Instruction

EURIA-Gastrin

Gastrin radioimmunoassay

Document No.E-23-0026-11 RUO

February, 2016

For Research Use Only. Not for use in diagnostic procedures.

REF **MD 302 RUO**

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INTRODUCTION

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.

PHYSIOLOGICAL CONSIDERATIONS

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.

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.

PRINCIPLE OF THE METHOD

The use of these reagents is for assay of gastrin in human serum. Gastrin in serum is assayed by a competitive radioimmunoassay using a rabbit antiserum raised against a gastrin 17 albumin conjugate. Gastrin in standards 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 standards and samples. Antibody-bound ^{125}I-gastrin is separated from the unbound fraction using the double antibody - polyethyleneglycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum used in this assay crossreacts with gastrin-34 and the sulphated forms of gastrin-17 and gastrin-34.
For professional use within a laboratory. The result shall not be used for clinical diagnosis or patient management.

PRECAUTIONS

For research use only. Not for use in diagnostic procedures.

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.

COMPOSITION OF THE REAGENT KIT

The reagents provided in each kit are sufficient for 100 tubes.

1. Anti-gastrin (Reagent A)

Rabbit antiserum raised against synthetic human gastrin-17 conjugated to bovine serum albumin, 21 mL antiserum. Diluent: 0.05 M phosphate buffer, pH 7.4, 0.25% human serum albumin and 0.05% sodium azide. Colour: Yellow.
For 100 tubes.

2. ^{125}I-Gastrin (Reagent B)

Contains 66 KBq or 1.8 μCi at reference date. Synthetic human gastrin-17 is iodinated.
The monoiodinated form is purified by HPLC.
Specific activity: 1700-2100 μCi/nmol (62-77 MBq/nmol). Lyophilized in 2.5 mL 0.5M phosphate buffer, pH 7.4, with 2.5% human serum albumin and 0.5% sodium azide.
Contains 0.12 mL normal rabbit serum. Colour: Blue.
Reconstitution in 25 mL distilled water.

3. Double antibody-PEG (Reagent C)

50 mL diluted goat anti-rabbit Ig antiserum in 0.05 M phosphate buffer, pH 7.4, 0.25% human serum albumin and 0.05% sodium azide.
Contains 5.0% (w/v) polyethylene glycol 6000. Colour: Red

4. Assay buffer (Reagent D)

40 mL 0.05 M phosphate buffer, pH 7.4, with 0.25% human serum albumin and 0.05% sodium azide.

5. Gastrin standard (Reagent E)

Lyophilized. 5.00 mL standard after reconstitution. Concentration : 500 pmol/L.
The standard is produced from synthetic human gastrin-17. Diluted in 0.05 M phosphate buffer, pH 7.4, 0.25% human serum albumin, 0.05% sodium azide.
Reconstitution in 5.00 mL distilled water.

6. Controls (Reagent F-G)

Lyophilized serum pools with low (normal) and high concentration of gastrin. 1.00 mL of each control after reconstitution.

EQUIPMENT REQUIRED BUT NOT PROVIDED

Disposable test tubes 11-13 x 55 mm, polystyrene.

Pipettes with disposable tips, 100, 200 and 500 μL.

A repeating pipette, e.g. Eppendorf Multipipette, for volumes 200 and 500 μL will facilitate the dispensing of the reagents.

Vortex mixer.

Centrifuge, capable for min 1700 x g (refrigerated centrifuge is preferred).

Well-type gammacounter.

REAGENT PREPARATION AND STORAGE

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 lyophilized 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 lyophilized 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.

Reagent A: Anti-gastrin
Ready for use. Store at 2-8° C.

Reagent B: ^{125}I-gastrin
Reconstitute with 25 mL distilled water. Store at 2-8° C.

Reagent C: Double antibody-PEG
Ready for use. Mix thoroughly before use. Store at 2-8° C.

Reagent D: Assay buffer
Ready for use. Store at 2-8° C.

Reagent E: Gastrin standard
Reconstitute with 5.00 mL distilled water. For preparation of working standards, see radioimmunoassay procedure.
Store at -18° C or lower if reused.

Reagent F-G: Controls
Reconstitute each vial with 1.00 mL distilled water.
Store at -18° C or lower if reused.

SPECIMEN COLLECTION

Subjects 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.

ASSAY PROCEDURE

Reconstitute the reagents as specified.
Reagents should be brought to room temperature, prior to use. Accuracy in all pipetting steps is essential. All tests (standards, controls and samples) should be performed in duplicate. A complete assay includes:

Standards (St-tubes): 7 different concentrations, 0, 15.6, 31.2, 62.5, 125, 250 and 500 pmol/L.

Controls (C-tubes): Low and high.

Samples (P-tubes).

Tubes for determining the ***non-specific binding (NSB-tubes).***
Tubes for determining the ***total radioactivity (TOT-tubes).***
For an overview, see page 10.

PERFORMANCE

- Reconstitute the lyophilized reagents according to the instructions on page 5 and allow the reagents to reach room temperature.
- Prepare the gastrin working standards by dilution of the Gastrin standard 500 pmol/L (Reagen E) with assay buffer (Reagent D) according to the following example:
  a. Reagent E after reconstitution = 500 pmol/L
  b. 1.0 mL standard 500 pmol/L + 1.0 mL assay buffer = 250 pmol/L
  c. 1.0 mL standard 250 pmol/L + 1.0 mL assay buffer = 125 pmol/L
  d. 1.0 mL standard 125 pmol/L + 1.0 mL assay bufer = 62.5 pmol/L
  e. 1.0 mL standard 62.5 pmol/L + 1.0 mL assay buffer = 31.2 pmol/L
  f. 1.0 mL standard 31.2 pmol/L + 1.0 mL assay buffer = 15.6 pmol/L
  g. Assay buffer = 0 pmol/L
  (Store the standards at –20° C or lower if reused).

- Pipette 100 μL of standards, controls and samples in their respective tubes. Pipette 300μL assay buffer (Reagent D) into NSB-standard-tubes.

- Pipette 200 μL of ^{125}I-Gastrin (Reagent B) into all tubes. The TOT-tubes are capped and kept aside.

- Pipette 200 μL anti-Gastrin (Reagent A) into all tubes **except** NSB and TOT.

- Vortex the tubes carefully and incubate for 60 min at room temperature (20-25° C).

- Add 500 µL of well mixed double antibody-PEG (Reagent C) into all tubes **except** TOT. Vortex carefully and incubate 30-60 min at room temperature.

- Centrifuge for 15 minutes at minimum 1700 x g, temperature 4° C.

- Decant the supernatant immediately after centrifugation, and count the radioactivity in the precipitates in a gamma counter.

CALCULATIONS

- Subtract the average count rate (CPM) of the NSB-standard from the count rate (CPM) of the replicates of the standards, controls and samples.

- A standard curve is generated by plotting the bound fraction, B/TOT against the concentrations of the gastrin standards. An example of a standard curve is given on page 10.

- Interpolate the gastrin concentrations of the controls and samples from the generated standard curve.

- The standard curve and the calculation of the concentrations in samples can be done by a computer method. A spline method may be used.

ASSAY CHARACTERISTICS

Sensitivity
The lowest detectable concentration was 5 pmol/L. The figure corresponds to a decrease in binding of two x SD of the bound radioactivity in the zero-concentration standard.

Accuracy
A mean recovery of 97.6% was achieved when known amounts of gastrin in the range 65-222 pmol/L were added to serum samples.

Precision
Intra assay variation

Level	Coefficient of variation (%CV)	N
41 pmol/L	3.0%	20
135 pmol/L	2.2%	20

Inter assay variation (total variation)

Level	Coefficient of variation (%CV)	N
47 pmol/L	7.5%	17
165 pmol/L	6.2%	17

Specificity
The following cross reactions have been found:

Compound	Cross reaction
Gastrin-17	100.0%
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%

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

QUALITY CONTROL

In order for the laboratory to completely monitor the consistent performance of the radioimmunoassay there are some important factors which must be checked.

1. The found concentrations of the control sera
(Reagent Fand G) are 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 25 000 CPM (-5%, +20%) at the reference date (counter efficiency = 80%).

3. Maximum binding (Bo/TOT)
Calculate for each assay the % bound radioactivity in the zero-standard: $\frac{Bo}{TOT} \times 100$.

4. Non-specific binding (NSB/TOT)
Calculate for each assay the % non-specific binding $\frac{NSB}{TOT} \times 100$.

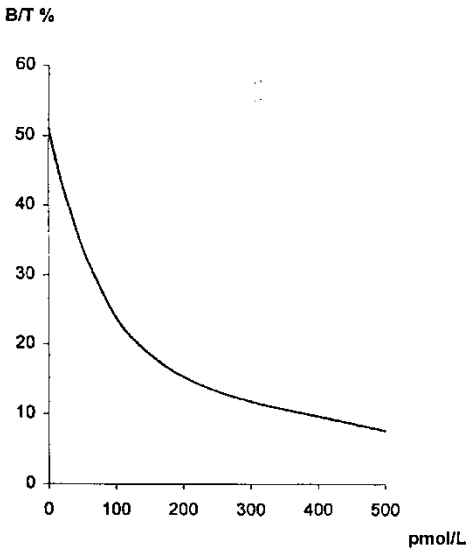
$\frac{NSB}{TOT} \times 100$ is less than 5%.

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

OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	Assay buffer (D)	^{125}I-gastrin with NRS (B)	Anti-Gastrin (A)		Double antibody PEG (C)	
TOT	1- 2	-	-	200 µL	-	Vortex-mix and incubate for 60 min. at room tempera-ture.	-	Vortex-mix and incubate for 30-60 min. at room tempera-ture. Centrifuge 15 min. at 1700 x g. Decant and count the radioactivi-ty of the precipitates.
NSB_{St}	3- 4	-	300 µL	200 µL	-		500 µL	
Stand 0	5- 6	100 µL	-	200 µL	200 µL		500 µL	
Stand 15.6	7- 8	100 µL	-	200 µL	200 µL		500 µL	
Stand 31.3	9-10	100 µL	-	200 µL	200 µL		500 µL	
Stand 62.5	11-12	100 µL	-	200 µL	200 µL		500 µL	
Stand 125	13-14	100 µL	-	200 µL	200 µL		500 µL	
Stand 250	15-16	100 µL	-	200 µL	200 µL		500 µL	
Stand 500	17-18	100 µL	-	200 µL	200 µL		500 µL	
Control low	19-20	100 µL	-	200 µL	200 µL		500 µL	
Control high	21-22	100 µL	-	200 µL	200 µL		500 µL	
Sample 1	23-24	100 µL	-	200 µLL	200 µL		500 µL	

EXAMPLE OF GASTRIN STANDARD CURVE



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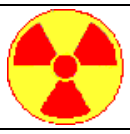
SYMBOLS USED ON LABELS

	Batch code.
	Catalogue number.
	Use by date.
	Temperature limit.
	Date of manufacture.
	Contains radioactive substances.
	Biological risks.
	Consult instructions for use.
	Manufacturer.
 **100**	Contains sufficient for 100 tests.

REAG A Ab	Anti-gastrin.
REAG B Ag ^{125}I	^{125}I-gastrin.
REAG C DAB	Double antibody – PEG solution.
REAG D BUF AS	Assay buffer.
REAG E CAL 500	Gastrin standard 500 pmol/L.
REAG F CONTROL	Control, level 1 (normal).
REAG G CONTROL	Control, level 2 (high).

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