IVD

CE

GASTRIN - RIA

KIPEMD302

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

History

Summary of change:

Previous Version: 200615	Current Version: 220303
Sensitivity chapter with analytical sensitivity	Update of the Detection Limit with LoB, LoD, LoQ Updated Precision data (Intra and Inter assay variation) Addition of Dilution test Addition of Interference data

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Read entire protocol before use.

Gastrin-RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of gastrin in human serum.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Gastrin-RIA

B. Catalog number : KIPEMD302 : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
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III. CLINICAL BACKGROUND

1. Biological activities

Gastrin and the vagal nerves are the main regulators of gastric acid secretion. However, other factors than gastrin contribute to the gastric acid secretion. The main site for gastrin production is the antropyloric mucosa of the stomach. A few gastrin producing cells may also be found in the duodenum and pancreas.
Gastrin occurs in many different forms in human serum. An amidated C-terminal is essential for the biological activity of the gastrins.
Progastrin is cleaved from preprogastrin. It has been shown that progastrin is partially sulphated in the tyrosine residues. The progastrin is enzymatically cleaved to the main circulating forms of biologically active gastrin: gastrin-34 and gastrin-17, which occur in sulphated an non-sulphated forms. Small amount of gastrin-52 (also named component 1), gastrin-14 (mini-gastrin) and even smaller fragments have been detected in serum.

2. Clinical application

Gastrin is one of the best studied gut hormones. It occurs in the circulation in several different forms, among those gastrin-34 and gastrin-17, sulphated and non-sulphated.
The determination of gastrin is useful in the diagnosis of gastrin-producing tumours and of achylia with or without pernicious anemia. In all these clinical situations the serum gastrin concentration is high. Treatment with powerful antisecretagogues may cause a rise in the serum gastrin concentration, because of an impaired acid feedback inhibition of gastrin release. Measurement of serum gastrin can thus be used to monitor the treatment with antisecretagogues.

IV. PRINCIPLES OF THE METHOD

Gastrin in serum is assayed by a competitive radioimmunoassay using a rabbit antiserum raised against a gastrin 17 albumin conjugate. Gastrin in calibrators and samples compete with ^{125}I-labelled gastrin-17 in binding to the antibodies. ^{125}I-gastrin binds in a reverse proportion to the concentration of gastrin in calibrators and samples. Antibody-bound ^{125}I-gastrin is separated from the unbound fraction using the double antibody - polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum used in this assay cross-reacts with gastrin-34 and the sulphated forms of gastrin-17 and gastrin-34.
For professional use within a laboratory.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
[ANTISERUM] Rabbit antiserum raised against synthetic human gastrin-17 conjugated to bovine serum albumin. Diluent: phosphate buffer, human serum albumin and sodium azide (<0.1%).	1 vial 21mL	Blue	**Ready** for use
Ag | ^{125}I TRACER: ^{125}Iodine labelled Gastrin in phosphate buffer with human serum albumin and NaN_3.	1 vial lyophilised 66 kBq	Red	**Add** 25 mL distilled water
[Ab|PEG] Double antibody-PEG: Goat anti-rabbit Ig antiserum in phosphate buffer with human serum albumin and sodium azide. (<0.1%). Contains polyethylene glycol	1 vial 50 mL	Green	**Ready** for use
[ASS|BUF] Assay buffer : phosphate buffer containing human serum albumin and sodium azide, (<0.1%).	1 vial 40 mL	Black	**Ready** for use
[CAL] Gastrin Calibrator in phosphate buffer containing human serum albumin and sodium azide (<0.1%).	1 vial lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on the vial label
[CONTROL|N] Control - N = 1 or 2 Lyophilised controls with two different levels of gastrin.	2 vials lyophilised	Silver	**Add** 1 mL distilled water

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Disposable test tubes 11-13 x 55 mm, polystyrene.
2. Pipettes with disposable tips, 100, 200 and 500 µL.
3. A repeating pipette, e.g. Eppendorf Multipipette, for volumes 200 and 500 µL will facilitate the dispensing of the reagents.
4. Vortex mixer.
5. Centrifuge, capable for min 1700 x g (refrigerated centrifuge is preferred).
6. Well-type gamma counter.

VII. REAGENT PREPARATION

- **A. Antiserum :** Ready for use. Store at 2-8° C.
- **B. ^{125}I-gastrin:** Reconstitute with 25 mL distilled water. Store at 2-8° C.
- **C. Double Antibody-PEG:** Ready for use. Mix thoroughly before use. Store at 2-8° C.
- **D. Assay buffer:** Ready for use. Store at 2-8° C.
- **E. Gastrin calibrator:** Reconstitute with distilled water by the volume stated on vial label. For preparation of working calibrators, see radioimmunoassay procedure.
  Store at -18° C or lower if reused.
- **F. Controls:** Reconstitute each vial with 1 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 stability of the reagents is indicated on the labels of the vials. For lyophilised reagents, the expiry date is valid for the unreconstituted reagents. The reconstituted reagents are stable for 8 weeks if stored properly.
The water used for reconstitution of lyophilised reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the content in a vial by gentle inversion and avoid foaming.

IX. SPECIMEN COLLECTION

Patients should be fasting at least ten hours prior to sample collection. Vein blood is collected in tubes without additives. The sample is cooled in an ice-bath and allowed to clot. Serum is separated by centrifugation at +4° C.
The serum should be frozen within 4 hours and stored at -18° C or lower until assayed. Repeated freezing and thawing should be avoided.

X. PROCEDURE

A. Handling notes

Reconstitute the reagents as specified.
Reagents should be brought to room temperature, prior to use. Accuracy in all pipetting steps is essential. All tests (calibrators, controls and samples) should be performed in duplicate. A complete assay includes:
Calibrators: 7 concentrations, 0, 15.6, 31.2, 62.5, 125, 250 and 500 pmol/L.
Controls: Low and high.
Samples.
Tubes for determining the non-specific binding (NSB-tubes).
Tubes for determining the total radioactivity (TOT-tubes).

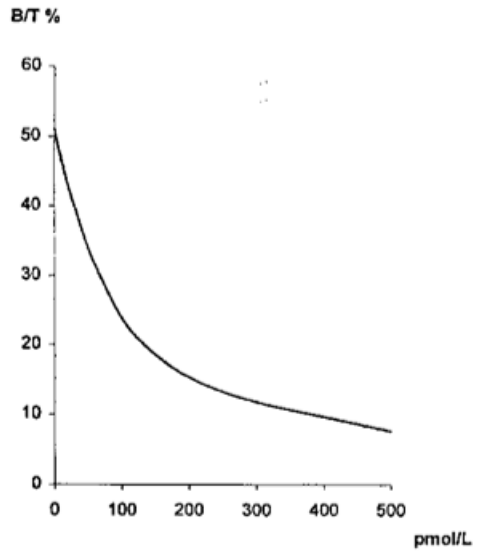
B. Procedure

1. Reconstitute the lyophilised reagents according to the instructions and allow the reagents to reach room temperature.
2. Prepare the gastrin working calibrators by dilution of the Gastrin Calibrator 500 pmol/L with assay buffer according to the following example:
   a. Gastrin calibrator after reconstitution = 500 pmol/L
   b. 1.0 mL calibrator 500 pmol/L + 1.0 mL assay buffer = 250 pmol/L
   c. 1.0 mL calibrator 250 pmol/L + 1.0 mL assay buffer = 125 pmol/L
   d. 1.0 mL calibrator 125 pmol/L + 1.0 mL assay bufer = 62.5 pmol/L
   e. 1.0 mL calibrator 62.5 pmol/L + 1.0 mL assay buffer = 31.2 pmol/L
   f. 1.0 mL calibrator 31.2 pmol/L + 1.0 mL assay buffer = 15.6 pmol/L
   g. Assay buffer = 0 pmol/L
   (Store the calibrators at –20° C or lower if reused).
3. Pipette 100 µL of calibrators, controls and samples in their respective tubes.
4. Pipette 300 µL assay buffer into NSB-tubes.
5. Pipette 200 µL of ^{125}I-Gastrin into all tubes. The TOT-tubes are capped and kept aside.
6. Pipette 200 µL anti-Gastrin into all tubes except NSB and TOT.
7. Vortex the tubes carefully and incubate for 60 min at room temperature (18-25° C).
8. Add 500 µL of well mixed double antibody-PEG into all tubes except TOT. Vortex carefully and incubate 30-60 min at room temperature.
9. Centrifuge for 15 minutes at minimum 1700 x g, temperature 4° C.
10. Decant the supernatant immediately after centrifugation, and count the radioactivity in the precipitates in a gamma counter.

XI. CALCULATION OF RESULTS

- Subtract the average count rate (CPM) of the NSB from the count rate (CPM) of the replicates of the calibrators, controls and samples.
- A calibration curve is generated by plotting the bound fraction, B/TOT against the concentrations of the gastrin calibrators.
- Interpolate the gastrin concentrations of the controls and samples from the generated calibration curve.
- The calibration curve and the calculation of the concentrations in samples can be done by a computer method. A spline method may be used.

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



XIII. PERFORMANCE AND LIMITATIONS

A. Limit of Detection

The LoB (limit of blank) was calculated by measuring the blank 21 times and was calculated as the mean + 2 standard deviations of the distribution of the test values . The LoB was calculated to be 3.97 pmol/L.
The LoD (limit of detection) was calculated as the LoB + 1.65 standard deviation of a low concentration sample tested in 10 different runs. The LoD was calculated to be 8.06 pmol/L.
The LoQ (limit of quantification) was calculated by testing 5 low values samples, in 10 different runs. The LoQ is 11.95 pmol/L.

B. Precision

Intra assay variation

Level	Coefficient of variation (% CV)	N
42.6 pmol/L	4.2%	20
178.5 pmol/L	3.9%	20

Inter assay variation (total variation)

Level	Coefficient of variation (% CV)	N
42.0 pmol/L	7.7%	13
172.6 pmol/L	4.9%	13

C. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concentration (pmol/L)	Measured Concentration (pmol/L)
A	1/1	92.61	92.61
	1/2	46.31	49.66
	1/4	23.15	26.44
	1/8	11.58	11.12
	1/16	5.79	4.75
B	1/1	106.04	106.04
	1/2	53.02	53.06
	1/4	26.51	30.15
	1/8	13.26	19.32
	1/16	6.63	11.07
C	1/1	55.96	55.96
	1/2	27.98	32.57
	1/4	13.99	14.17
	1/8	7.00	6.21
	1/16	3.50	4.58

Samples were diluted with the assay buffer.

D. Specificity

The following cross reactions have been found:

Compound	Cross reaction (%)
Gastrin-17	100
Gastrin-17, sulphated	83
Gastrin-34	61
CCK-8	36
Gastrin 1-14	< 0.1
Gastrin releasing peptide	<0.01%
Vasoactive intestinal peptide	<0.01%
Motilin	<0.01%
Glucagon	<0.01%
Somatostatin 14	<0.01%
C-peptide	<0.01%

E. Interference

The effect of potential interfering substances on samples using the DIAsource Gastrin RIA test was evaluated. Different levels of Haemoglobin, Bilirubin, and Triglyceride were tested on samples with different Gastrin concentrations. Our acceptance criteria was to have interference of less than 10%. The tested substances did not affect the performance of the DIAsource Gastrin RIA test.

Substance	Gastrine concentration (pmol/L)	Concentration of Interferent (mg/dL)	Mean % Variation
Haemoglobin	35.3	500	-5.9%
		250	
	154.7	500	
		250	
Bilirubin	35.3	100	-2.7%
		50	
	154.7	100	
		50	
Triglyceride	38.6	250	+5.3%
		125	
		62.5	
	146.3	250	
		125	
		62.5	

XIV. INTERNAL QUALITY CONTROL

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

1. 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-gastrin in this kit will give 25000 CPM (-5, +20%) 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 is less than 5%.

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 gastrin in human serum: ≤60 pmol/L (fasting level obtained with this procedure).
Mean value: 25 pmol/L ± 10 pmol/L (1SD).
Range: 11-54 pmol/L.

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 are 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 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 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.

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrators (0-6)	Controls	Samples
Calibrators	-	-	**100 µL**	-	-
Controls	-	-	-	**100 µL**	-
Samples	-	-	-	-	**100 µL**
Assay diluent	-	**300µL**	-	-	-
^{125}I Tracer	**200 µL**				
Antiserum	-	-	**200 µL**		
Vortex-mix and incubate for 60 min at 18-25°C					
Double antibody PEG	-	**500 µL**			
Vortex-mix and incubate for 30-60 min at 18-25°C					
Centrifuge 15 min (1700 g) at 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/

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