2
	SD	1.00	5.34	8.39	15.63	51.67
	%CV	58%	22%	18%	16%	14%
Lot F040802	Mean	2.1	28.6	56.3	107.9	406.0
	SD	2.30	6.16	9.02	18.69	72.15
	%CV	112%	22%	16%	17%	18%

Within-Lab Reproducibility

Lot F030701							
Sample	Concentration (U/mL) ↓	Within-Run			Day-to-Day		
		Mean	SD	%CV	Mean	SD	%CV
1	0	3.8	0.90	24%	3.7	3.7	21%
2	23.4	26.7	3.34	13%	26.9	26.9	12%
3	46.8	43.4	7.44	17%	47.7	47.7	18%
4	95.6	101.7	14.47	14%	91.9	91.9	20%
5	374.4	345.8	59.51	17%	378.2	378.2	17%

Lot F040801							
Sample	**Concentration (U/mL) ↓**	**Within-Run**			**Day-to-Day**		
		Mean	SD	%CV	Mean	SD	%CV
1	0	0.8	0.94	115%	1.7	1.00	58%
2	23.4	24.3	4.56	19%	23.9	5.34	22%
3	46.8	50.5	2.56	5%	47.2	8.39	18%
4	95.6	90.1	12.00	13%	95.3	15.63	16%
5	374.4	359.3	45.84	13%	376.2	51.67	14%
Lot F040802							
Sample	**Concentration (U/mL) ↓**	**Within-Run**			**Day-to-Day**		
		Mean	SD	%CV	Mean	SD	%CV
1	0	0.2	0.29	149%	2.1	2.30	112%
2	23.4	25.3	5.02	20%	28.6	6.16	22%
3	46.8	55.2	9.87	18%	56.3	9.02	16%
4	95.6	96.8	11.33	12%	107.9	18.69	17%
5	374.4	360.5	57.63	16%	406.0	72.15	18%

Operator-to-Operator Reproducibility

Lot F040801		**Concentration (U/ml)**				
		0	**23.4**	**46.8**	**95.6**	**374.4**
Operator A	**Mean**	1.9	19.9	49.8	94.9	350.5
	SD	0.93	2.31	6.68	13.12	52.81
	CV%	50%	12%	13%	14%	15%
Operator B	**Mean**	1.1	15.1	30.1	73.8	310.8
	SD	1.76	5.82	1.16	15.90	65.90
	CV%	166%	38%	4%	22%	21%

Reader-to-Reader Reproducibility

Lot F040801		**Concentration (U/ml)**				
		0	**23.4**	**46.8**	**95.6**	**374.4**
Reader 996E	**Mean**	1.9	19.9	49.8	94.9	350.5
	SD	0.93	2.31	6.68	13.12	52.81
	CV%	50%	12%	13%	14%	15%
Reader 6F26	**Mean**	2.5	18.7	45.8	94.7	355.0
	SD	1.14	1.54	4.94	13.35	46.28
	CV%	46%	8%	11%	14%	13%
Reader 45CC	**Mean**	4.2	25.4	56.0	110.2	395.4
	SD	1.30	1.78	6.22	13.50	52.13
	CV%	31%	7%	11%	12%	13%

Analytical Sensitivity

- Limit of Blank (LoB) = Avg. Blank + (SD of blank) *1.645 = 6.4 U/mL
- Limit of Detection (LoD) calculated at 16.6 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 F040801	Mean (U/mL)	SD	%CV
Low Positive (12 U/mL)	13.5	3.83	28%
Low Positive (18 U/mL)	17.8	5.41	30%
Low Positive (24 U/mL)	21.8	3.77	17%
Low Positive (30 U/mL)	27.2	5.06	19%
Lot F040802	**Mean (U/mL)**	**SD**	**%CV**
Low Positive (12 U/mL)	18.7	5.13	27%
Low Positive (18 U/mL)	24.8	6.16	25%
Low Positive (24 U/mL)	31.9	6.79	21%
Low Positive (30 U/mL)	42.2	6.54	15%

LoQ Lot F040801 = 24 U/mL
LoQ Lot F040802 = 30 U/mL

Test result display on Insudex® IAA reader

Result	Report
<30 U/mL	Not detected, result <30 U/mL
>30 U/mL	IAA detected, Positive, report result

Analytical Specificity

The high, clinically feasible levels listed for bilirubin (20 mg/dL), hemoglobin (500 mg/dL), insulin (10 mg/mL) and intralipid (1000 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. Insulin interference was observed at 0.25 mg/mL.

Effect of potential interferents

Potential Interferents	Replicate	Analyte Concentration Tested (U/mL) → Analyte Concentration Observed (U/mL) ↓			
		0	**23**	**47**	**187**
None (Control)	**R1**	0	16	54	131
	R2	2	18	45	136
	R3	4	18	38	198
	R4	1	28	49	163

	R5	2	14	48	137
	Average	1.7	18.8	46.9	153
	SD	1.5	5.2	6.1	28.2
	%CV	-	27.5	12.9	18.4
Bilirubin (20 mg/dL)	**R1**	4	15	35	117
	R2	1	24	61	120
	R3	1	23	39	125
	R4	3	15	34	135
	R5	1	15	36	121
	Average	2.2	18.4	40.9	123.7
	SD	1.2	4.6	11.2	7.2
	%CV	-	24.8	27.5	5.8
Hemoglobin (500 mg/dL)	**R1**	1	17	50	137
	R2	1	14	38	124
	R3	0	19	42	141
	R4	2	15	40	125
	R5	0	12	54	128
	Average	0.7	15.5	44.7	130.8
	SD	0.7	2.6	7	7.4
	%CV	-	16.9	15.5	5.7
Intralipid (1000 mg/dL)	**R1**	3	18	52	159
	R2	3	20	70	129
	R3	3	21	49	142
	R4	3	18	55	136
	R5	5	28	52	137
	Average	3.5	21	55.5	140.8
	SD	0.7	3.9	8.3	11.3
	%CV	-	18.7	14.9	8
Insulin (10 mg/mL)	**R1**	0	3	7	29
	R2	0	1	9	21
	R3	0	7	13	20
	R4	2	8	13	21
	R5	3	7	10	22
	Average	1.1	5	10.6	22.4
	SD	1.2	2.7	2.4	3.6
	%CV	-	54.4	22.9	16

Dilution and Recovery

Insudex® IAA calibrators were diluted with IAA antibody negative serum and assayed in the Insudex® IAA test. The results are shown in the table below. The recoveries ranged from 88% to 122.0 % with a mean recovery of 103%.

		Expected Concentration (U/mL) →				
	Replicate #	**0**	**23.4**	**46.8**	**95.6**	**374.4**
Observed Concentration (U/mL) ↓	**R1**	2.2	30.7	38.7	72.8	402.6
	R2	2.7	25.0	55.3	86.1	386.8
	R3	3.7	30.1	43.9	92.4	393.3
	Average	2.9	28.6	46.0	83.8	394.2
	SD	0.76	3.17	8.49	10.01	51.67
	%CV	27%	11%	18%	12%	13%
	% Recovery	-	122%	98%	88%	105%

Hook Effect

A high IAA antibody positive serum sample was diluted in IAA negative serum to determine if there was any hook effect. No hook effect was observed for back calculated concentrations of up to 748 U/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:

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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