IL-6

Porrima

Interleukin-6 (CLIA)

REF: PM037703 **100**

Intended use
The Porrima IL-6 assay is a Chemiluminescent Immunoassay (CLIA) for the quantitative determination of Interleukin-6 (IL-6) in **Human Serum**. For professional use only.

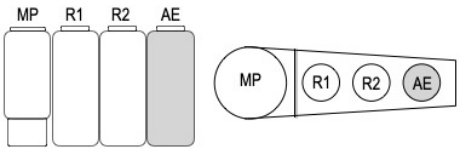
Summary
References[1-4]
IL-6, a key mediator for inflammation and an early alarm signal of infection that becomes elevated as part of the inflammatory response, has emerged as a valuable biomarker in the management of sepsis.
In a study of 1,032 patients with severe trauma, patients who subsequently developed septic complications had the highest IL-6 levels on day 1 following injury. Similarly, in a study of 50 patients following major surgery, IL-6 levels were correlated with the development of septic complications during the first 5 days following surgery (area under the curve [AUC] 0.82; 95% CI: 0.66 – 0.98), with a sensitivity of 90 % and selectivity of 58%. Furthermore, when IL-6 levels and clinical indicators were combined, sensitivity and selectivity increased to 100% and 79%, respectively.
Early peak IL-6 levels correlate significantly with the development of SIRS and sepsis. The degree of elevation in IL-6 levels can be used to differentiate SIRS from severe sepsis and septic shock, with higher IL-6 levels correlating with increased severity.
As a marker for systemic inflammation, high IL-6 levels may be predictive of future organ dysfunction. In addition, continually elevated IL-6 levels have been reported to be predictive of mortality in patients with sepsis.

Test principle
Two-step Sandwich principle. Total duration of assay: **25 minutes**
In the first incubation, sample and paramagnetic microparticles coated with monoclonal anti-IL-6 antibody are added into a reaction vessel. After incubation, microparticle is magnetically captured while other unbound substances are removed by washing.
In the second incubation, add monoclonal anti-IL-6 antibody-acridinium labeled conjugate to reaction mixture to form a sandwich complex, the complex becomes bound to the solid phase while other unbound Conjugate are removed by washing. Pre-Trigger and Trigger Solutions are then added to the reaction mixture, the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of IL-6 in the sample and the RLUs detected by the Porrima system.

Materials provided

MP	1 x 5.0 mL, anti-IL-6 antibody (mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizers with preservative.
AE	1 x 10 mL, monoclonal anti-IL-6 antibody (mouse)-acridinium ester labeled conjugate in MES buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
C0	Calibrator, 1 vial, 1.0 mL, ready to use.
C1	Calibrator, 1 vial, 1.0 mL, ready to use.
C2	Calibrator, 1 vial, 1.0 mL, ready to use.

The position of each reagent component is shown in the figure below (front view on the left and top view on the right):



Materials required (but not provided)
- Porrima Chemiluminescent Immunoassay Analyzer
- CT2201 DiaSino ControlSet: 2 levels, L and H
- TR7701 Porrima TriggerPack: ①Pre-Trigger Solution, ②Trigger Solution
- WB7701 Porrima Wash Buffer (20X)
- RV7701 Porrima Reaction Vessels
- PW7701 Porrima Probe Wash Buffer
- SC7701 Porrima Sample Tubes (D13x75mm, D13x100mm or D16x100mm can be acceptable on Porrima system)

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/ face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Reagent handling
The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability
- Store at 2-8°C. Do not freeze.
- Store the Porrima reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.
- The Porrima IL-6 reagent kit can be stored onboard and used for a maximum of 28 days after opening at 2-8°C.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Centrifuge the specimens after clot formation is complete. Transfer the supernatants into tubes for storage or test within two hours after centrifugation.
- Specimens should be tested as soon as possible after sample collection. If testing is not completed within 8 hours, specimens should be tightly capped and refrigerated at 2-8°C. If testing will be delayed for more than 72 hours, specimens should be frozen at -20°C or below.
- Avoid repeated freeze and thaw cycles.

Assay procedure
- For optimal performance of this assay, operators should read the Porrima CLIA system operation manual carefully, to get sufficient information such as operation instructions, sample preservation and management, safety precaution, and maintenance. Prepare all required materials for the assay as well.
- Before loading the reagent kit on the machine for the first time, unopened reagent bottle should be inverted gently for at least 30 times to resuspend the microparticles that have settled during shipment or storage.
- Visually inspect the bottle to ensure the microparticles have been resuspended. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely resuspended.
- If the microparticles cannot be resuspended, **DO NOT USE**. Contact DiaSino Technical Support for help. Do not invert opened reagent bottle.
- This assay requires 100 µL of sample for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration
Traceability: The Porrima IL-6 CLIA has been standardized against the NIBSC (National Institute for Biological Standards and Control) 1st IS 89/548 Standard.
The specific information of master calibration curve of IL-6 CLIA reagent kit is stored in the barcode attached in the reagent pack. It's used together with calibrators for the calibration of the specific reagent lot. When performing the calibration, scan the information of master calibration curve from the barcode into the system first, and then use the calibrators at two levels. Valid calibration curve is required before any IL-6 test. Recalibration is recommended every 28 days, or when a new reagent lot is used, or the quality controls are out of specified range. For detailed instruction of calibration, refer to the system operations manual.

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Quality control
For quality control, use DiaSino ControlSet. In addition, other suitable control material can be used.
Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Examine the assay system referring to the system operation manual. If the quality control results are still out of the specified range, please contact DiaSino Technical Support for help.

Calculation
The analyzer automatically calculates the analyte concentration of each sample.

Limitations - interference
- The false positive results include cross-reactions with some components of serum from individual to antibodies; and non-specific adhesion of some components in human blood that have similar epitopes to capture and detector antibodies.
- In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of IL-6 antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions.
- Plasma using anticoagulants (e.g. heparin or citrate) other than EDTA has not been evaluated in Porrima IL-6 assay and thus should not be used.
- Other factors may interfere with Porrima IL-6 assay and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
0.5-3000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.5 pg/mL. Values above the measuring range are reported as
> 3000 pg/mL.

Lower detection limit
0.5 pg/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n = 21).

Expected values
0-7.0 pg/mL
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human Serum 1	5.17	0.39	7.64	0.42	8.13
Human Serum 2	74.38	4.06	5.46	4.75	6.39
Human Serum 3	262.96	10.97	4.17	13.73	5.22
ControlSet 1	46.52	2.67	5.75	3.32	7.14
ControlSet 2	351.76	15.93	4.53	22.02	6.26

Method comparison
A comparison of the Porrima IL-6 assay (y) with the Roche Elecsys IL-6 (x) using clinical samples gave the following correlations:
Number of samples measured: 58
Linear regression
y = 1.016x + 0.178
r = 0.9725

Analytical specificity
The Porrima IL-6 assay does not show any significant cross-reactivity with the following substances, tested with IL-6 concentrations of approximately 1 pg/mL and 3 pg/mL (maximum tested concentration):

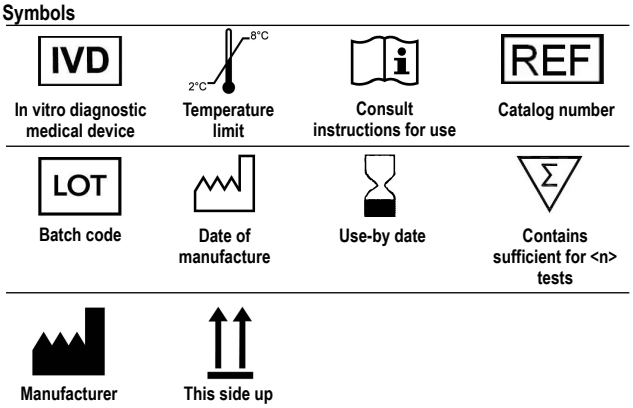
Substances	Non-interfering concentrations (pg/mL)
Interleukin-1α	3000
Interleukin-1β	3000
Interleukin-2	3000
Interleukin-3	3000
Interleukin-4	3000
Interleukin-8	3000
Interferon-γ	3000
TNF-α	3000

Functional sensitivity
0.60 pg/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

1. Barker, S.B., "Determination of Protein Bound Iodine."
2. *Journal Biological Chemistry*, 173, 175, (1984).
3. Caldwell, G et al, "A new Strategy for Thyroid Test in the Routine Laboratory Tests." *Lancet*, I, 1117 (1985).
4. Young DS, Pestaner LC, and Gilberman U, "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry*, 21, 3660 (1975)

Symbols



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