ARCHITECT

SYSTEM

en

ProGRP

REF 1P45

F5-Y208-2/R2

ProGRP

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	SN	Serial Number
IVD	*In Vitro* Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	SAMPLE CUPS	Sample Cups
	Expiration Date	SEPTUM	Septum
2°C 8°C	Store at 2-8˚C	REPLACEMENT CAPS	Replacement Caps
		REAGENT LOT	Reagent Lot
	Consult instructions for use	ASSAY CD-ROM	Assay CD-ROM
		CONTROL NO.	Control Number
	Manufacturer	WARNING: SENSITIZER	Warning: Sensitizer

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

Abbott

WARNING: The concentration of Pro-gastrin-releasing-peptide (ProGRP) in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining ProGRP levels serially is changed, additional sequential testing should be carried out. Before changing assay methods, the laboratory MUST confirm baseline values for patients being serially monitored.

The use of plasma specimens is recommended.
Endogenous proteases generated during the clotting process can degrade ProGRP in serum. Special care is needed for handling serum specimens. ProGRP is significantly more stable in plasma than in serum. The difference in stability of ProGRP in serum and plasma may result in differences in the values of ProGRP in serum and plasma samples. (Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section for detailed information.) It is recommended that independent clinical decision points be established for ProGRP levels in serum and plasma. It is important to report the results of ProGRP testing in conjunction with the type of sample matrix used. The same type of sample matrix must be used when using serial ProGRP samples to monitor the response to therapy or to detect disease progression.

NAME

ARCHITECT ProGRP

INTENDED USE

The ARCHITECT ProGRP assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of ProGRP in human serum and plasma on the ARCHITECT *i* System. The ARCHITECT ProGRP assay is to be used to aid in the differential diagnosis of lung cancer in conjunction with other clinical methods. The ARCHITECT ProGRP assay is used in conjunction with other clinical methods in the management of patients with small cell lung cancer.

SUMMARY AND EXPLANATION OF TEST

Gastrin-releasing peptide (GRP) is a neuropeptide hormone that is frequently produced by small cell lung carcinoma (SCLC) cells.[1] The measurement of GRP in blood was evaluated as a tumor marker of SCLC, but it was not possible to measure due to its extreme instability. Instead, an assay for the measurement of ProGRP (31-98), a carboxy-terminal region common to three types of human ProGRP splice variants, was developed and it was proven that serum ProGRP (31-98) levels served as a reliable marker in SCLC patients.[2-5] ARCHITECT ProGRP assay measures ProGRP (31-98) in serum and plasma.

ProGRP is one of several molecules, such as neuron-specific enolase (NSE) and chromogranin A, which are associated with neuroendocrine derived tissues and tumors. Increased levels of serum ProGRP have been reported in several neuroendocrine-derived tumor types, including small cell lung cancer, carcinoids, undifferentiated large cell carcinomas of the lung with neuroendocrine features, medullary thyroid carcinoma,[6] other neuroendocrine malignancies,[6] and in a subset androgen-independent prostate cancer with neuroendocrine features.[7,8] Molina *et al*[9,10] have reviewed the literature and also reported their evaluation of serum ProGRP levels in patients without malignancy, with non-lung malignancies and with lung malignancies.

ProGRP is useful for the differential diagnosis of lung masses as small cell carcinoma vs. non-small cell carcinoma. It has been reported to be the most sensitive marker for SCLC compared to benign diseases of the lung.[11] Additive information in the histological diagnosis of lung cancer is provided by serum NSE.[4,9,12,13] It is useful in the monitoring of response to therapy and for the detection of recurrent disease.[12-14]

ProGRP elevates in early stage of the SCLC. However, since the incidence of SCLC in the general population is low, ProGRP assay should not be used as a screening test.[15] ARCHITECT ProGRP assay testing is not recommended as a screening procedure in the general population.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT ProGRP assay is a two-step immunoassay for the quantitative determination of ProGRP (31-98) in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample, assay diluent, and anti-ProGRP coated paramagnetic microparticles are combined. ProGRP present in the sample binds to the anti-ProGRP coated microparticles. After washing, anti-ProGRP acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of ProGRP in the sample and the RLUs detected by the ARCHITECT *i* System optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests

ARCHITECT ProGRP Reagent Kit (1P45)

- **MICROPARTICLES** 1 Bottle (6.6 mL) Anti-ProGRP (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.04% solids. Preservative: ProClin 300.
- **CONJUGATE** 1 Bottle (5.9 mL) Acridinium-labeled anti-ProGRP (mouse, monoclonal) conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 106 ng/mL. Preservative: ProClin 300.
- **ASSAY DILUENT** 1 Bottle (2.9 mL) ProGRP Assay Diluent containing TRIS buffer. Preservative: ProClin 300.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- **Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.**

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[16] Biosafety Level 2[17] or other appropriate biosafety practices[18,19] should be used for materials that contain or are suspected of containing infectious agents.
- Microparticles, Conjugate and Assay Diluent of this kit contain methylisothiazolones, which are components of ProClin. These components are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

R43 May cause sensitization by skin contact.
S24 Avoid contact with skin.
S35 This material and its container must be disposed of in a safe way.
S37 Wear suitable gloves.
S46 If swallowed, seek medical advice immediately and show this container or label.

- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT ProGRP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

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The ARCHITECT ProGRP Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT ProGRP Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT ProGRP assay file must be installed on the ARCHITECT *i* System from the ARCHITECT *i* Assay CD-ROM before performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Endogenous proteases generated during the clotting process can degrade ProGRP in serum. Special care is needed for handling serum specimens. ProGRP is significantly more stable in plasma than in serum.[20] The difference in stability of ProGRP in serum and plasma may result in differences in the values of ProGRP in serum and plasma samples.

Specimen Types

- **The use of plasma specimens for the ARCHITECT ProGRP assay is recommended.**

The specimen collection tubes listed below were verified for use with the ARCHITECT ProGRP assay. Other specimen collection tubes have not been tested with this assay.

- Human serum (including serum collected in serum separator tubes) **Serum collection tubes that contain a thrombin based clotting acceleration agent must not be used because this agent causes degradation of ProGRP.**
- Human plasma collected in:
  - EDTA
  - Sodium Heparin
  - Lithium Heparin
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT ProGRP assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL)
  - obvious microbial contamination
  - cadaver specimens or any other body fluids
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- **Avoid the use of serum specimens that have been subjected to more than 3 cumulative hours of room temperature (15-30°C) exposure. This includes clot time, preparation for analysis time, and storage time. Serum specimens exposed to more than 3 cumulative hours of room temperature (15-30°C) may produce inaccurate results.**
  - **To minimize the degradation of ProGRP in serum specimens, minimize the specimen time at room temperature (15-30°C) and test as soon as possible after sample collection.**
  - **Serum specimens should be processed immediately following adequate clotting, or stored at 2-8°C.**
  - **Serum specimens must be tested immediately after removal from storage at 2-8°C.**

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥ 10000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, or
  - they were frozen and thawed.

  Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Serum specimens should be processed immediately following adequate clotting, or stored at 2-8°C.
- Specimens should be tested within the following times to avoid degradation of ProGRP in specimens. If testing will be delayed longer than the time shown below, remove serum or plasma from the clot, red blood cells, or separator gel and store frozen (≤ -15°C). Frozen specimens should be tested within 7 days. For long term storage, samples should be stored at ≤ -70°C. Specimens stored at ≤ -70 °C for 12 months were tested and shown to have stable concentrations.
- Avoid more freeze/thaw cycles than shown below.

Serum

Storage		On board
Room temperature (15-30°C)	3 hours*	1 hour
2-8°C	3 hours	1 hour
≤ -15°C	7 days**	1 hour
	(1 freeze/thaw cycle)	

Plasma

Storage		On board
Room temperature (15-30°C)	8 hours*	3 hours
2-8°C	24 hours	3 hours
≤ -15°C	7 days**	3 hours
	(3 freeze/thaw cycles)	

* **Cumulative time** including clot time, preparation for analysis time, and storage time.

** For long term storage, samples should be stored at ≤ -70°C.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens should be shipped on dry ice. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 1P45 ARCHITECT ProGRP Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- 8K30 ARCHITECT *i* System **ASSAY CD-ROM**
- 1P45-02 ARCHITECT ProGRP Calibrators
- 1P45-11 ARCHITECT ProGRP Controls or other control material
- 7D82-50 ARCHITECT *i* **MULTI-ASSAY MANUAL DILUENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT ProGRP Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT ProGRP Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present before running the test.

Handling of serum samples:

- **All ARCHITECT ProGRP serum samples must be priority loaded. Priority loading of serum samples prevents analyte degradation that will impact assay results.** For information on priority loading of samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Priority: 150 µL for the first ARCHITECT ProGRP test plus 50 µL for each additional ARCHITECT ProGRP test from the same sample cup.
- Serum samples must not be on board for > 1 hour.

Handling of plasma samples:

- Priority loading is not required for plasma samples.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT ProGRP test plus 50 µL for each additional ARCHITECT ProGRP test from the same sample cup.
  - Plasma samples must not be on board for > 3 hours.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - Confirm Calibrators and Controls are not frozen before use. Mix ARCHITECT ProGRP Calibrators and Controls by gentle inversion before use.
  - To obtain the recommended volume requirements for the ARCHITECT ProGRP Calibrators and Controls, hold the bottles **vertically** and dispense **7** drops of each calibrator or **4** drops of each control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedure.

Specimen Dilution Procedures

Specimens with a ProGRP concentration of > 5000 pg/mL will be flagged as "> 5000.00" and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

- If using the Automated Dilution Protocol, the system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.
- Manual dilutions should be performed as follows:
  - It is recommended that dilutions not exceed 1:10.
  - Add 50 µL of the patient specimen to 450 µL of ARCHITECT *i* Multi-Assay Manual Diluent in case of 1:10 dilution.

- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result should be greater than 50 pg/mL before the dilution factor is applied.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT ProGRP calibration, test calibrators A, B, C, D, E, and F in duplicate. A single sample of each ProGRP control level must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0 - 5000 pg/mL.
- Once an ARCHITECT ProGRP calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT ProGRP assay is that a single sample of each control be tested once every 24 hours each day of use. If laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. The ARCHITECT ProGRP Control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT ProGRP assay belongs to method group 1.

RESULTS

The ARCHITECT ProGRP assay uses a 4 Parameter Logistic Curve fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range (Reportable Range)

The measurement range of the ARCHITECT ProGRP assay is 3 pg/mL to 5000 pg/mL. For patient specimens with a ProGRP assay value exceeding 5000 pg/mL refer to the **Specimen Dilution Procedures** section of the package insert.

LIMITATIONS OF THE PROCEDURE

- If the ProGRP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Serum and plasma samples from the same individual may give different results. Report the results in conjunction with the sample type.
- Serum specimens from the same patient may produce decreased results compared to plasma specimens. The same type of sample matrix must be used when using serial ProGRP samples to monitor the response to therapy or to detect disease progression.
- Increased serum concentrations of ProGRP have been observed in patients with renal dysfunction. There is a significant correlation between serum ProGRP levels and serum creatinine concentrations in patients with renal dysfunction.[21] The evaluation of serum creatinine levels should be considered in cases of high serum ProGRP levels that are not consistent with diagnostic and clinical characteristics of the patient.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[22,23] Specimens containing HAMA may produce anomalous values when tested with assay kits (such as ARCHITECT ProGRP) that employ mouse monoclonal antibodies.[22]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[24] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- The ARCHITECT ProGRP assay should not be used as a cancer screening test.
- **ProGRP levels, regardless of value, should not be interpreted as absolute evidence for the presence or absence of malignant disease. In patients with suspected or known cancer, other tests and procedures must also be considered for diagnosis and good management.**
- The concentration of ProGRP in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.

EXPECTED VALUES

Paired serum and EDTA plasma samples from 194 apparently healthy individuals with no known lung disease or kidney problems were tested. There were 170 men and 24 women ranging in age from 20 to 63 years with a mean age of 45.7 years. Serum or plasma sample tubes were held for 30 minutes to allow the serum to clot, then centrifuged for 10 minutes and serum or plasma were separated. Samples were stored at ≤ -70°C until testing. The ProGRP concentration in the samples was tested on ARCHITECT immediately after thawing, mixing and centrifuging. The results of the testing are shown below.

- In this study, 95.4% (185/194) of the apparently healthy subjects had ProGRP values for serum sample of 63 pg/mL or less at one laboratory. Median of serum values was 36.7 pg/mL.
- In this study, 95.4% (185/194) of the apparently healthy subjects had ProGRP values for plasma sample of 65 pg/mL or less at one laboratory. Median of plasma values was 39.1 pg/mL.

The expected values for serum ProGRP will be affected by the conditions of collection including length of time and temperature of storage. It has been reported that the 95% of 100 serum samples from apparently healthy individuals tested with the ARCHITECT ProGRP assay at the University of Munich was 37.7 pg/mL or less.[25]

It is recommended that each laboratory establish its own expected reference range for the population of interest and the collection procedures used within the laboratory.

The distribution of ARCHITECT ProGRP values determined in 498 paired serum and EDTA plasma specimens from apparently healthy individuals, patients with benign lung diseases, and patients with small cell and non-small cell lung cancer are shown in the following tables.*

Distribution of ARCHITECT ProGRP Values in Serum

		Percent (%)				
	Number of Subjects	0 - 46 (pg/mL)	46 - 70 (pg/mL)	70 - 100 (pg/mL)	100 - 200 (pg/mL)	≥ 200 (pg/mL)
Apparently healthy subjects[a]	194	77.8	18.6	3.6	0.0	0.0
Nonmalignant disease[b]						
All	97	70.1	18.6	8.2	3.1	0.0
(Cr normal[c]	82	76.8	17.1	6.1	0.0	0.0)
Malignant disease[b]						
NSCLC	104	76.9	15.4	3.8	3.8	0.0
SCLC	103	29.1	11.7	5.8	11.7	41.7

Distribution of ARCHITECT ProGRP Values in EDTA Plasma

		Percent (%)				
	Number of Subjects	0 - 46 (pg/mL)	46 - 70 (pg/mL)	70 - 100 (pg/mL)	100 - 200 (pg/mL)	≥ 200 (pg/mL)
Apparently healthy subjects[a]	194	71.6	24.7	3.6	0.0	0.0
Nonmalignant disease[b]						
All	97	55.7	30.9	7.2	6.2	0.0
(Cr normal[c]	82	63.4	29.3	4.9	2.4	0.0)
Malignant disease[b]						
NSCLC	104	68.3	20.2	6.7	3.8	1.0
SCLC	103	18.4	20.4	3.9	12.6	44.7

[a] The samples collected from apparently healthy individuals were not evaluated for renal dysfunction.

[b] The 304 patient specimens were tested at a different laboratory from the laboratory where the healthy individuals were tested. For the paired serum and EDTA plasma samples from the patients, sample tubes were clotted for at least 30 minutes. Then the tubes were immediately centrifuged for 10 minutes and serum or plasma were separated. Samples were frozen until testing. ProGRP concentration in samples was tested on ARCHITECT immediately after thawing, mixing and centrifuging.

[c] Cr normal: Creatinine in serum for male ≤ 1.04 mg/dL, female ≤ 0.79 mg/dL.

* Representative data; results in individual laboratories may vary from these data.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT ProGRP assay is designed to have precision of ≤ 10% total CV across the range of 25 to 5000 pg/mL of ProGRP.

A study was performed with the ARCHITECT ProGRP assay based on guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A2. Three controls, serum and plasma panels were assayed, using three lots of reagents, in replicates of two at two separate times per day for 20 days. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.*

Sample	Reagent Lot	n	Mean (pg/mL)	Within Run SD	Within Run %CV	Total SD	Total %CV
Control L	1	80	36.6	1.5	4%	1.5	4%
	2	80	39.8	1.4	4%	1.6	4%
	3	80	39.4	1.7	4%	1.7	4%
Control M	1	80	151.4	6.1	4%	6.8	4%
	2	80	159.0	5.0	3%	5.5	3%
	3	80	155.2	6.1	4%	6.2	4%
Control H	1	80	2591.7	87.8	3%	96.0	4%
	2	80	2556.7	60.3	2%	77.2	3%
	3	80	2523.0	55.5	2%	71.8	3%
Serum Panel 1	1	80	57.9	2.5	4%	2.8	5%
	2	80	60.8	2.0	3%	2.6	4%
	3	80	59.9	1.9	3%	2.2	4%
Serum Panel 2	1	80	243.7	9.3	4%	10.5	4%
	2	80	252.0	9.2	4%	9.8	4%
	3	80	247.1	8.4	3%	9.0	4%
Serum Panel 3	1	80	3555.2	91.4	3%	115.0	3%
	2	80	3515.7	79.3	2%	127.4	4%
	3	80	3443.5	91.5	3%	111.8	3%
Plasma Panel 1	1	80	53.4	1.9	4%	2.0	4%
	2	80	56.3	2.2	4%	2.4	4%
	3	80	55.4	2.2	4%	2.4	4%
Plasma Panel 2	1	80	233.0	8.9	4%	9.6	4%
	2	80	236.6	10.0	4%	11.7	5%
	3	80	231.3	8.8	4%	8.8	4%
Plasma Panel 3	1	80	3725.9	116.1	3%	125.5	3%
	2	80	3548.2	104.1	3%	109.3	3%
	3	80	3572.6	99.5	3%	116.6	3%

* Representative data; results in individual laboratories may vary from these data.

Recovery

The ARCHITECT ProGRP assay is designed to have a mean recovery of 100 ± 10%.

A study was performed where known concentrations of ProGRP were added to 8 aliquots of human serum and plasma. The concentration of ProGRP was determined using the ARCHITECT ProGRP assay and the resulting percent recovery was calculated. Data from this study are summarized in the following table.*

Specimen	Endogenous Level (pg/mL)	ProGRP Added (pg/mL)	Value Obtained (pg/mL)	% Recovery[a]
Plasma 1	23.0	52.1	74.4	99%
Plasma 1	23.0	153.6	171.0	96%
Plasma 2	6.7	52.1	58.0	98%
Plasma 2	6.7	153.6	145.8	91%
Serum 1	1.5	52.1	48.4	90%
Serum 1	1.5	153.6	134.8	87%
Serum 2	2.1	52.1	52.0	96%
Serum 2	2.1	153.6	145.0	93%
Mean				94%

[a] $\% \text{ Recovery} = \frac{\text{Value Obtained - Endogenous Level}}{\text{ProGRP Added}} \times 100$

* Representative data; results in individual laboratories may vary from these data.

Dilution Linearity

The ARCHITECT ProGRP assay is designed to have a mean recovery of ± 10% of the expected result.

A dilution linearity study was performed using specimens with undiluted values that ranged between 45 and 4543 pg/mL. These specimens were diluted manually using ARCHITECT ProGRP Calibrator A (0 pg/mL) at various dilution factors. Data from this study are summarized in the following table.*

Sample	Dilution Factor	Mean Expected Value (pg/mL)	Mean Observed Value (pg/mL)	% Recovery[a]
Plasma 1	Undiluted	4543.1	4543.1	100%
	9:10	4088.8	3888.3	95%
	7:10	3180.2	3227.4	101%
	5:10	2271.6	2400.3	106%
	3:10	1362.9	1453.1	107%
	1:10	454.3	460.0	101%
Plasma 2	Undiluted	491.0	491.0	100%
	9:10	441.9	438.1	99%
	7:10	343.7	348.6	101%
	5:10	245.5	249.9	102%
	3:10	147.3	150.8	102%
	1:10	49.1	49.6	101%
Plasma 3	Undiluted	45.0	45.0	100%
	9:10	40.5	39.6	98%
	7:10	31.5	30.7	97%
	5:10	22.5	21.9	97%
	3:10	13.5	13.2	97%
	1:10	4.5	4.1	91%
Serum 1	Undiluted	4461.7	4461.7	100%
	9:10	4015.5	3682.7	92%
	7:10	3123.2	3025.1	97%
	5:10	2230.8	2306.4	103%
	3:10	1338.5	1437.9	107%
	1:10	446.2	469.0	105%
Serum 2	Undiluted	501.3	501.3	100%
	9:10	451.2	448.0	99%
	7:10	350.9	346.5	99%
	5:10	250.6	254.6	102%
	3:10	150.4	155.5	103%
	1:10	50.1	51.7	103%
Serum 3	Undiluted	63.1	63.1	100%
	9:10	56.7	54.3	96%
	7:10	44.1	41.5	94%
	5:10	31.5	29.6	94%
	3:10	18.9	17.4	92%
	1:10	6.3	5.8	91%

[a] % Recovery = $\frac{\text{Mean Observed Value}}{\text{Mean Expected Value}}$ x 100

* Representative data; results in individual laboratories may vary from these data.

Autodilution Verification

The ARCHITECT ProGRP assay is designed to have a mean recovery of 100 ± 10% by autodilution.

Recovery performance was evaluated for the autodilution method of the ARCHITECT ProGRP assay by testing specimens with undiluted values that ranged between 4010 and 4138 pg/mL. Data from this study are summarized in the following table.*

Sample	Mean Undiluted Value (pg/mL)	Mean Observed Value (pg/mL)	% Recovery [a]
Serum 1	4038.0	3741.4	93%
Serum 2	4111.2	3749.4	91%
Serum 3	4111.0	3863.3	94%
Plasma 1	4138.3	3765.8	91%
Plasma 2	4127.7	3706.5	90%
Plasma 3	4010.7	3675.1	92%

[a] % Recovery = $\frac{\text{Mean Observed Value}}{\text{Mean Undiluted Value}}$ x 100

* Representative data; results in individual laboratories may vary from these data.

Sensitivity

Functional Sensitivity

The ARCHITECT ProGRP assay is designed to have a functional sensitivity of ≤ 3 pg/mL at a total CV of 20%.

In a study, serum panels ranging in concentration from 0.17 - 5.26 pg/mL were tested in replicates of ten over five days on two instruments using three reagent lots and one set of calibrators for a total of 300 replicates per panel. The total %CVs (combining variance components for replicate, run, instrument, day and reagent lot) were calculated and plotted against the mean concentration. A reciprocal curve was fitted through the data and the functional sensitivity value was calculated as the concentration corresponding to the 20% CV level of the fitted curve. The lowest ARCHITECT ProGRP assay value exhibiting a 20% CV was calculated to be 0.89 pg/mL.*

* Representative data; results in individual laboratories may vary from these data.

Limit of Detection

The ARCHITECT ProGRP assay is designed to have a limit of detection of ≤ 3 pg/mL.

A study was conducted based on guidance from the CLSI, Protocol EP17-A2. The LoD was calculated to be 0.50 pg/mL.*

* Representative data; results in individual laboratories may vary from these data.

Specificity

The specificity of the ARCHITECT ProGRP assay is designed to have ≤ 10% cross-reactivity when tested with gastrin-releasing peptide (GRP) at a concentration of 100 ng/mL.

A study was performed with the ARCHITECT ProGRP assay based on guidance from the CLSI, Protocol EP7-A2. Aliquots of human serum and plasma, containing ProGRP across the range of 25 to 75 pg/mL, were supplemented with GRP at a concentration of 100 ng/mL and tested for ProGRP. Cross-reactivity of GRP was calculated to be less than 0.01%.*

* Representative data; results in individual laboratories may vary from these data.

Interference

Potential interference in the ARCHITECT ProGRP assay from hemoglobin, bilirubin, triglycerides, red blood cells, and total protein is designed to be ≤ 10%.

A study based on guidance from the CLSI, Protocol EP7-A2 was performed for the ARCHITECT ProGRP assay. Specimens with ProGRP levels between 4.3 and 224.6 pg/mL were supplemented with the following potentially interfering compounds. The average amount of interference observed during the study ranged from 1% to 6%.*

Potentially Interfering Substance	Potentially Interfering Substance Concentration	Mean % Interference
Triglycerides	3000 mg/dL	5%
Bilirubin	20 mg/dL	1%
Protein (High)	12 g/dL	4%
Protein (Low)	3 g/dL	4%
Hemoglobin	500 mg/dL	6%
Red Blood Cells	0.4 %	5%

* Representative data; results in individual laboratories may vary from these data.

Evaluation of Drug Interference

Potential interference in the ARCHITECT ProGRP assay from the following drugs is designed to be ≤ 10% at the levels indicated.

A study based on guidance from the CLSI, Protocol EP7-A2 was performed for the ARCHITECT ProGRP assay. Specimens with ProGRP levels spiked between 6.8 to 230.7 pg/mL were supplemented with various drugs.

The average amount of interference observed during the study ranged from 1% to 3%.*

Drug	Drug Concentration	Drug	Drug Concentration
Carboplatin	110.8 mg/L	Ifosfamide	0.8 mg/L
Cisplatin	7.2 mg/L	Irinotecan	11.5 mg/L
Cyclophosphamide	4.1 mg/L	Paclitaxel	13.5 µg/L
Docetaxel	4.6 mg/L	Topotecan	0.2 mg/L
Doxorubicin	0.4 mM	Vincristine	0.1 mg/L
Etoposide	80 mg/L	Vinorelbine	2.0 mg/L
Gemcitabine	43.7 mg/L		

* Representative data; results in individual laboratories may vary from these data.

Evaluation of Other Potential Interferents

Potential interference in the ARCHITECT ProGRP assay from HAMA and rheumatoid factor (RF) is designed to be ≤ 10%.

In a study, the ARCHITECT ProGRP assay was evaluated by testing specimens with HAMA and RF to further assess the clinical specificity. Specimens positive for HAMA and specimens positive for RF were evaluated for % interference with ProGRP levels spiked between 72.2 and 114.6 pg/mL. Mean % interference is summarized in the following table.*

Other Potential Interferents	Number of Specimens	Mean % Interference
HAMA Positive Serum	15	3%
HAMA Positive Plasma	5	5%
RF Positive Serum	10	6%

* Representative data; results in individual laboratories may vary from these data.

Method Comparison

The ARCHITECT ProGRP assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient of ≥ 0.9 when compared to Fujirebio Serum-Labo ProGRP (ELISA) and tested with serum specimens.*

A study was performed with the ARCHITECT ProGRP assay, where regression analysis was performed using the Passing-Bablok method.[a,26] In this evaluation, the NSCLC and SCLC specimens of 5000 pg/mL or lower were tested. Data from this study are summarized in the following table.**

Number of Specimens	Slope (95% CI)[a]	Intercept (95% CI)[a]	Correlation Coefficient (r)
204	1.00	0.51	0.99
	(0.98 to 1.03)	(-0.96 to 2.00)	

[a] A linear regression method with no special assumptions regarding the distribution of samples and measurement errors.

* Serum samples were used in the Method Comparison study in which the comparison assay, Fujirebio Serum-Labo ProGRP (ELISA), requires the use of this sample type. Serum specimens may exhibit decreased values due to degradation of ProGRP by endogenous proteases generated during the clotting process.

** Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

In this evaluation, specimen concentration ranged from 12.3 pg/mL to 4005 pg/mL with the ARCHITECT ProGRP assay and from 9.7 pg/mL to 3604 pg/mL with the Fujirebio Serum-Labo ProGRP (ELISA).

Correlation between Serum and Plasma Specimens

A study was performed to compare serum and EDTA plasma pair specimen results with the ARCHITECT ProGRP assay, where regression analysis was performed using the Passing-Bablok method.[a,26] In this evaluation, the NSCLC, SCLC and benign lung disease specimens were tested. Data from this study are summarized in the following table.*

Number of Specimens	Slope (95% CI)[a]	Intercept (95% CI)[a]	Correlation Coefficient (r)
301	1.14	1.31	0.99
	(1.12 to 1.16)	(0.39 to 2.20)	

[a] A linear regression method with no special assumptions regarding the distribution of samples and measurement errors.

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

In this evaluation, specimen concentration ranged from 12.3 pg/mL to 4005 pg/mL with serum and from 16.6 pg/mL to 4336 pg/mL with plasma.

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