IVD

CE

Glucagon - RIA

RB310

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

Previous Version: **191011-1**	**Current Version:** **200224-1**
No IVD logo	IVD logo added
LOT	Version :
No history	History added
	Addition of the following sentence at the end of the English IFU: "Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/"

CE

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Read entire protocol before use.

Glucagon RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of glucagon in human plasma.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Glucagon RIA

B. Catalog number : RB310 : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. CLINICAL BACKGROUND

A. Biological activities

Glucagon is a 29 amino acids straight chain peptide produced in the pancreatic α-cells (1,2). Glucagon is cleaved out from preproglucagon with 159 amino acids. The amino acid sequence of glucagon is found in glicentin, a 69 amino acid peptide (3). Glicentin has been proposed to be a biosynthetic intermediate for pancreatic and gut glucagon.
Increases in the plasma glucagon level affect glucose production first by stimulating a transient phase of glycogenolysis and then a prolonged period of glyconeogenesis (4,5).
A sustained increase in the glucagon level continues to modulate hepatic glucose production (6).
Glucagon also plays a role in the amino acid metabolism. Elevation of glucagon in plasma decreases amino acids whereas glucagon deficiency increases amino acids (7,8,9). The amino acid sequence of human pancreatic glucagon: His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr.

B· Clinical application

Glucagon is involved in carbohydrate, fat and protein metabolism. Basal amounts of glucagon are essential for the maintenance of normoglycemia and a physiological role for glucagon is to prevent hypoglycemia.
Pancreatectomy do not cause totally glucagon deficiency. However, the concentrations in plasma are significantly lower than in normals (7,10).
Since glucagon in diabetics has been found elevated absolutely or relatively to insulin, it has been proposed that glucagon contributes essentially to the development of the hyperglycemia and keto acidosis found in diabetes (11,12,13). Elevated levels of glucagon in plasma are found in patients with A-cell tumors (8).
The test should not be relied upon as the sole basis of decisions on clinical therapy, but should be used in combination with clinical symptoms and the results of other available tests.

IV. PRINCIPLES OF THE METHOD

Glucagon in plasma is assayed by the competitive radioimmunoassay using a rabbit antiserum raised against a glucagon-albumin conjugate. Glucagon in calibrators and samples compete with ^{125}I-labelled glucagon in binding to the antibodies in a two steps incubation.
^{125}I -glucagon binds in a reverse proportion to the concentration of glucagon in calibrators and samples . Antibody-bound ^{125}I -glucagon is separated from the unbound fraction using double antibody solid phase. The radioactivity of the bound fraction is measured in a gamma counter.
The antiserum used in this assay shows less than 0.1% cross reaction with gut-GLI (14).
For professional use within a laboratory.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
Ab Anti-glucagon : Rabbit antiserum raised against porcine glucagon, conjugated to human serum albumin in glycin buffer with sodium azide and aprotinin.	1 vial lyophilised	Blue	**Add** 52 ml distilled water
Ag | ^{125}I TRACER: ^{125}Iodine labelled glucagon in glycin buffer with human serum albumin, sodium azide and aprotinin.	1 vial lyophilised 28 kBq	Red	**Add** 52 ml distilled water
DASP Double antibody solid phase : Anti-rabbit-Ig coupled to cellulose particles in phosphate buffer with human serum albumin, NaCl, NaN_3, EDTA and Tween 80.	1 vial 11 mL	Green	**Ready** for use
ASS BUF Assay diluent : glycin buffer containing human serum albumin, sodium azide and aprotinin. To be used for the preparation of glucagon working calibrators and instead of antiserum in non-specific binding control tubes.	1 vial 50 mL	Black	**Ready** for use
CAL Glucagon calibrator in glycin buffer containing human serum albumin, sodium azide (<0.1%) and aprotinin.	1 vial lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on vial label
CONTROL N Controls - N = 1 or 2 Contains sodium azide.(<0.1%).	2 vials lyophilised	Silver	**Add** 2 mL distilled water

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Disposable test tubes of polystyrene: 11-13x55 mm
3. Pipettes with disposable tips: 200 and 500 µL
4. Pipettes 1 mL and 5 mL (for calibrator preparation)
5. Vortex mixer
6. Centrifuge, refrigerated, giving a minimum of 1700 x g.
7. Gamma counter

VII. REAGENT PREPARATION

A. Anti-Glucagon : Reconstitute with 52 mL of distilled water. Store at 2-8°C.
B. ^{125}I-Glucagon : Reconstitute with 52 mL of distilled water. Store at -18° C or lower if reused.
C. Double antibody solid phase : Ready for use. Stir continuously during pipetting this reagent. Store at 2-8° C.
D. Assay diluent : Ready for use. Store at 2-8° C.
E. Glucagon calibrator : Reconstitute with distilled water by the volume stated on vial label. Store at -18° C or lower if reused.
F. Controls : Reconstitute with 2.00 mL distilled water. Store at -18° C or lower if reused.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

Store all reagents at 2-8° C before reconstitution and use.
The water used for reconstitution of the lyophilized reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the contents in the vials by gentle inversion and avoid foaming.
The stability for each reagent is found on the label of the vial. For the lyophilized reagents the expiry date is valid for the unreconstituted reagents. The reconstituted reagents are stable for 10 weeks (or to the expiry date for the labelled glucagon) when stored.

IX. SPECIMEN COLLECTION

Vein blood is collected in tubes containing EDTA and aprotinin. The sample is cooled in an ice-bath immediately. Plasma is separated by centrifugation (refrigererated centrifuge is preferred). The plasma should be frozen within 2 hours and stored at -18° C or lower until assayed. Repeated freezing and thawing must be avoided.

X. PROCEDURE

A. Handling notes

Reconstitute the reagents as specified. Accuracy in all pipetting steps is essential.
All tests (calibrators, samples and controls) should be performed in duplicate.
A complete assay includes:
Calibrator : 7 concentrations: 0, 4.7, 9.4, 18.8, 37.5, 75, 150 pmol/L
(= 0, 16.3, 32.6, 65.3, 131, 261, 522 pg/mL).
Controls : Two controls with known concentrations of glucagon for quality control.
Sample
Tubes for determination of the *non-specific binding*
Tubes for determination of the *total radioactivity* added

B. Procedure

1. Reconstitute the reagents according to the instructions.
2. Prepare the glucagon working calibrators by dilution of the 300 pmol/L calibrator with the assay diluent according to the following:
   a/ 1.00 mL calibrator 300 pmol/L + 1.00 mL assay diluent = 150 pmol/L
   b/ 1.00 mL calibrator 150 pmol/L + 1.00 mL assay diluent = 75 pmol/L
   c/ 1.00 mL calibrator 75 pmol/L + 1.00 mL assay diluent = 37.5 pmol/L
   d/ 1.00 mL calibrator 37.5 pmol/L + 1.00 mL assay diluent = 18.8 pmol/L
   e/ 1.00 mL calibrator 18.8 pmol/L + 1.00 mL assay diluent = 9.4 pmol/L
   f/ 1.00 mL calibrator 9.4 pmol/L + 1.00 mL assay diluent = 4.7 pmol/L
   g/ Assay diluent = 0 pmol/L
   Store the calibrator solutions a-g and the 300 pmol/L calibrator at -18° C or lower if reused.
3. Pipette 200 µL of the calibrators a-g, samples and controls in their respective tubes (duplicates).
4. Pipette 200 µL of the assay diluent in the NSB-tubes for calibrator.
5. Pipette 500 µL anti-glucagon in all tubes except the NSB- and TOT-tubes.
6. Pipette 500 µL assay diluent in the NSB-tubes.
7. Vortex-mix and incubate for 20-24 hours at 2-8° C.
8. Pipette 500 µL ^{125}I-Glucagon in all tubes. The TOT-tubes are sealed and kept aside.
9. Vortex-mix and incubate for 20-24 hours at 2-8° C.
10. Add 100 µL double antibody solid phase to all tubes except the TOT-tubes. Stir continuously during pipetting this reagent
11. Vortex-mix and incubate for 30-60 minutes at 2-8° C.
12. Centrifuge the tubes for 15 minutes at +4° C (1700 x g).
    Note: The correct centrifugation force is important for accurate performance.
13. Decant the liquid immediately after centrifugation.
    Note: The accurateness and coherency in handling of supernatants are crucial for the assay precision.
14. Count the radioactivity of the pellets in a gamma counter. The counting time should be at least 2 minutes.

XI. CALCULATION OF RESULTS

1. Substract the average count rate (CPM) of the non-specific binding tubes for calibrator from the count rate (CPM) of the replicates of the calibrator tubes, the sample tubes and control tubes.
2. A calibration curve is generated by plotting the bound CPM (in CPM or % B/TOT) against the concentration of the glucagon calibrators.

3. Interpolate the glucagon concentrations in the samples and controls from the generated calibration curve.
4. The calibration curve and the calculation of the concentrations of the samples can be done by a suitable computer program. A spline algorithm may be used.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Tube no	Type of tube	Concentration pmol/L	CPM (raw)	B/TOT x 100
1	NSBst	-	684	5.5%
2	"		653	5.3%
3	TOT	-	12301	B-NSB / TOT-NSB x 100
4	"		12347	
5	Cal	0.0	6253	50.7%
6	"	"	6203	50.3%
7	Cal	4.7	5827	47.3%
8	"	"	5775	46.9%
9	Cal	9.4	5267	42.7%
10	"	"	5384	43.7%
11	Cal	18.8	4661	37.8%
12	"	"	4729	38.4%
13	Cal	37.5	3379	27.4%
14	"	"	3310	26.9%
15	Cal	75	1777	14.4%
16	"	"	1760	14.3%
17	Cal	150	1050	8.5%
18	"	"	1081	8.8%

Control parameters

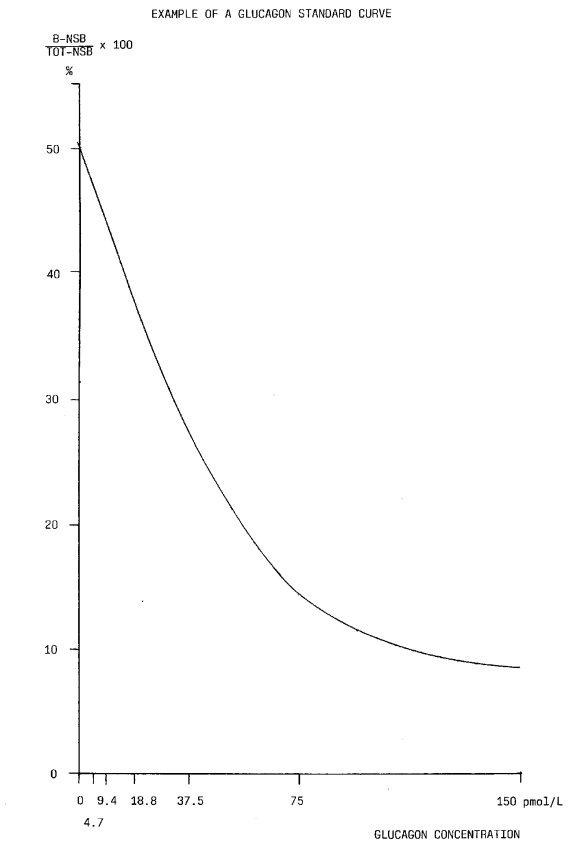
Bo/TOT x 100 : 48.0 %

NSB/TOT x 100 : 5.4 %

ED 80 : 14.5 pmol/L

ED 50 : 39.8 pmol/L

ED 20 : 100 pmol/L



XIII. PERFORMANCE AND LIMITATIONS

A. Sensitivity

The lowest detectable concentration in the assay is 3 pmol/L.
This figure corresponds to a decrease in binding of 2 x SD of the bound radioactivity in the zero-calibrator.

B. Precision

Intra assay variation:

Level	Coefficient of variation (%CV)	N
16.4 pmol/L	8.1	30
60.1 pmol/L	4.5	30

Total variation (sum of intra- and inter assay variation):

Level	Coefficient of variation (%CV)	N
25.4 pmol/L	6.8	6
22.0 pmol/L	7.4	6
23.0 pmol/L	8.3	5
73.9 pmol/L	3.9	6
97.9 pmol/L	5.6	6

C. Accuracy

The recovery was 97.6% when known amounts of glucagon were added to plasma samples.

D. Specificity

The following cross reactions have been found:

Peptide	Cross reaction
Glucagon, pancreatic, human	100.0%
Gut GLI	<0.1%
Secretin	<0.02%
Cholecystokinin -39	<0.02%
Vasoactive intestinal peptide	<0.02%
Gastric inhibitory peptide	<0.02%
GLP1	<0.1%
Oxyntomodulin	<0.1%

E. Correlation
Glucagon assay correlates with WHO 69/194 calibrator.

F. Interference
Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

XIV. INTERNAL QUALITY CONTROL

In order to enable the laboratory to completely monitor the consistent performance of the assay, the following important factors should be checked.

1. The found concentrations of the controls
The found concentrations of the controls should be within the limits given on the labels of the vials.

2. Total counts
Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I-glucagon in this kit will give 10.500 CPM
(-5, +30%) at the reference date (counting efficiency: 80%).

3. Maximum binding (Bo/TOT)
Calculate for each assay the % bound radioactivity in the zero-calibrator:

$$\frac{Bo}{TOT} \times 100$$

4. Non-specific binding (NSB/TOT)
Calculate for each assay the % non-specific binding:

$$\frac{NSB}{TOT} \times 100$$

The non-specific binding should be less than 6%.

5. Slope of calibration curve
For example, monitor the 80, 50 and 20% points of the calibration curve for run to run reproducibility.

XV. REFERENCE INTERVALS

Normal level of glucagon in plasma after 12 hours fasting: <60 pmol/L (obtained with this method).It is recommended that users establish reference ranges for the populations served by their own laboratories.

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.
As the regulations may vary from one country to another, it is essential that the person responsible for the laboratory is familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this test.
This kit contains components of human origin. They have been tested by immunoassay for hepatitis B surface antigen, antibodies to HCV and for antibodies to HIV-1 and HIV-2 and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives, should be observed.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. Steps should be taken to ensure the proper handling of the radioactive material, according to local and/or national regulations. Only authorized personnel should have access to the reagents.

The following precautions should be observed when handling radioactive materials:

- Radioactive material should be stored in specially designed areas, not normally accessible to unauthorized personnel.
- Handling of radioactive material should be conducted in authorized areas only.
- Care should be exercised to prevent ingestion and contact with the skin and clothing.
  Do not pipette radioactive solutions by mouth.
- Drinking, eating or smoking should be prohibited where radioactive material is being used.
- Hands should be protected by gloves and washed after using radioactive materials.
- Work should be carried out on a surface covered by disposable absorbing material.
- Spills of radioactive material should be removed immediately, and all contaminated materials disposed as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

The reagents in this kit contain sodium azide. Contact with copper or lead drain pipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbing suspected of being contaminated with these explosive deposits should be rinsed thoroughly with 10% sodium hydroxide solution.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrator (0-6)	Controls	Samples
Calibrator	-	-	**200 µl**	-	-
Controls	-	-	-	**200 µl**	-
Samples	-	-	-	-	**200 µl**
Anti-glucagon	-	-	**500 µl**		
Assay diluent	-	**500 µl**	-	-	-
Vortex-mix and incubate for 20-24 hours at 2-8°C.					
^{125}I Tracer	**500 µl**				
Vortex-mix and incubate for 20-24 hours at 2-8°C.					
Double antibody solid phase	-	**100 µl**			
Vortex-mix and incubate for 30-60 min at 2-8°C.					
Centrifuge 15 min (1700 g; 4°C)					
Decant and count the radioactivity of the precipitates					

Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/

DIAsource Catalogue Nr : RB310	Revision nr : 200224-1

Revision date : 24/02/2020

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