Glucagon RIA

For the quantitative determination of glucagon in human plasma

For *In Vitro* Diagnostic use within the United States of America. This product is for Research Use Only outside of the United States of America.

Catalog Number: 38-GLUHU-R100

Size: 100 Tubes

Version: 230123- ALPCO 3.3

1. Intended Use

The Glucagon RIA is a radioimmunoassay for the quantitative measurement of glucagon in human plasma. For *In Vitro* Diagnostic use within the United States of America. This product is for Research Use Only outside of the United States of America.

2. Introduction

Glucagon is a straight chain peptide of 29 amino acids produced in 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 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 is: 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.

3. Clinical Considerations

Glucagon is involved in carbohydrate, fat, and protein metabolism. Basal levels of glucagon are essential for the maintenance of normoglycemia and a physiological role for glucagon is to prevent hypoglycemia. Pancreatectomy does not result in total glucagon deficiency. However, the concentrations in plasma afterward are significantly lower than in normal individuals (7, 10).

In diabetics, glucagon has been found to be elevated absolutely or relatively to insulin, thus 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 for decisions on clinical therapy and should be used in combination with clinical symptoms and results of other available tests.

4. Principle of the Assay

Glucagon in plasma is assayed by 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-step 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 a 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-reactivity with gut-GLI (14).

5. Reagents Provided

Reagent	Quantity	Color Code	Reconstitution
Anti-glucagon: rabbit antiserum raised against porcine glucagon, conjugated to human serum albumin in glycine buffer with sodium azide and aprotinin	1 vial lyophilized	Blue	Add 52 mL distilled water
Tracer: ^{125}Iodine labelled glucagon in glycine buffer with human serum albumin, sodium azide, and aprotinin	1 vial lyophilized 28 kBq	Red	Add 52 mL distilled water
Double antibody solid phase: anti-rabbit-Ig coupled to cellulose particles in phosphate buffer with human serum albumin, NaCl, NaN_3, and Tween 80	1 vial 11 mL	Green	Ready for use
Assay diluent: glycine buffer containing human serum albumin, sodium azide, and aprotinin. To be used for the preparation of glucagon working calibrators and in place of antiserum in non-specific binding control tubes	1 vial 50 mL	Black	Ready for use
Glucagon calibrator in glycine buffer containing human serum albumin, sodium azide (<0.1%) and aprotinin	1 vial lyophilized	Yellow	Reconstitute with distilled water using volume stated on the vial label
Controls - N = 1 or 2 contains sodium azide (<0.1%)	2 vials lyophilized	Silver	Add 2 mL distilled water

6. Materials Required but Not Provided

The following material is required, but not provided with the kit:

- Distilled water
- Disposable polystyrene test tubes: 11-13x55 mm
- Pipettes with disposable tips: 200 and 500 µL
- 1.00 mL and 5.00 mL pipettes (for calibrator preparation)
- Vortex mixer
- Centrifuge, refrigerated, giving a minimum of 1700 x g
- Gamma counter
- Timer

7. 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 while 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.

8. Storage and Stability 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 vial contents by gentle inversion and avoid foaming.

The stability for each reagent is found on the label of the vial. The lyophilized reagents are stable until the expiry date stated on the label. The reconstituted reagents are stable for 10 weeks (or to the expiry date for the labelled glucagon) when stored accordingly.

9. Sample Collection

Venous blood is collected in tubes containing EDTA and aprotinin. The sample is cooled in an ice-bath immediately. Plasma is separated by centrifugation (refrigerated 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.

10. Assay 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 different controls with known concentrations of glucagon for quality control.
- ***Samples****:*
  - Tubes for determination of the ***non-specific binding (NSB)***
  - Tubes for determination of the ***total radioactivity*** added

B. Procedure

1. Reconstitute the reagents according to the instructions.
2. Prepare the glucagon working standards by dilution of the 300 pmol/L calibrator with the assay diluent according to the following:
   a/ 1.00 mL standard 300 pmol/L + 1.00 mL assay diluent = 150 pmol/L
   b/ 1.00 mL standard 150 pmol/L + 1.00 mL assay diluent = 75 pmol/L
   c/ 1.00 mL standard 75 pmol/L + 1.00 mL assay diluent = 37.5 pmol/L
   d/ 1.00 mL standard 37.5 pmol/L + 1.00 mL assay diluent = 18.8 pmol/L
   e/ 1.00 mL standard 18.8 pmol/L + 1.00 mL assay diluent = 9.4 pmol/L
   f/ 1.00 mL standard 9.4 pmol/L + 1.00 mL assay diluent = 4.7 pmol/L
   g/ Assay diluent = 0 pmol/L
   Note: Store the standard solutions (a-g) and 300 pg/mL standard at -18°C or lower if they are to be reused.
3. Pipette 200 µL of the standards a-g, samples, and controls into their respective tubes (in duplicate).
4. Pipette 200 µL of the assay diluent into the NSB-tubes.
5. Pipette 500 µL anti-glucagon into all tubes except the NSB- and TOT-tubes.
6. Pipette 500 µL assay diluent into the NSB-tubes.
7. Vortex-mix and incubate for 20-24 hours at 2-8°C.
8. Pipette 500 µL ^{125}I-Glucagon into 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 while 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 (1700xg).
    Note: The correct centrifugation force is important for accurate performance.
13. Decant the liquid immediately after centrifugation.
    Note: Accuracy and attention to the 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.

11. Calculation of Results

1. Subtract the average count rate (CPM) of the non-specific binding tubes from the count rate (CPM) of the replicates of the calibrator tubes, the sample tubes, and control tubes.
2. A standard curve is generated by plotting the bound CPM (in CPM or % B/TOT) against the concentration of the glucagon standards.
3. Interpolate the glucagon concentrations in the samples and controls from the generated standard curve.
4. The standard curve and the calculation of the concentrations of the samples can be done by a suitable computer program. A spline algorithm may be used.

12. Typical Data

The following data are for illustration only and should not be used over the real time standard curve.

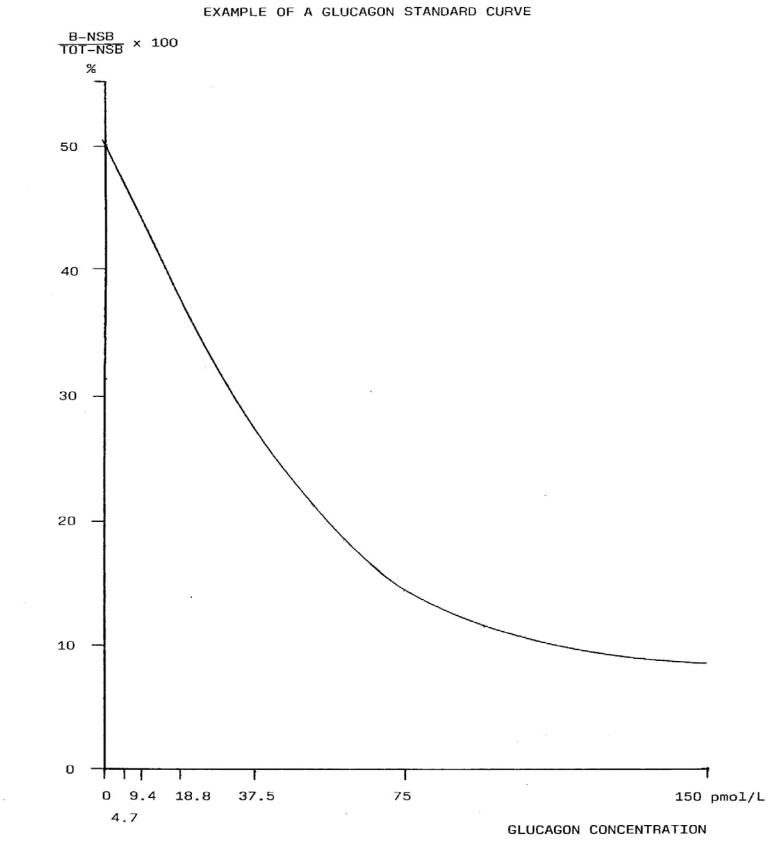
Tube #	Type of tube	Concentration pmol/L	CPM (raw)	$\frac{B}{TOT} \times 100$
1	NSB_{st}	-	684	5.5%
2	"		653	5.3%
3	TOT	-	12301	$\frac{B-NSB}{TOT-NSB} \times 100$
4	"		12347	
5	Cal	0	6253	50.7%
6	"	"	6203	50.3%
7	Cal	4.7	5827	47.3%
8	"	"	5775	46.9%
9	Cal	9.4	5267	42.7%
10	"	"	5384	43.7%
11	Cal	18.8	4661	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

- $\frac{Bo}{TOT}$ x 100 = 48.0 %

ED 80: 14.5 pmol/L
ED 50: 39.8 pmol/L
ED 20: 100 pmol/L

$\frac{NSB}{TOT}$ x 100 = 5.4 %



13. 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-standard.

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. Analytical Specificity

The following cross-reactivity has been determined:

Peptide	Cross-Reactivity
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%
GLP-1	<0.1%
Oxyntomodulin	<0.1%

E. Correlation

The Glucagon RIA assay correlates with WHO 69/194 standard.

F. Interference

Samples displaying cloudiness, hemolysis, gross lipemia or containing fibrin may give inaccurate results.

14. Quality Control

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

a. The concentrations of the controls

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

b. 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%).

c. Maximum binding (Bo/TOT)

Calculate for each assay the % bound radioactivity in the zero-calibrator:

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

d. 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%.

e. Slope of standard curve

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

15. Reference Values

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.

16. Precautions and Warnings

Safety

For in vitro diagnostic use only in the United States. This product is for Research Use Only outside of the United States of America.

Since 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) radiation. 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 designated 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 and other kit reagents by mouth.
- Drinking, eating, and smoking should be prohibited where radioactive material and immunoassay reagents are being used.
- Hands should be protected by gloves and washed after using kitmaterials.
- 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 of as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

The reagents in this kit contain sodium azide. Contact with copper or lead drainpipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewer, 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.

17. References

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18. Summary of the Assay Procedure

	Total count	**NSB**	**Standards (0-6)**	**Controls**	**Samples**
Standard	-	-	200 µL	-	-
Controls	-	-	-	200 µL	-
Samples	-	-	-	-	200 µL
Anti-glucagon	-	-	500 µL		
Assay diluent	-	700 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 mins at 2-8°C					
Centrifuge 15 min (1700 xg; 4°C)					
Decant and count the radioactivity of the precipitates					