Procalcitonin (PCT) Multi-Purpose (MPR) Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL401PC	1 x 44 ml	PCAL R1	2 - 8°C
	1 x 14 ml	PCAL R2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination PCT in human serum, EDTA or lithium heparin plasma. Measurement of PCT in conjunction with other laboratory findings and clinical assessments aids in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock.

SUMMARY AND EXPLANATION:

Procalcitonin (PCT) is a 116 amino acid protein, the prohormone of calcitonin. Whereas hormonally active calcitonin is produced exclusively in the C-cells of the thyroid gland after specific intracellular proteolytic procession of the prohormone PCT, PCT is ubiquitously and uniformly expressed in multiple tissues throughout the body in response to sepsis[1]. In healthy conditions, the PCT levels in circulation are very low (< 0.05 ng/ml). Elevated circulating levels of PCT are important indicators in response to microbial infections and a powerful tool in the early detection of sepsis[2-8]. Elevated PCT may not always be caused by systemic bacterial infection[2]. If there is a disagreement between the laboratory findings and the clinical signs, additional tests should be performed.

PRINCIPLE OF THE TEST:

Glenbio's PCT Assay is based on a latex enhanced immunoturbidimetric assay. PCT proteins in the sample bind to the specific anti-PCT antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of PCT in the sample. The instrument calculates the PCT concentration of a sample by interpolation of the obtained signal of a 6-point calibration curve.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request. The reagents contain sodium azide (<0.1%) as a preservative. Do not ingest. Avoid contact with skin and eyes.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Do not mix reagents from different lots.
- Refer to local legal requirements for safe waste disposal.

Note: The PCT Assay is not indicated to be used as a stand-alone diagnostic assay and should be used in conjunction with clinical signs and symptoms of infection and other diagnostic evidence. It is not indicated to be used as an aid in decision making on antibiotic therapy for patients[11]. Certain patient characteristics, such as severity of renal failure or insufficiency, may influence procalcitonin values and should be considered as potentially confounding clinical factors when interpreting PCT values. Increased PCT levels may be observed in severe illness such as polytrauma, burns, major surgery, prolonged or cardiogenic shock.

COMPONENT COMPOSITION:

Reagent 1	100 mM Tris-buffer solution, ready to use
Reagent 2	Suspension of anti-human PCT antibody coated latex particles (0.2%), ready to use

REAGENT PREPARATION AND STABILITY:

Reagents 1 & 2 are liquid stable and ready to use.

If stored at 2 – 8°C and handled properly, components are stable until expiry date stated on the label.

TYPE OF SPECIMEN:

The Glenbio PCT Assay is formulated for use with serum or lithium heparin and EDTA plasma. For monitoring purpose, the same sample matrix should always be used. PCT increases about 3 hours after bacterial infection, reaching maximum values after 6-12 hours with a half-life of 25 to 30 hours [2, 3, 8]. PCT is relatively stable in both plasma and serum samples, and require no special requirements for pre-analytical sample handling. Samples stored at 2-8°C are stable for three days and also stable at room temperature for 24 hours. When stored at -20°C, serum samples are stable for 6 months and plasma samples are stable for 2 months. 4 cycles of freeze/thaw has <10% loss of PCT in sample. Turbid samples must be clarified by centrifugation.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalogue No.	Description	Catalogue No.
PCT Controls	GL4011	General Laboratory Equipment	N/A
PCT Calibrator	GL4012	Analyser & Consumables	N/A
0.9% Saline is needed as Cal 0	N/A		N/A

Assay procedure:
Refer to relevant application sheets. Refer to user's manual for instructions on instrument start-up, loading components and samples, calibration, sample testing procedures, calculating and reporting results.

Calibration:
Using recommended Calibrator, calibrate the assay:
- Bi-weekly.
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the analyser in use.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory QC Programme.

RESULTS:

Results are printed out in ng/mL.

Note: samples with values greater than 52 ng/mL should be manually diluted 1:4 with 0.9% saline and rerun. Multiply results by dilution factor 5.

In agreement with the literature[2-4, 9-10] the clinical sensitivity results obtained with Glenbio PCT Assay using samples from patients admitted to ICU revealed that a concentration < 0.5 ng/mL represents a low risk of severe sepsis and/or septic shock; a concentration > 2 ng/mL represents a high risk of severe sepsis and/or septic shock. Nevertheless, concentrations < 0.5 ng/mL do not exclude local infection or a systemic infection in its initial stages (< 6 hours). Furthermore, increased PCT levels can occur without infection. PCT concentrations between 0.5 and 2.0 ng/mL should be interpreted considering the patient's history. It is recommended to retest PCT within 6-24 hours if any concentrations < 2 ng/mL are obtained.

NORMAL RANGE:

To establish the reference interval of normal population for Glenbio PCT Assay, serum samples from 216 apparently healthy adults ≥ 21 years of age were tested using the PCT Assay according to CLSI C28-A3 guideline. The central 95% reference interval was established to be from 0.02 to 0.30 ng/mL.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linear up to 52 ng/mL.

Detection Limits:
Limit of Blank (LoB) – 0.06 ng/mL.
Limit of Detection (LoD) – 0.16 ng/mL.
Limit of Quantitation (LoQ) – 0.20 ng/mL.

Prozone:
No high dose hook effect was observed up to 300 ng/mL PCT. PCT was recovered ≥ 10 ng/mL when PCT up to 2700 ng/mL.

Precision:

Sample	Mean ng/mL	Within-Run SD CV%	Between-Run SD CV%	Between-day SD CV%	Between-lot SD CV%	Total SD CV%
1	0.27	0.034 12.3%	0.027 10.0%	0.019 6.9%	0.047 17.3%	0.047 17.3%
2	0.48	0.035 7.3%	0.033 6.8%	0.031 6.4%	0.057 11.8%	0.057 11.9%
3	1.80	0.062 3.4%	0.032 1.8%	0.059 3.2%	0.09 5.0%	0.091 5.0%
4	5.30	0.085 1.6%	0.140 2.6%	0.130 2.5%	0.21 3.9%	0.209 4.0%
5	23.56	0.058 2.5%	0.482 2.0%	0.891 3.8%	1.20 5.1%	1.168 5.0%
6	47.65	0.069 1.5%	0.712 1.5%	0.657 1.4%	1.18 2.5%	1.196 2.5%
Con 1	1.16	0.046 4.0%	0.038 3.3%	0.031 2.7%	0.07 5.8%	0.068 5.8%
Con 2	18.30	0.477 2.6%	0.126 0.7%	0.751 4.1%	0.88 4.8%	0.899 4.9%

Interfering substances
The following substances normally present in the samples produced less than 10% deviation when tested at levels equal to the concentrations listed below:

Interference Substances	Concentration
Ascorbic acid	129 mg/dL
Free Bilirubin	30 mg/dL
Bilirubin Conjugated	30 mg/dL
Hemoglobin	750 mg/dL
Triglyceride	750 mg/dL
Rheumatoid Factor	75 IU/mL
Albumin	4 g/dL
Human Calcitonin	60 ng/mL
Human Katacalcin	10 ng/mL
Human alpha-CGRP	10 µg/mL
Human beta-CGRP	10 µg/mL
Human Anti-mouse IgG (HAMA)	350

The following therapeutic drugs showed no significant interference (< ± 10%) up to the concentrations summarized below:

Tested Drugs	Concentration
Imipenem	0.5 mg/mL
Cefotaxime	180 mg/dL
Noradrenalin	4 µg/mL
Dobutamine	22.4 µg/mL
unfractionated Heparin	16,000 U/L
Furosemide	4 mg/dL
Vancomycin	3 mg/mL
Dopamine	26 mg/dL

Method Comparison:

The method comparison of the assay was evaluated following CLSI EP9-A2 protocol. A total of 219 serum samples ranging from 0.21-51.26 ng/mL were tested at three different sites by three different operators on three Olympus (Beckman) AU400 chemistry analyzers. The results were compared with a predicate assay, the percentage of concordance between the two assays at cutoffs 0.5 ng/mL and 2.0 ng/mL are 94.5% and 95.0% respectively.

Results with a cut-off at 0.5 ng/mL (3x3 table):

Glenbio PCT	Predicate PCT			Total
	≤ 0.5 ng/ml	0.5 ng/mL< PCT≤ 2.0 ng/ml	> 2.0 ng/mL	
≤ 0.5 ng/ml	29	7	0	36
0.5 ng/mL< PCT≤ 2.0 ng/ml	4	96	7	107
> 2.0 ng/mL	1	3	72	76
Total	34	185		219

Negative percent agreement (NPA) =85.3% (29/34), 95% CI: 69.9 to 93.6%
Positive percent agreement (PPA) = 96.2% (178/185), 95% CI: 92.4 to 98.2%

Revision: 02 Issued on: 26 July 2021

Results with a cut-off at 2.0 ng/mL:

Glenbio PCT	Predicate PCT			Total
	≤ 0.5 ng/ml	0.5 ng/mL< PCT≤ 2.0 ng/ml	> 2.0 ng/mL	
≤ 0.5 ng/ml	29	7	0	36
0.5 ng/mL< PCT≤ 2.0 ng/ml	4	96	7	107
> 2.0 ng/mL	1	3	72	76
Total	140		79	219

Negative percent agreement (NPA) = 97.1% (136/140), 95% CI: 92.9 to 98.9%
Positive percent agreement (PPA) = 91.1% (72/79), 95% CI: 82.8 to 95.6%

The results of regression analysis are summarized in the following tables (Regression analysis were similar for all sites):

Parameter	Regular Linear	Regular Deming (λ = 1)	Weighted Deming (λ = 1)*	Passing Bablock
n	219	219	219	219
Slope	1.041	1.050	0.866	0.944
95% CI	1.023 to 1.059	1.032 to 1.068	0.796 to 0.917	0.882 to 0.984
Intercept	-0.225	-0.259	0.100	0.001
95% CI	-0.376 to -0.075	-0.410 to -0.109	-0.055 to 0.146	-0.090 to 0.057
R^2	0.9837	0.9837	0.9837	0.9837
Sample Range	0.21-51.26 ng/mL	0.21-51.26 ng/mL	0.21-51.26 ng/mL	0.21-51.26 ng/mL

*The Weighted Deming regression is a modification of Deming regression assuming the ratio of the coefficient of variation (CV), rather than the ratio of variances (SD), is constant across the measuring interval. Therefore the fit is very influenced by the low concentration points, not much by high concentration points. (Reference CLSI EP09-A3).

Regular regression:

Parameter	Site 1	Site 2	Site 3	Total
n	49	53	117	219
Slope	1.051	1.051	1.023	1.041
95% CI	1.005 to 1.096	1.010 to 1.093	1.006 to 1.041	1.023 to 1.059
Intercept	-0.318	-0.239	-0.172	-0.225
95% CI	-0.809 to 0.172	-0.627 to 0.150	-0.287 to -0.056	-0.376 to -0.075
R2	0.9785	0.9807	1.023	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

Deming regression:

Parameter	Site 1	Site 2	Site 3	Total
n	49	53	117	219
Slope	1.063	1.062	1.028	1.050
95% CI	1.017 to 1.109	1.021 to 1.104	1.010 to 1.045	1.032 to 1.068
Intercept	-0.384	-0.287	-0.184	-0.259
95% CI	-0.876 to 0.108	-0.677 to 0.102	-0.300 to -0.069	-0.410 to -0.109
R2	0.9785	0.9807	0.9916	0.9837
Sample Range	0.23-51.26 ng/mL	0.24-50.60 ng/mL	0.21-51.11 ng/mL	0.21-51.26 ng/mL

Clinical Sensitivity:

Clinical sensitivity studies were conducted by testing 116 serum samples from patients on their first day of ICU admission. Patient selection criteria: Patients with trauma, surgery, burns or prolonged or severe cardiogenic shock were excluded. Patient age ranged from 21 to 93. Patients were classified based on the ACCP/SCCM consensus criteria: 4 of No Infection patients (I), 26 of SIRS patients (II), 18 of Sepsis (III), 36 of Severe sepsis patients (IV), and 32 of Septic shock patients (V).
The PCT levels of the group patients with no infection or SIRS or sepsis versus severe sepsis or septic shock with cutoffs at 0.5 ng/mL and 2.0 ng/mL were as follows:

Results with a cut-off at 0.5 ng/mL:

Glenbio PCT level	Classification results					
	No Infection/SIRS/Sepsis			Severe Sepsis/Septic Shock		Total
	No Infection	SIRS	Sepsis	Severe Sepsis	Septic Shock	
≤0.5 ng/mL	2	15	4	0	0	21
0.5 ng/mL < PCT ≤ 2.0 ng/ml	2	10	11	2	0	25
>2.0 ng/mL	0	1	3	34	32	70
Sub-total	4	26	18	36	32	116
Total	48			68		116

NPA-negative percent agreement (Specificity), 43.8% (21/48); 95% CI: 30.7 to 57.7%
PPA-positive percent agreement (Sensitivity), 100% (68/68); 95% CI: 94.7 to 100%

Results with a cut-off at 2.0 ng/mL:

Glenbio PCT level	Classification results					
	No Infection/SIRS/Sepsis			Severe Sepsis/Septic Shock		Total
	No Infection	SIRS	Sepsis	Severe Sepsis	Septic Shock	
≤0.5 ng/mL	2	15	4	0	0	21
0.5 ng/mL < PCT ≤ 2.0 ng/ml	2	10	11	2	0	25
>2.0 ng/mL	0	1	3	34	32	70
Sub-total	4	26	18	36	32	116
Total	48			68		116

NPA-negative percent agreement (Specificity), 91.7% (44/48); 95% CI: 80.4 to 96.7%
PPA-positive percent agreement (Sensitivity), 97.1% (66/68); 95% CI: 89.9 to 99.2%

BIBLIOGRAPHY:

1. Müller B, et al., Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab 2001; 86(1):396-404.
2. Meisner M. Procalcitonin (PCT) – A new, innovative infection parameter. Biochemical and clinical aspects. Thieme; Stuttgart, New York, 2000; ISBN 3-13-105503-0.
3. Christ-Crain M, et al., Procalcitonin in bacterial infections – hype, hope or more or less? Swiss Med Wkly 2005; 135: 451-60.
4. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992; 101: 1644-55
5. Christ-Crain M, Jaccard-Stolz D, Bingisser R, Gencay MM, Huber PR, Tamm M, Muller B. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. Lancet 2004; 363:600-7.
6. Christ-Crain M, Stolz D, Bingisser R, Muller C, Miedinger D, Huber PR, Zimmerli W, Harbarth S, Tamm M, Muller B. Procalcitonin Guidance of Antibiotic Therapy in Community-acquired Pneumonia: A Randomized Trial. Am J Respir Crit Care Med 2006; 174:84-93.
7. Stolz D, Christ-Crain M, Bingisser R, Leuppi J, Miedinger D, Muller C, Huber P, Muller B, Tamm M. Antibiotic treatment of exacerbations of COPD: a randomized, controlled trial comparing procalcitonin-guidance with standard therapy. Chest 2007; 131:9-19.
8. Schuetz P, et al., Long-term stability of procalcitonin in frozen samples and comparison of Kryptor and VIDAS automated immunoassays. Clin Biochem 2010; 43(3):341-4.
9. Muller B. et al., Calcitonin precursors are reliable markers of sepsis in medical intensive care unit. Crit Care Med 2000; 28(4): 977-983.
10. Harbarth S. et al., Diagnostic value of procalcitonin, interleukin-6 and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Resp Crit Care Med 2001; 164: 396-402.
11. Schuetz, P. et al., Procalcitonin to guide initiation and duration of antibiotic treatment in acute respiratory infections: an individual patient data meta-analysis. Clin Infect Dis 2012: 55(5): 651-662.

SYMBOLS:

The following symbols are used in the labelling of Glenbio Ltd. systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	CE	CE Mark - Device comply with the Directives 98/79/EC
	Storage temperature		Consult Instruction for Use
	Expiry Date (Last day of the month)		Manufactured By

GLENBIO LTD
10 Kilbegs Road, Antrim, Co. Antrim, BT41 4NN
Tel/Fax: +44(0)2879659842
Email: info@glenbio.com
Web: www.glenbio.com

EC REP GLENBIO IRELAND LTD
17b Fota Business Park, Carrigtwohill, Co. Cork,
T45 PK77, Ireland

* For Reagent Instrument Application Settings please contact: **applications@glenbio.com**

Revision: 02 Issued on: 26 July 2021