Insudex® C-Peptide

Product Information Sheet
Instructions for use

INSUDEX®

Diabetes Detection Redefined

REF 2013

CE IVD

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INTENDED USE

The Insudex® C-Peptide test kit is an immunoassay for the quantitative determination of C-peptide in human serum or fingerstick whole blood.

The assay is intended for use as an aid in the diagnosis and treatment of patients with abnormal insulin secretion, including diabetes mellitus.

INTRODUCTION, BACKGROUND, AND CLINICAL SIGNIFICANCE

In the beta cells of the pancreas, the proinsulin molecule is cleaved to form insulin and C-peptide. C-peptide, a polypeptide consisting of 31 amino acids (Mw 3020), is stored in the secretory granules of the beta cells and released into circulation in equimolar amounts with insulin. C-peptide plays an important role in the synthesis and functionality of insulin. C-peptide ensures the correct structure of insulin by aligning the A and B chains of the insulin molecule so that the correct interchain disulfide bonds form. Cleavage of C-peptide from proinsulin exposes the B chain C-terminal end that allows insulin to interact with the insulin receptors.[1,2,3]
Insulin determinations in insulin-treated diabetics present special problems because of interferences by exogenous insulin and circulating antibodies to insulin. C- peptide assays are not subject to significant interference from the insulin antibodies detected in patients on insulin

therapy. The determination of C-peptide provides an assessment of endogenous insulin secretory reserves in patients with diabetes mellitus and is considered a more reliable indicator of insulin secretion than insulin itself. Insulin and C-peptide are secreted in equimolar quantities into the portal blood supply that passes through the liver. The liver extracts a considerable and variable amount of insulin; however, almost all of the C-peptide emerges from the liver and enters the systemic circulation. C-peptide has a half-life of ~35 minutes as compared to the half-life of insulin which is ~5 to 10 minutes. C-peptide is excreted in the urine.[1,2]

Serum and urine C-peptide determinations, in conjunction with blood glucose and insulin levels, aid in the differential diagnosis of hypoglycemia. C -peptide levels can be important in diagnosing overzealous insulin treatment since C-peptide does not exist in commercial insulin preparations. In these cases, insulin levels are elevated and C-peptide levels are low. Elevated insulin levels and low C-peptide levels are also observed in patients with detectable insulin autoantibodies and postprandial hyperglycemia. C-peptide levels are better indicators of beta-cell function and capacity than peripheral insulin levels. C-peptide has no insulin-like action but can be measured in the blood in patients receiving insulin to estimate residual beta-cell function. C-peptide levels increase in insulinomas and beta-cell tumors.[1,2,4]

SUMMARY OF THE TEST

The device consists of: Test strip in a plastic cartridge housing in a foil pouch with desiccant, a vial containing sample buffer, a mixing vial, the Insudex® C-Peptide Test Calibration Card and these instructions. The test strip has a mouse monoclonal anti-C-Peptide antibody immobilized at the Test Line and an anti-Mouse IgG immobilized at the Control Line. Another mouse monoclonal anti-C-Peptide antibody is immobilized on colloidal gold nanoparticles dried down on a separate membrane.

PRINCIPLES OF THE TEST

The Insudex® C-Peptide device is a two-site sandwich immunoassay. When a diluted sample is applied to the Test Cartridge Sample Application Port, colloidal gold nanoparticles coated with an anti-C-peptide monoclonal antibody are rehydrated and interact with C-peptide in the sample resulting in colloidal gold-C-peptide complexes. These complexes migrate via capillary action to the test membrane where another anti-C-peptide monoclonal antibody is bound at the Test Line allowing capture of the colloidal gold-C-peptide complexes forming a reddish-purple line. The color intensity of the Test Line measured by the Insudex® Reader is proportional to the concentration of C-peptide in the sample. The Reader, using lot-specific calibration data, converts this color reading into a test result that is displayed on the screen.

MATERIALS PROVIDED

The Insudex® C-Peptide Test is available in 20 test kits.
Each box contains:

- Insudex® C-Peptide Test Cartridge in foil pouch with desiccant (1 per test)
- Vial containing Sample Buffer (1 vial with 1 mL Sample Buffer per test)
- Empty tube for mixing sample with buffer (1 per test)
- Instructions for use (this document) (1 per box)
- Insudex® C-Peptide Calibration Card (2 per box, 1 for serum and 1 for fingerstick whole blood)

MATERIALS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Insudex® Reader, REF 2005
- Pipettes capable of delivering 15 µL, 120 µL and 135 µL (additionally, 30 µL for fingerstick)
- Timer
- Quality Control Materials – Please refer to the Product Insert for the Insudex® C-Peptide Control Kit for directions for use.
- Lancet

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.
- 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.
- If the preservative bag is missing, DO NOT USE. Discard test device and use a new test device.
- Do not use any test device if the cartridge pouch has been perforated.

- 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® C-Peptide Test is designed for use with serum or fingerstick whole blood.
- If testing frozen serum samples, allow specimen to thaw completely and thoroughly mix prior to sampling.

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 (15 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 15 µL serum to the mixing tube and mix by aspirating the sample and Sample Buffer mixture up and down 6-8 times.
6. 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 120 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® C-Peptide 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 ‘ACTIVATE BARCODE READ’. The barcode reader will start the scanning using a light that will disappear when the barcode has been read. If the confirmed cartridge doesn’t correspond to the configuration currently loaded in the reader, an error message will be displayed. In such a case, use a cartridge of the proper type, or load a new configuration corresponding to the cartridge you will use.
10. 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.
11. Open the foil pouch, remove the cartridge and place it on a flat surface.
12. Pipette 120 µL of the diluted sample into the sample well of the cartridge.
13. 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.
14. Press the ‘Timed Scan’ option.
15. 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.
16. 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.
17. After completing the test, discard the cartridge into a biohazard container.

EXPECTED RESULTS

The Insudex C-Peptide test is calibrated against the WHO 1st International Standard for Human C-Peptide code 13/146, established in 2015 from the National Institute for Biological Standards and Control (NIBSC).

Every lot of Insudex C-Peptide has a bar coded label containing specific information for calibration of the particular reagent lot.

Calculation: The reader automatically calculates the C-Peptide concentration of each sample in ng/mL using the calibration curve data contained on the Test Calibration Card.

Conversion factors
ng/mL (µg/L) x 0.33333 = nmol/L
ng/mL x 333.33 = pmol/L
nmol/L x 3.0 = ng/mL
pmol/L x 0.003 = ng/mL

Results are reported in ng/mL. The literature reports normal adult fasting levels as 0.78-1.89ng/mL. 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

Insudex® C-Peptide test was compared to the Siemens ADVIA Centaur CpS assay on 200 subjects. The sample cohort included:

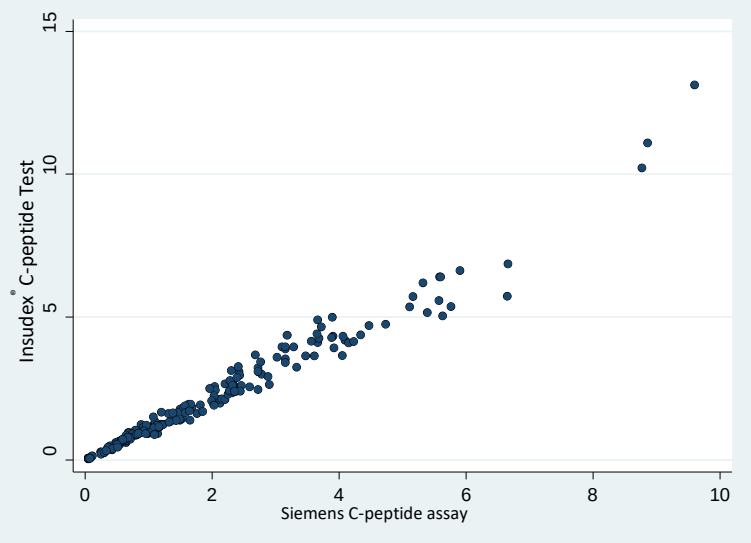
- 155 healthy controls
- 22 Type 1 diabetics (newly diagnosed and/or confirmed Type 1 diabetes)
- 23 Type 2 diabetics

Table 1. Comparison between Insudex® C-Peptide test and Siemens ADVIA Centaur CpS test

	# Samples	Median	25th percentile	75th percentile
Insudex® C-peptide Test, ng/ml	200	1.64	0.87	3.10
Siemens CpS test, ng/ml	200	1.49	0.71	2.72

Statistical method used for comparison: Pearson correlation coefficient (PCC), also referred to as Pearson's r, is used to assess the correlation between Insudex® C-peptide Test against the predicate Siemens CpS test values. PCC is a statistic that measures linear correlation between two variables X and Y. It has a value ranging from -1 to 1 (-1<r<1), where 1 is total positive linear correlation, 0 is no linear correlation, and −1 is total negative linear correlation.

Figure 1. Correlation between results obtained in the Insudex C-Peptide assay and the Siemens CpS assay



The correlation coefficient r=0.9831

PERFORMANCE CHARACTERISTICS

Precision

Table 2. Lot-to-Lot Reproducibility

		Target Sample Concentration (ng/mL)						
		0.00	**0.17**	**0.34**	**0.95**	**3.18**	**6.35**	**11.42**
Lot#CPP200101	**Mean**	0.01	0.18	0.35	0.94	3.31	6.73	10.85
	SD	0.01	0.02	0.02	0.04	0.10	0.31	0.74
	% CV	103.64	12.59	5.14	4.22	3.12	4.67	6.86
Lot#CPP200102	**Mean**	0.003	0.18	0.37	0.98	3.02	6.41	11.99
	SD	0.004	0.03	0.05	0.02	0.25	0.23	1.99
	% CV	141.52	14.17	14.14	1.58	8.21	3.65	16.60
Lot#CPP200103	**Mean**	0.01	0.17	0.37	0.98	3.26	6.64	10.21
	SD	0.01	0.01	0.03	0.06	0.19	0.24	1.24
	% CV	68.36	3.65	8.20	5.88	5.73	3.60	12.16

Table 3. Within-Lab Reproducibility

Lot CPP200101		**Within-Run**			**Day-to-Day**		
Sample	**Concentration (ng/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**
1	**0.0**	0.01	0.01	103.64	0.01	0.01	187.21
2	**0.2**	0.18	0.02	12.59	0.18	0.03	15.93
3	**0.3**	0.35	0.02	5.14	0.35	0.04	11.32
4	**1.0**	0.94	0.04	4.22	0.97	0.08	8.47
5	**3.2**	3.31	0.10	3.12	3.21	0.26	7.99
6	**6.4**	6.73	0.31	4.67	6.29	0.49	7.82
7	**11.4**	10.85	0.74	6.86	11.41	1.23	10.76
Lot CPP200102		**Within-Run**			**Day-to-Day**		
Sample	**Concentration (ng/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**
1	**0.0**	0.003	0.004	141.52	0.005	0.01	197.64
2	**0.2**	0.18	0.03	14.17	0.17	0.02	12.95
3	**0.3**	0.37	0.05	14.14	0.34	0.00	10.21
4	**1.0**	0.98	0.02	1.58	0.93	0.00	7.49
5	**3.2**	3.02	0.25	8.21	3.16	0.23	7.21
6	**6.4**	6.41	0.23	3.65	6.39	0.51	7.9
7	**11.4**	11.99	1.99	16.60	11.76	1.42	12.05
Lot CPP200103		**Within-Run**			**Day-to-Day**		
Sample	**Concentration (ng/mL) ↓**	**Mean**	**SD**	**% CV**	**Mean**	**SD**	**% CV**
1	**0.0**	0.01	0.01	68.36	0.01	0.01	94.87
2	**0.2**	0.17	0.01	3.65	0.17	0.02	13.53
3	**0.3**	0.37	0.03	8.20	0.33	0.04	10.55
4	**1.0**	0.98	0.06	5.88	0.97	0.09	9.55
5	**3.2**	3.26	0.19	5.73	3.17	0.26	8.20
6	**6.4**	6.64	0.24	3.60	6.37	0.56	8.82
7	**11.4**	10.21	1.24	12.16	11.07	1.48	13.34

Table 4. Operator-to-Operator Reproducibility

		Sample Concentration (ng/mL)						
		0	**0.17**	**0.34**	**0.95**	**3.18**	**6.35**	**11.42**
Technician A	**Mean**	0.01	0.18	0.35	0.94	3.31	6.73	10.85
	SD	0.01	0.02	0.02	0.04	0.10	0.31	0.74
	% CV	103.64	12.59	5.14	4.22	3.12	4.67	6.86
Technician B	**Mean**	0.01	0.18	0.35	0.97	3.17	6.02	10.74
	SD	0.01	0.02	0.04	0.06	0.26	0.48	0.46
	% CV	115.22	12.84	11.01	6.01	8.12	7.99	4.32

Table 5. Reader-to-Reader Reproducibility

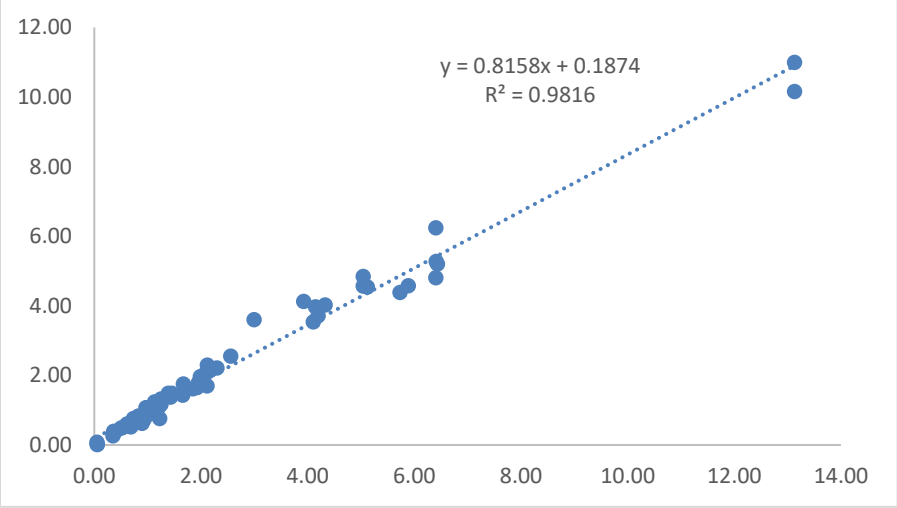
		Sample Concentration (ng/mL)						
		0	**0.17**	**0.34**	**0.95**	**3.18**	**6.35**	**11.42**
Reader 393	**Mean**	0.01	0.18	0.35	0.94	3.31	6.73	10.85
	SD	0.01	0.02	0.02	0.04	0.10	0.31	0.74
	% CV	103.64	12.59	5.14	4.22	3.12	4.67	6.86
Reader 9150	**Mean**	0.00	0.17	0.34	0.91	3.30	6.74	11.09
	SD	0.003	0.02	0.02	0.04	0.12	0.27	0.76
	% CV	130.77	9.54	6.01	4.47	3.72	4.05	6.86
Reader 47C6	**Mean**	0.02	0.19	0.36	0.94	3.26	6.62	10.68
	SD	0.01	0.02	0.02	0.04	0.11	0.29	0.58
	% CV	73.83	12.84	5.45	4.26	3.29	4.38	5.47

Table 6. Site-to-Site reproducibility

		Observed Concentration (ng/mL)						
Lab		**0.0**	**0.2**	**0.3**	**1.0**	**3.2**	**6.4**	**11.4**
1	**Mean**	0.003	0.19	0.35	0.97	3.24	6.40	11.16
	SD	0.01	0.02	0.03	0.08	0.21	0.43	0.99
	% CV	167.36	10.51	7.33	8.16	6.50	6.69	8.84
2	**Mean**	0.03	0.19	0.31	0.90	2.91	5.24	9.17
	SD	0.02	0.01	0.03	0.09	0.30	0.56	1.03
	% CV	44.61	7.65	8.83	9.63	10.28	10.63	11.22

The correlation graph for the data from the two laboratories that tested the 61 neat clinical serum samples is shown below in Figure 2. The regression co-efficient is 0.98 and the correlation is y = 0.8158x + 0.1874.

Figure 2. Site-to-Site Correlation



Analytical Sensitivity

- Limit of Blank (LoB) = Avg. Blank + (SD of blank) *1.645 = 0.006 + 0.009*1.645= 0.021

- Limit of Detection (LoD) calculated at 0.05 ng/mL as LoB + (SD of low concentration sample) *1.645- the higher of the three calculations

LoD Lot # CPP200101 = 0.006 +(0.020)*1.645 = 0.039
LoD Lot # CPP200102 = 0.005 +(0.012)*1.645 = 0.025
LoD Lot # CPP200103 = 0.021 +(0.016)*1.645 = 0.048

- 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 tables are below.

Table 7. Limit of Quantitation Summary Data

Lot #	Standard (ng/mL)	Mean (ng/mL)	SD	%CV
CPP200101	0.17	0.17	0.02	11.72
	0.34	0.33	0.02	7.36
	0.42	0.42	0.03	7.68
CPP200102	0.17	0.16	0.01	7.64
	0.34	0.32	0.03	7.98
	0.42	0.42	0.03	6.19
CPP200103	0.17	0.17	0.02	9.68
	0.34	0.32	0.02	7.82
	0.42	0.42	0.04	9.35

LoQ Lot # CPP200101 = 0.17 ng/mL
LoQ Lot # CPP200102 = 0.16 ng/mL
LoQ Lot # CPP200103 = 0.17 ng/mL

The Limit of Quantitation (LoQ, analytical) was calculated to be 0.17 ng/mL as this is where the %CV did not exceed 20%.

Analytical Specificity

The high, clinically feasible levels listed for bilirubin (24 mg/dL), hemoglobin (250 mg/dL), triglycerides (1000 mg/dL), insulin (375 µU/mL), proinsulin (1.25 ng/mL), glucagon (2500 pg/mL), calcitonin (500 pg/mL), somatostatin (12.5 ng/mL and secretin (6250 ng/mL) represent their levels in serum or plasma, prior to the 1:10 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.

Table 8. Effect of potential interferents

Potential interferents ↓	Analyte concentration (expected (ng/mL) → observed (ng/mL) ↓)					
	Replicate #	**0**	0.16	0.88	3.42	10.44
None (Control)	R1	0.02	0.15	0.95	3.42	9.60
	R2	0.01	0.15	0.88	3.44	12.29
	R3	0.01	0.18	0.93	3.38	9.84
	Avg	0.01	0.16	0.92	3.41	10.58
	Std dev	0.003	0.02	0.04	0.03	1.49
	% CV	23	12.60	4.01	0.86	14.08
Bilirubin (24 mg/dL)	R1	0.07	0.21	0.80	3.07	10.45
	R2	0.04	0.19	0.90	2.90	11.28
	R3	0.04	0.20	0.83	3.33	11.36
	Avg	0.05	0.20	0.84	3.10	11.03
	Std dev	0.02	0.01	0.05	0.22	0.51
	% CV	33.38	6.36	6.03	6.95	4.60
	% Recovery		121.57	95.53	90.64	105.61
Calcitonin (500 pg/mL)	R1	0.00	0.17	0.85	2.96	9.71
	R2	0.00	0.18	0.87	3.23	9.94
	R3	0.01	0.14	0.86	3.40	9.80
	Avg	0.003	0.16	0.86	3.20	9.82
	Std dev	0.003	0.02	0.01	0.22	0.11
	% CV	107.45	11.12	0.75	6.88	1.16
	% Recovery		100.94	97.63	93.48	93.97
Glucagon (2500 pg/mL)	R1	0.03	0.13	0.86	3.44	10.44
	R2	0.00	0.16	0.81	3.65	12.11
	R3	0.02	0.14	0.88	3.29	10.60
	Avg	0.02	0.14	0.85	3.46	11.05
	Std dev	0.01	0.02	0.04	0.18	0.92
	% CV	89.05	13.04	4.25	5.26	8.35
	% Recovery		88.31	96.95	101.25	105.78
Hemoglobin (250 mg/dL)	R1	0.00	0.16	0.86	3.61	9.98
	R2	0.04	0.15	0.84	3.57	12.94
	R3	0.03	0.17	0.85	3.45	10.18
	Avg	0.02	0.16	0.85	3.54	11.03
	Std dev	0.02	0.01	0.01	0.09	1.65
	% CV	89.59	4.80	1.42	2.43	14.96
	% Recovery		98.42	96.67	103.64	105.63
Insulin (375 µU/mL)	R1	0.04	0.18	0.75	2.93	10.61
	R2	0.00	0.13	0.84	2.81	10.05
	R3	0.00	0.17	0.87	3.23	10.11
	Avg	0.01	0.16	0.82	2.99	10.26

Potential interferents ↓	Analyte concentration (expected (ng/mL) → observed (ng/mL) ↓)					
	Replicate #	**0**	0.16	0.88	3.42	10.44
	Std dev	0.02	0.03	0.07	0.22	0.31
	% CV	160.00	15.69	7.97	7.22	3.02
	% Recovery		99.66	93.00	87.47	98.22
Intralipid (1000 mg/dL)	R1	0.04	0.15	0.89	3.15	12.33
	R2	0.04	0.23	0.95	3.59	11.23
	R3	0.03	0.19	0.90	3.23	9.35
	Avg	0.03	0.19	0.91	3.32	10.97
	Std dev	0.01	0.04	0.03	0.23	1.51
	% CV	23.77	19.33	3.79	7.03	13.74
	% Recovery		117.28	103.69	97.21	105.05
Proinsulin (1.25 ng/mL)	R1	0.01	0.15	0.85	3.25	8.99
	R2	0.03	0.20	0.89	3.20	12.04
	R3	0.02	0.15	0.87	3.58	11.64
	Avg	0.02	0.17	0.87	3.35	10.89
	Std dev	0.01	0.03	0.02	0.21	1.65
	% CV	34.82	17.92	2.39	6.18	15.18
	% Recovery		102.19	98.55	97.90	104.25
Secretin (6250 ng/mL)	R1	0.04	0.18	0.89	3.30	10.09
	R2	0.02	0.19	0.90	3.68	9.90
	R3	0.05	0.17	0.82	3.65	11.35
	Avg	0.04	0.18	0.87	3.54	10.45
	Std dev	0.02	0.01	0.05	0.21	0.79
	% CV	45.54	6.87	5.28	5.98	7.52
	% Recovery		110.01	98.68	103.67	100.04
Somatostatin 14 (12.5 ng/mL)	R1	0.00	0.15	0.74	3.23	11.61
	R2	0.01	0.16	0.86	3.03	10.06
	R3	0.00	0.13	0.79	3.56	10.91
	Avg	0.004	0.15	0.80	3.27	10.86
	Std dev	0.004	0.02	0.06	0.27	0.78
	% CV	123.73	11.66	7.27	8.11	7.15
	% Recovery		89.83	90.41	95.67	103.96
Somatostatin 28 (12.5 ng/mL)	R1	0.00	0.17	0.79	3.11	10.28
	R2	0.00	0.17	0.82	3.30	9.81
	R3	0.01	0.12	0.80	3.45	12.27
	Avg	0.002	0.15	0.80	3.29	10.79
	Std dev	0.003	0.03	0.01	0.17	1.31
	% CV	155.26	19.11	1.61	5.24	12.12
	% Recovery		94.10	91.16	96.19	103.29

Dilution and Recovery

For evaluating linearity, four serum pools at different C-Peptide concentrations were diluted in C-Peptide depleted normal human serum to obtain 6-8 dilutions each across the measuring range of the assay. The concentrations in the range tested were 0 to 11 ng/mL. These samples were tested in triplicates in the Insudex® C-Peptide Test, as per directions.

Table: 9 A	Sample C1, expected concentration →, Observed Concentration ↓ (ng/mL)						
Samples →	**1.50**	**1.20**	**0.90**	**0.60**	**0.45**	**0.30**	**0.15**
R1	1.75	1.49	0.98	0.72	0.53	0.37	0.22
R2	1.59	1.35	0.98	0.66	0.52	0.36	0.22
R3	1.83	1.28	0.91	0.69	0.57	0.36	0.17
Avg	1.73	1.37	0.96	0.69	0.54	0.36	0.20
Std dev	0.12	0.11	0.04	0.03	0.03	0.01	0.03
%CV	7.09	7.96	4.44	4.30	4.78	2.04	14.93

Table: 9 B	Sample D, expected concentration →, Observed Concentration ↓ (ng/mL)					
Samples →	**3.18**	**2.54**	**1.91**	**1.27**	**0.64**	**0.32**
R1	2.72	2.41	1.74	1.28	0.59	0.30
R2	2.99	2.27	1.49	1.06	0.59	0.30
R3	2.85	2.24	1.62	1.27	0.57	0.31
Avg	2.85	2.30	1.62	1.20	0.58	0.30
Std dev	0.14	0.09	0.12	0.12	0.01	0.01
%CV	4.84	3.97	7.71	10.15	2.24	2.88

Table: 9 C	Sample E1, expected concentration →, Observed Concentration ↓ (ng/mL)						
Samples →	**6.35**	**5.72**	**4.45**	**3.18**	**2.54**	**1.91**	**1.27**
R1	6.50	5.66	4.10	3.11	2.42	1.88	1.20
R2	5.21	4.80	3.77	3.19	2.14	1.71	1.12
R3	5.15	4.75	3.90	2.89	2.27	1.69	1.22
Avg	5.62	5.07	3.92	3.06	2.27	1.76	1.18
Std dev	0.76	0.51	0.17	0.15	0.14	0.10	0.05
%CV	13.56	10.13	4.27	4.97	6.22	5.91	4.35

Table: 9 D	Sample E2, expected concentration →, Observed Concentration ↓ (ng/mL)						
Samples →	**10.28**	**9.14**	**7.99**	**6.85**	**5.71**	**4.57**	**3.43**
R1	10.47	7.11	6.73	6.02	5.63	3.88	2.89
R2	8.30	7.11	6.16	7.05	4.59	3.97	3.20
R3	9.73	6.97	6.94	5.62	5.15	4.04	3.20
Avg	9.50	7.06	6.61	6.23	5.12	3.96	3.10
Std dev	1.10	0.08	0.40	0.74	0.52	0.08	0.18
%CV	11.60	1.10	6.10	11.84	10.23	1.94	5.82

Table 1: The correlation between expected and observed concentrations for samples in the linearity test.

Sample	Correlation	R^2
Sample C1	y = 1.1191x + 0.0193	R^2 = 0.9966
Sample D	y = 0.8871x + 0.0196	R^2 = 0.9973
Sample E1	y = 0.8674x + 0.1218	R^2 = 0.9974
Sample E2	y = 0.8408x + 0.1798	R^2 = 0.9524

Hook Effect

For evaluating hook effect, the test was conducted with three clinical specimens of very high concentration (up to ~160 ng/mL), by spiking reference standard material into a high sample of known concentration. Each specimen was tested in up to 128-fold serial dilutions. No high dose hook effect was seen up to 80 ng/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, and patient age.

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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

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